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. Our results demonstrate that the NHIS significantly reduces the risk of infection and bone damage while maintaining high precision in eye-tracking data, making it a superior choice for long-term studies.

Introduction

Head fixation is crucial in behavioral experiments involving nonhuman primates, particularly for tasks requiring precise eye movement tracking and any kind of neural recording or stimulation, like electrophysiological studies and optogenetic interventions. The foundational work of Evarts (Evarts 1968) and Mountcastle (Mountcastle et al. 1975) marked the advent of long-term head fixation techniques. They employed 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 overuse of antibiotics. Moreover, the cap's inadequate adhesion to healthy bone could foster bacterial colonization, leading to bone necrosis and implant failure.

Subsequent research has spurred the development of alternative head fixation mechanisms in response to these drawbacks. Innovations from various laboratories (Betelak et al. 2001; Evarts 1968; Isoda et al. 2005; Lemon 1984; Foeller and Tychsen 2002; Pigarev, Saalmann, and

Vidyasagar 2009) 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 continue to 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, removing the head post typically renders the skull unsuitable 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 (Isoda et al. 2005; Pigarev, Saalmann, and Vidyasagar 2009; Srihasam et al. 2010; Amemori et al. 2015; Slater et al. 2016; Davis et al. 2009; Drucker et al. 2015). These methods have successfully achieved rigid head fixation while reducing invasiveness; however, minor skin incisions remain necessary during installation.

Completely non-invasive head fixation methods have been developed, such as helmets made from thermoplastics or flexible plastic and bar clamps (Amemori et al. 2015; Drucker et al. 2015) , but they 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, some non-invasive helmet designs involve vacuuming the subject's head(Srihasam et al. 2010), which can be uncomfortable and potentially anxiety-inducing, prolonging and complicating the training phase. Lastly, these systems are wanting for easy reproducibility to accommodate 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) explicitly tailored 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(A. Shahbazi et al. 2018), we engineered a 3D-printable mask system. This mask system is uniquely designed with specific cutouts to accommodate eye-tracking equipment, reward delivery mechanisms, and physiology experiment hardware.

This mask system can be customized for different levels of head immobilization based on project requirements. Research that requires eye-tracking and fixation, such as electrophysiology recordings (Wang et al. 2023; Roussy et al. 2022; Hallum et al. 2017; Boutte et al. 2017; Ma et al. 2022), open chamber implant and intracranial recording probe, or optogenetic stimulation projects (E. Shahbazi et al. 2024, 2023; E. Shahbazi, Ma, and Afraz 2023; Lafer-Sousa et al. 2023) typically necessitate continuous immobilization with minimal head movements. For this, we provided a method to develop a specific mask based on each monkey's face to minimize head movement and facilitate suitable data collection. This can optionally be paired with a back piece to reduce head movement and secure continuous immobilization further; the back piece can alternatively be left off for use cases where uninterrupted head restraint is not necessary.

For projects that instead only rely on monkeys' free eye movement or other behavioral responses, we provided a less restrictive model with specific sizes (small, medium, or large) based on the average monkey face in different sexes and weights. This less form-fitting model has yet to be robustly tested but shows promise.

Method

We conducted experiments and gathered data involving two adult male Rhesus monkeys (Macaca mulatta) named Nd and Gr (Nd, 8.65 kg, and Gr, 14 kg). The first monkey, Nd, has been implanted with OptoArrays (Blackrock Neurotech - 530 nm wavelength) on the virally transduced area which AAV5-CaMKIIa-C1V1(t/t)-EYFP (nominal titer: 8 × 1012 particles/ml) has been injected in the cortex using a custom-made injection array consisting of four 31-gauge needles arranged in a 2 × 2 mm square. Gr has been implanted with two multi-electrode Utah Array in the central IT cortex.

3D printing technology has seen significant advancements in recent years, making printing increasingly feasible with various materials, including metals, plastics, and silicones. For this project, we used the Stratasys F370 3D printer, which offers a print precision of up to 0.127mm (0.005 in.). The material selected for printing was Acrylonitrile Styrene Acrylate (ASA), known for its high durability and versatility in 3D printing applications. ASA exhibits excellent properties such as high impact resistance, robust mechanical performance, and strong resistance to UV rays, water, and various chemicals (Carlota 2023). Post-printing, the mask was double-coated with spraying resin to ensure it was suitable for contact with the subject's skin.

