3D-Printable Non-invasive Head Immobilization System for Non-Human Primates

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Abstract

We introduce a novel 3D-printed non-invasive head immobilization system (NHIS) for macaque monkeys, addressing the limitations of traditional head fixation methods in behavioral experiments. Traditional techniques, crucial for precise eye movement tracking and neural recording, often lead to biological complications like infection and bone softening. Our innovative NHIS, designed using CT/MRI scans and custom software FLoRIN, offers a tailored, comfortable fit for each subject. It accommodates essential experimental equipment such as eye-tracking and physiological monitoring devices, providing a more adaptable and efficient alternative for nonhuman primate research.

Introduction

Head fixation is crucial in behavioral experiments involving nonhuman primates, particularly for tasks requiring precise eye movement tracking and involving any kind of neural recording or stimulation, like electrophysiological studies and optogenetic interventions. The foundational work of Evarts [1] and Mountcastle [2] marked the advent of long-term head fixation techniques, employing stainless steel bolts anchored in the skull to affix an acrylic head cap, which, in turn, was secured to a primate chair for head immobilization.

While the Mountcastle method, predominantly composed of acrylic, proved cost-effective and relatively durable, it posed significant biological challenges. The expansive acrylic cap necessitated extensive removal of soft tissue, potentially disrupting bone healing and creating interfaces susceptible to infection. This required intensive maintenance and the potential use of antibiotics. Moreover, the cap's inadequate adhesion to healthy bone could foster bacterial colonization, leading to bone necrosis and implant failure.

In response to these drawbacks, subsequent research has spurred the development of alternative head fixation mechanisms. Innovations from various laboratories [1,3–7], have sought to eliminate the acrylic cap. Betelak et al., for instance, introduced a skull-anchored hardware solution that replaced the top section of the frontal bone with a surgically inserted skull cap featuring a replaceable Delrin plug.

Building on these advancements, Adams et al. introduced an improved design utilizing titanium and a K-shaped leg contoured during surgery to align with the skull's curvature. This system employs a 60 mm leg secured by 12 to 14 screw holes that need 2.7-mm-diameter × 8-mm-long titanium cortex screws, ensuring a robust connection to the skull. This contemporary approach has been rapidly adopted and is now prevalent in various research laboratories.

Although existing head stabilization techniques effectively immobilize the subject's head, they present long-term complications such as an increased risk of intracranial infection, necrosis, and bone softening around the head post due to the high torques applied during experiments. The dense clustering of 12-18 screws and the bone beneath the titanium hardware creates infection-prone conditions. Once infection ensues, leading to bone softening and potentially open wounds, the removal of the head post does not typically render the skull suitable for reimplantation, compromising device stability and integrity.

In response to these challenges, research has pivoted towards developing less invasive head fixation methods that do not rely on bolts screwed into the skull [4,7–12]. These methods have successfully achieved rigid head fixation while reducing invasiveness; however, minor skin incisions remain necessary during installation.

Non-invasive head fixation methods in non-human primates have several limitations. The requirement for numerous components to be individually attached, arranged, and calibrated for each subject poses a challenge, particularly when multiple monkeys share the same apparatus. Additionally, these methods necessitate molding the monkey's face under mild sedation, which can be cumbersome and time-consuming. Using helmets made from thermoplastic or plastic, filled with expandable foam for a snug fit, often restricts the concurrent use of devices necessary for optogenetics or electrophysiology ports. Furthermore, the process involves vacuuming the subject's head, which can be uncomfortable and potentially anxiety-inducing, prolonging and complicating the training phase. Lastly, these systems need easier reproducibility due to changes in the monkey's head size over time or after subsequent implantation, presenting an obstacle to consistent long-term use.

None of these methods facilitate experimental protocols involving the animal's voluntary participation, such as data collection at the cage side. Although various non-invasive head immobilization systems (NHIS) exist, their widespread adoption has been limited, likely due to the complexity of their construction and the challenge of adapting them to a range of research needs.

Here, we designed and implemented an innovative 3D-printed non-invasive head immobilization system (NHIS) tailored explicitly for macaque monkeys. This system is streamlined for easy production and customization. Utilizing CT/MRI scans of the monkey's head and our bespoke software, FLoRIN[13], we engineered a 3D-printable helmet. This helmet is uniquely designed with specific cutouts to accommodate eye-tracking equipment, reward delivery mechanisms, and physiological monitoring hardware.