The animals were positioned 57 cm for Nd and 61cm for Gr from a calibrated 32" screen (120 Hz, 1920 × 1080 IPS LCD, Cambridge Research System Ltd). Data was collected using a

customMonkeyLogic scripts on a Dell computer for Nd and Gr. Eye tracking was carried out with an Eyelink 1000 Plus (SR Research), at a frequency 1000 Hz.

A prior publication thoroughly documented the details of the complementary surgical and anesthesia procedures, postoperative care, and implantation methods(E. Shahbazi et al. 2024). All procedures were conducted in accordance with and approved by the National Institute of Mental Health Animal Use and Care Committee guidelines.

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. (A. Shahbazi et al. 2018), for reconstructing neurons and membranes from high-resolution microscopy images (Joesch et al. 2016; A. Shahbazi 2018), 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, as shown in Fig 1. a.

Notably, FLoRIN's 3D modeling accurately reflects the current physical state of the subject's head, including potential surgical implants. A significant advantage of using FLoRIN over other segmentation and 3D modeling tools like 3D Slicer is its Python package, which allows for plug-and-play functionality to convert a batch of MRI scans into 3D models seamlessly. Another aspect of our mask system design utilizes the Blender software package, where scene operators can be exported to Python code. This capability enables the creation of masks from MRI images with adjustable size parameters, providing greater customization and precision.

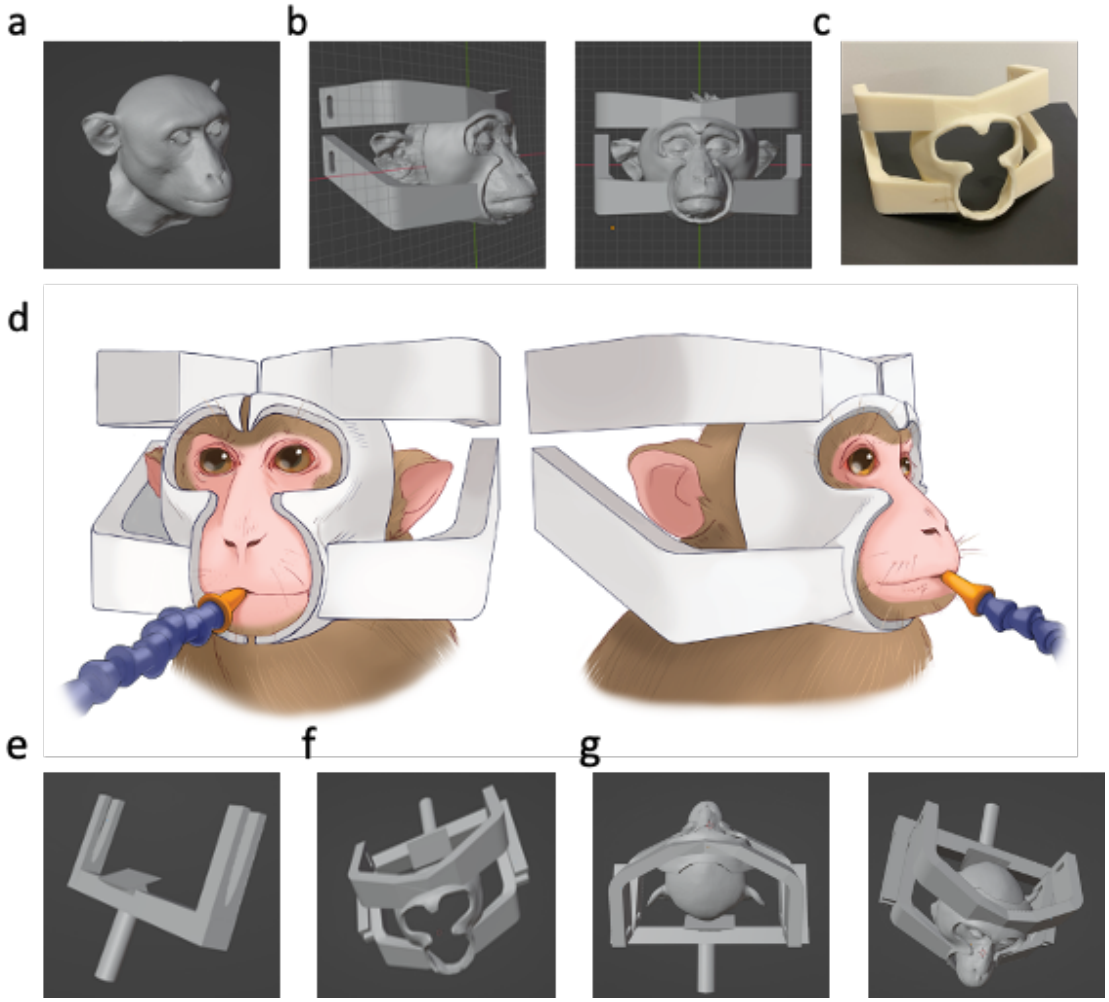

Figure 1 - Overview of NHIS Design and Implementation: a) 3D facial reconstruction using FLoRIN based on MRI imaging of the monkey, providing a detailed and accurate model of the subject's facial anatomy. b) Design of the Non-Invasive Head Immobilization System (NHIS) using Blender, illustrating the mask's structure and various connecting components. c) Completed 3D-printed mask, 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. e) the backpiece design f) Full mask system: include the backpiece connected to the mask g) the monkey 3D face inside the full mask system

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, which can be adjusted based on the single-piece or two-layered design and project flexibility for head movement. 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. Figure 1. b shows the 3D model of the mask with the monkey's head, and Fig 1. c shows the 3D printed mask.

A backpiece is designed to be attached to the mask to restrain the monkey's head from behind. It is adaptable to different monkey chairs and can be connected to the mask or the medial diapason-shaped connector. The backpiece is also fully customizable based on the monkey's implant position. Figure 1. e shows the backpiece, and Figure 1. f shows the back piece and the mask together. The monkey's head is in the full mask system, including the mask and backpiece shown in Figure 1. g.

As monkeys mature, their head and face sizes can increase due to changes during puberty and aging. To accommodate these changes, 3D-designed masks can be resized or modified based on updated scans, creating more accurate and well-fitting masks. We also utilized an average monkey face model, derived from over 100 monkey faces in our facility, to develop standard masks in small, medium, and large sizes, considering different ages and sexes. These standard masks are particularly suitable for behavioral projects that require the monkey's attention but allow for small head movements between trials. Also, masks can be updated to accommodate changing features by obtaining new CT scans or resizing the previous mask based on the observed physical changes.

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 headpost. This design ensures consistent face positioning, maintaining an accurate distance from the monitor and aligning eye positions identically to those achieved with a headpost.

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.