Method

Making a 3D base model

Our innovative approach involves creating a unique 3D-printed mask for each nonhuman primate, specifically designed to fit the individual subject's face. This method eliminates the need for physical molds or one-size-fits-all models. We use MRI imaging of the subject's head and neck to achieve this. We then employed FLoRIN, software developed initially by Shahbazi et al. [13], for reconstructing neurons and membranes from high-resolution microscopy images [14,15], which we have adapted for creating a precise 3D model of the subject's face, skull, and brain, ensuring a tailored and accurate fit for each mask.

Notably, FLORIN's 3D modeling accurately reflects the current physical state of the subject's head, accounting for potential surgical implants.

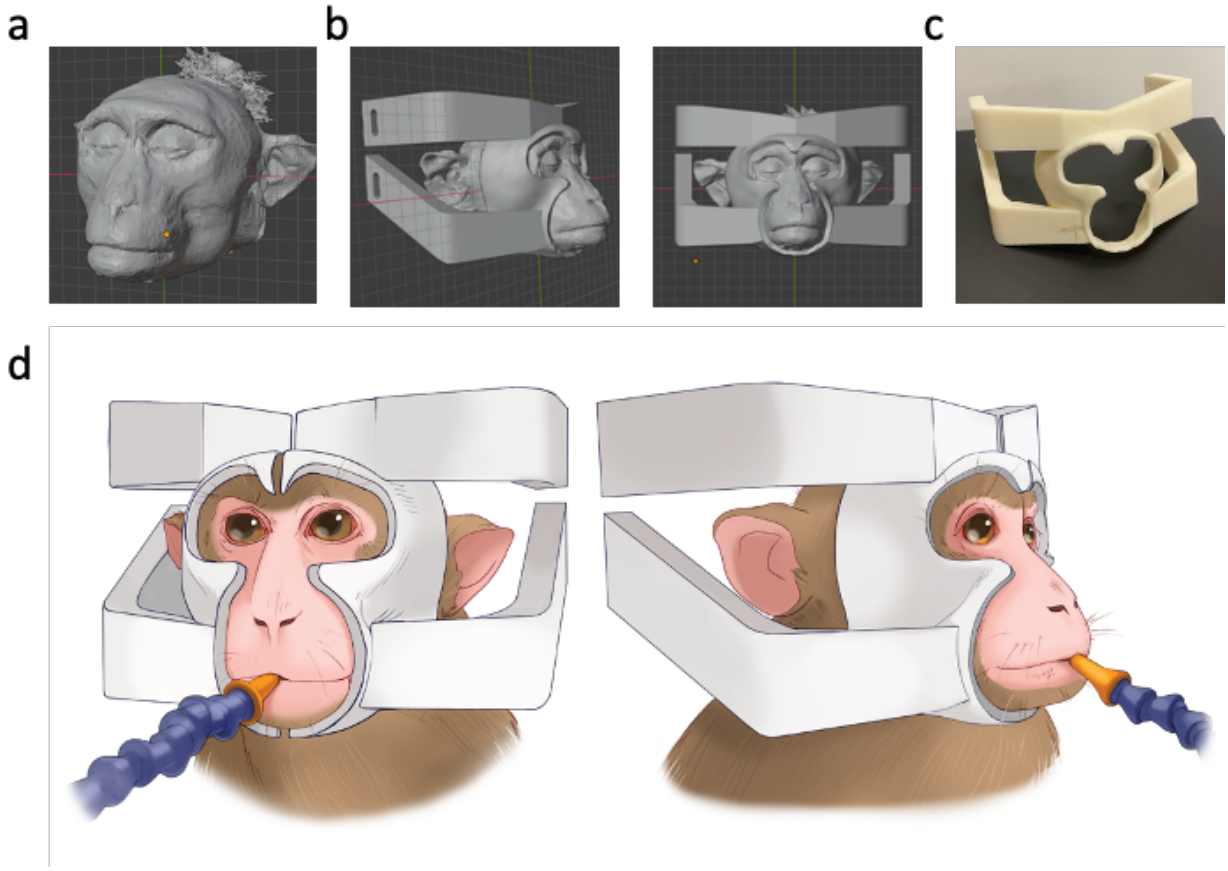

Figure 1 - Overview of NHIS Design and Implementation: a) 3D facial reconstruction using FLoRIN based on MRI imaging of the monkey, providing a detailed and accurate model of the subject's facial anatomy. b) Design of the Non-Invasive Head Immobilization System (NHIS) using Blender, illustrating the helmet's structure and various connecting components. c) Completed 3D-printed helmet, showcasing the final product ready for use. d) Illustrative depiction of the training's second phase, highlighting the monkey's voluntary engagement with the mask to obtain rewards, demonstrating the system's animal-friendly approach.