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

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

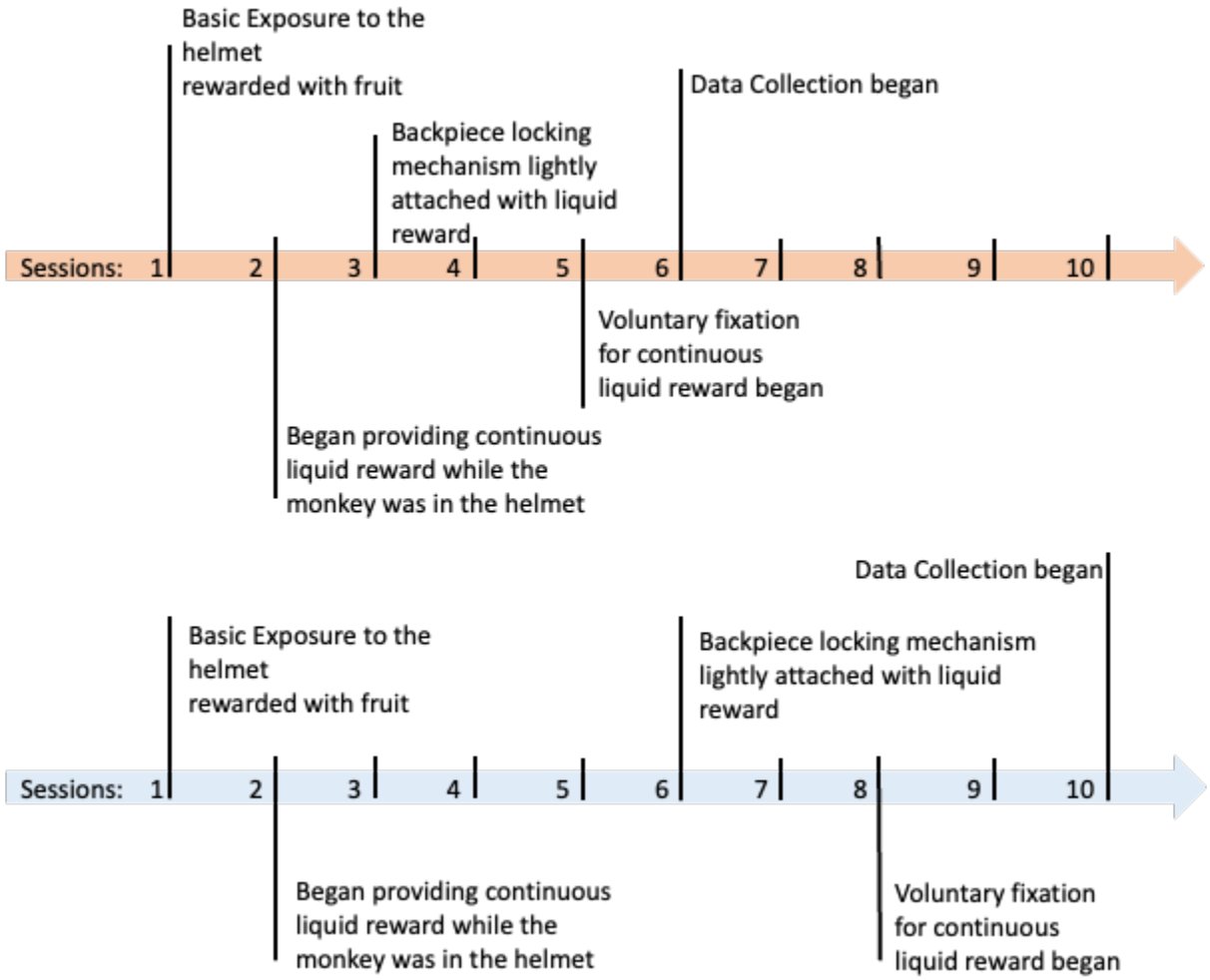

Figure 2—Behavioral training for mask system stabilization: The training process for mask system stabilization using our 3D-printed mask began with familiarizing the subjects with the mask and employing positive reinforcement. Subjects were then introduced to the backpiece and full head restriction (on the 3rd day for Gr and the 6th day for Nd). Following acclimation to the full mask system, subjects commenced voluntary fixation for continuous rewards, with data collection starting immediately thereafter (on the 6th day for Gr and the 10th day for Nd). The timelines for Gr and Nd are depicted in orange and blue, respectively.

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 mask-based restraint. Both subjects were tasked with fixating on 13 targets during the testing

procedure. Compliance with the fixation task was reinforced through liquid rewards, with eye movements recorded via an eye tracker.

Crucially, entry into the mask restraint system was entirely voluntary for the monkey, and at no point during this session was the locking backpiece of the mask engaged. This aspect of the study design was implemented to assess the effectiveness and compliance of the mask-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 mask-restrained monkey, we aimed to derive insights into the viability and reliability of our novel mask-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.

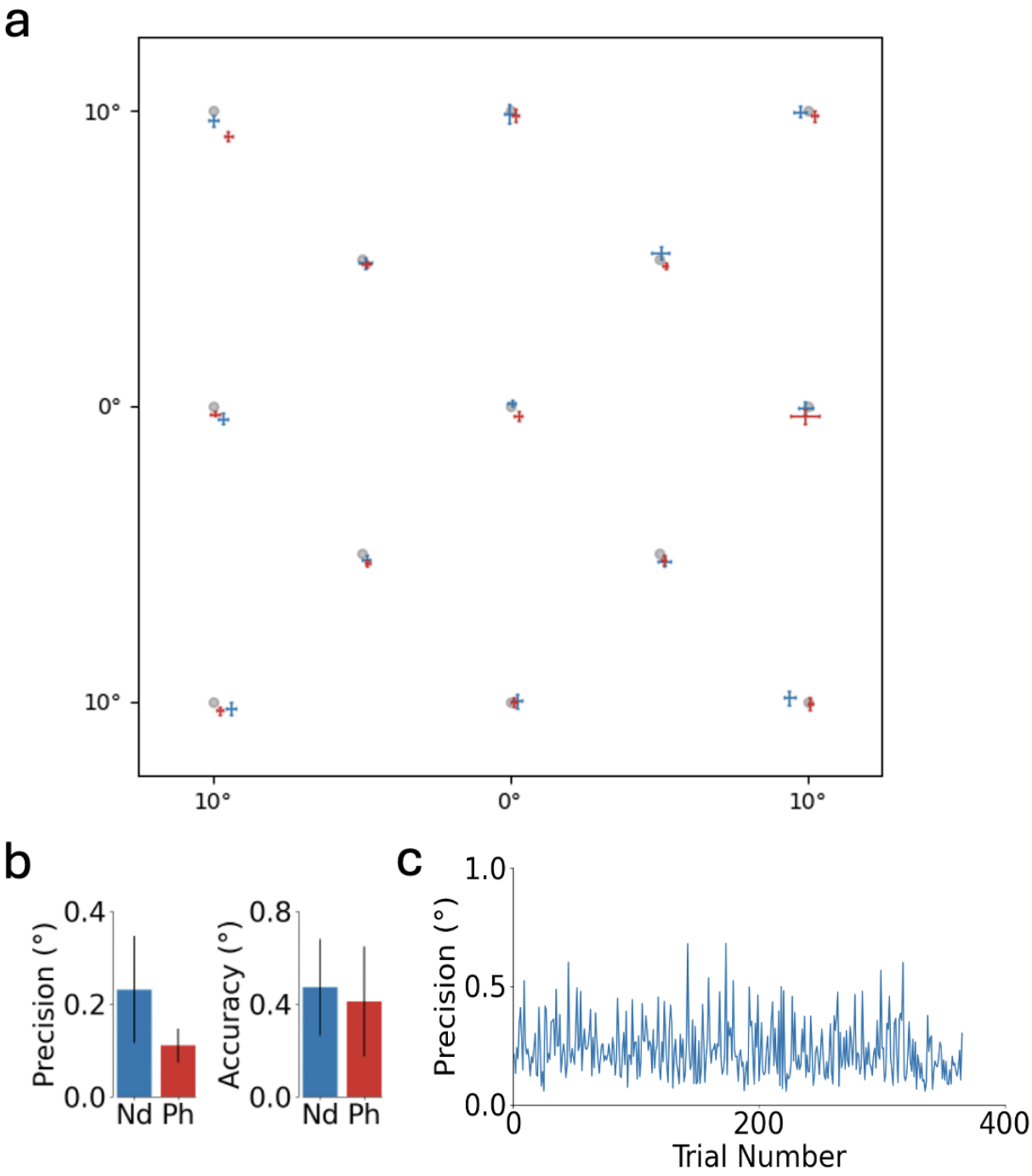

Figure 3–eye movement accuracy: 2 monkeys (Nd, mask-restrained, blue; and Ph, headposted, red) fixated on 13 targets throughout the testing procedure, receiving a liquid reward, recorded using an eye tracker. a) Fixation targets and mean fixation positions for monkeys Nd and Ph. Error bars show 1 SD. b) Gaze precision and accuracy for monkeys Nd and Ph during the 13-target fixation task, error bars shown 1 SD. c) Gaze precision for monkey Nd across trials in 13-target fixation task.

Data Collections

We started data collection immediately after the monkeys began voluntarily fixating on continuous liquid rewards while their heads were immobilized with the full mask (mask and backpiece).

Nd, who had an optogenetic implant in the V4 visual cortex, performed a cortical perturbation detection (CPD) task. In this task, the monkey had to fixate on a central dot for 500 ms to start the trial. Then, an image was shown for 1 second, during which, in half of the trials at random, his brain was stimulated for 200 ms halfway through the image presentation. After the image disappeared, the monkey was rewarded for correctly identifying whether the trial contained cortical stimulation by looking at one of two targets at the top and bottom of the screen for 250 ms. The average number of trials per session for another monkey for the same task with the headpost was 1200, and Nd with the mask did 1500 trials per session. Over 8 months, Nd completed around 100,000 trials.