Mask design

Blender, software that allows the design to be exported as code, facilitates mask creation. In Blender, we design a surface with strategically placed openings for the eyes and mouth, considering facial bone structures like the Maxillary bones and lower jaw. This design ensures subject comfort, allows free breathing and unobstructed vision, and accommodates liquid reward dispensers.

The designed surface is then conformed to the 3D facial model, maintaining a default distance of 1mm from the subject's face. This design features an upper part covering the forehead and frontal bone. A chin rest protects the edges around the Maxillary bone and lower jaw. Importantly, this design leaves the top of the skull unobstructed, facilitating the use of implanted ports for optogenetics, electrophysiology, or other cranial chambers and devices.

Results

Training phase:

Our 3D-printed mask, specifically tailored to each subject, is designed to attach to the primate chair using the exact mechanism as a traditional titanium head post. This design ensures consistent face positioning, maintaining an accurate distance from the monitor and aligning eye positions identically to those achieved with a head post.

The training process for the monkeys begins with a gradual introduction of the mask, attached to the chair, without any coercion for the subject to engage with it. This initial phase lets the subject familiarize with the mask's material, scent, and shape. On the second day, we incorporate positive reinforcement, offering treats and juice through a dispenser strategically positioned. Hence, the monkey voluntarily places its face into the mask to access these rewards. Within four days, the subjects willingly engaged with the mask for extended periods to receive juice and water.

On the sixth day of training, we introduced the backpiece of the non-invasive head immobilization system (NHIS), which secures the monkey's head in place. On the eighth day, the monkeys voluntarily begin maintaining eye fixation in exchange for continuous liquid rewards. Following ten days of initial exposure to the helmet, the monkeys are sufficiently acclimatized, allowing us to collect data.

Figure 2 in our study delineates this behavioral training timeline for helmet stabilization, providing a detailed overview of each step of the acclimatization and training process. This meticulous approach ensures that the monkeys are comfortable and willing participants in the experiments, facilitating reliable and ethical data collection.

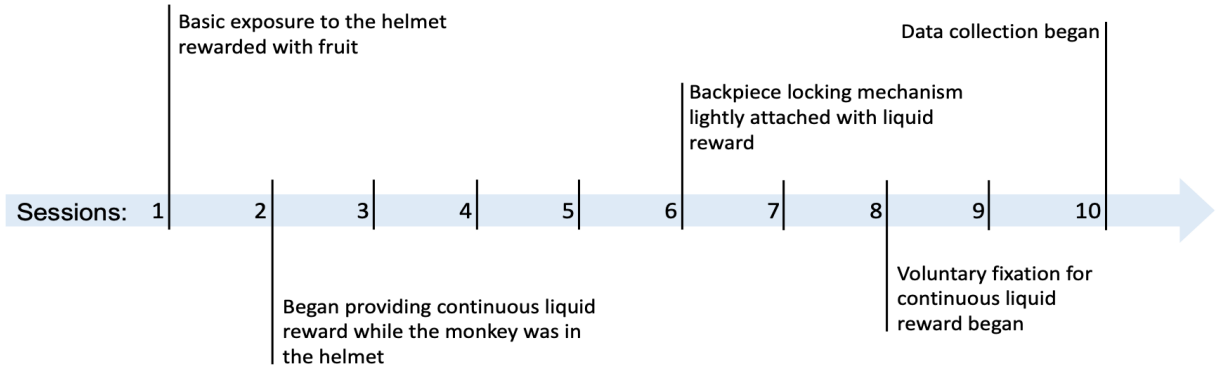

Figure 2- Behavioral training for helmet stabilization: The training with our 3D-printed mask starts by allowing the subjects to familiarize themselves with it, followed by positive reinforcement. Within four days, subjects voluntarily engaged with the mask for extended periods. By the tenth day, they are comfortable enough for data collection.

Testing Procedure:

In our study, we conducted a comparative analysis of eye-tracking data using two distinct restraint systems on two monkeys: one with a traditional headpost and the other using our helmet-based restraint. Over a one-hour session, both subjects were tasked with fixating on 13 targets. Compliance with the fixation task was reinforced through liquid rewards, with eye movements meticulously recorded via an eye tracker.

Crucially, entry into the helmet restraint system was entirely voluntary for the monkey, and at no point during this session was the locking backpiece of the helmet engaged. This aspect of the study design was implemented to assess the effectiveness and compliance of the helmet-based system under conditions that prioritize animal comfort and autonomy.

This comparative analysis evaluated the efficacy and accuracy of eye-tracking data obtained from two different restraint methods. By analyzing and contrasting the eye-tracking results from the headposted monkey and the helmet-restrained monkey, we aimed to derive insights into the viability and reliability of our novel helmet-based system in a controlled experimental setup. This comparison is vital for understanding each system's potential advantages and limitations in facilitating precise and ethical behavioral research with non-human primates.

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	Precision	Accuracy
Helmet Monkey	0.23 ± 0.11°	0.47 ± 0.21°
Head-post Monkey	0.11 ± 0.04°	0.41 ± 0.24°

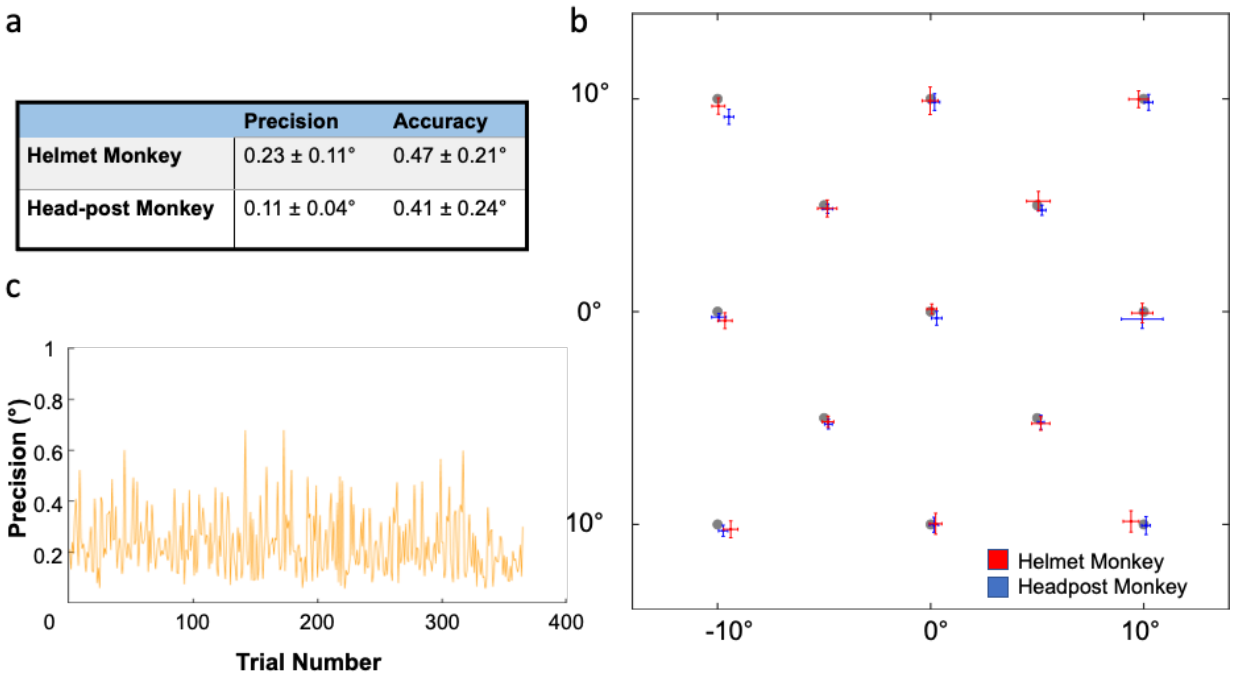

Figure 3 - eye movement accuracy: 2 monkeys (headposted and helmet-restrained) fixated on 13 targets throughout a 1-hour session, receiving a liquid reward, recorded using an eye tracker.

Discussion

In our study, the monkey stabilized with the helmet demonstrated eye-tracking precision and accuracy remarkably similar to that of the headposted counterpart. Notably, the helmet system allowed for an easier transition to data collection and presented no surgical complications, unlike the headposted method. Using a 3D-printable helmet model offers significant practical advantages, including ease of in-house production and the ability to readily replace or resize the helmet without re-molding with a sedated subject or retraining the monkey with a different size or model.

Furthermore, this novel non-invasive head immobilization system (NHIS) design affords additional space on the top of the skull, facilitating the placement of other research-related implants. This feature starkly contrasts with most full-head thermoplastic helmets, which often require vacuuming of the head and are typically one-size-fits-all, potentially allowing for minor movements. Such movements can render these helmets unsuitable for experiments requiring high-precision modalities like fMRI or other methodologies necessitating accurate eye-tracking. The helmet-based NHIS thus emerges as a superior alternative, offering enhanced stability and adaptability to various scientific investigations involving non-human primates.

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