The second monkey, Gr, had a Utah array implanted in his middle face patch and also performed the CPD task by viewing face and non-face images. The trial structure was similar to that of Nd's, and Gr averaged 1500 trials per session. He completed 30,000 trials in 3 months.

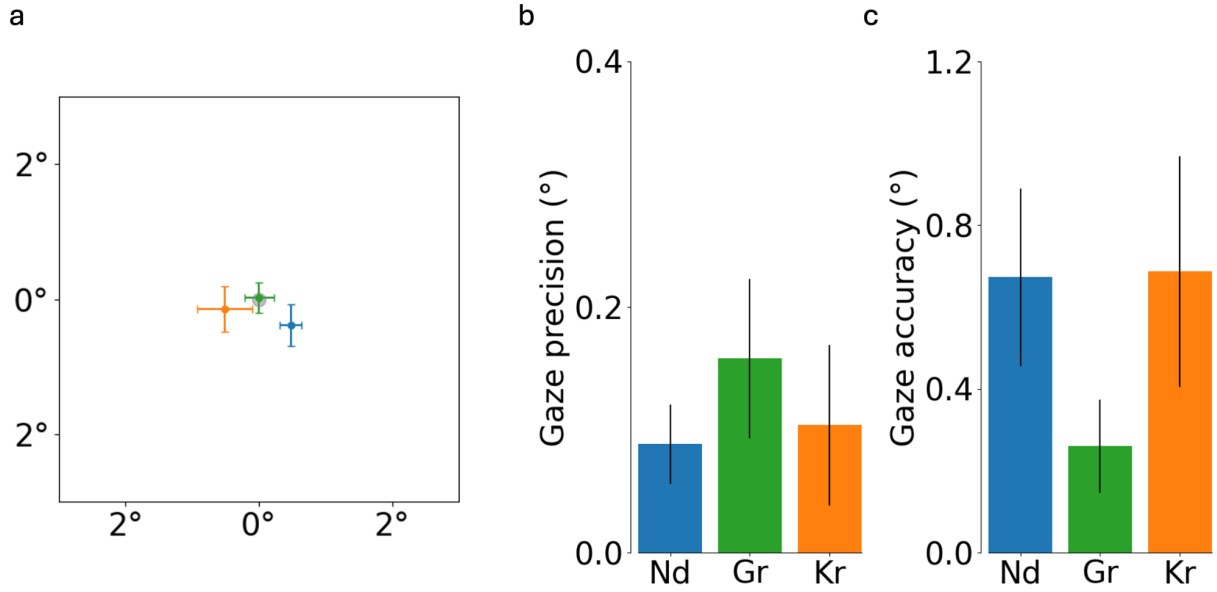

Figure 4 - Accuracy and Precision During Data Collection: a) Fixation target and mean gaze position for monkeys Nd, Gr, and Kr during central fixation in a two-alternative forced choice (2AFC) task. Monkeys Nd and Gr were head-restrained using our NHIS, while Kr was headposted as a control. Error bars show 1 SD. The data shown is from n=21718 trials. b) Gaze precision during the same behavioral task, error bars shown 1 SD. c) Gaze accuracy during the same behavioral task. Error bars show 1 SD.

We carefully measured the accuracy and precision during the active data collection, as shown in Fig 4. The gaze accuracy and precision for the two monkeys using our designed mask were comparable and statistically equivalent to those of a monkey with a headpost performing the same CPD task. This measurement indicates that our proposed head immobilization method offers accuracy and precision comparable to traditional methods, with additional benefits. These benefits include fewer health complications and a reduced risk of infection, fewer required surgeries, a faster project kick-off stage, and less pain for the monkeys during data collection hours.

Discussion

In our study, the monkey stabilized with the mask demonstrated eye-tracking precision and accuracy similar to that of the headposted counterpart. Notably, the mask system allowed for an easier transition to data collection and presented no surgical complications, unlike the headposted method. The NHIS offers significant practical advantages over traditional head fixation methods. The system can be quickly produced in-house using 3D printing technology, allowing for rapid customization and resizing without sedation or extensive downtime. It is beneficial for accommodating changes in the monkey's head size over time, thus ensuring a comfortable and consistent fit throughout the study.

Moreover, the NHIS design minimizes health complications commonly associated with traditional methods. By eliminating the need for surgical implants and reducing the risk of infection and bone damage, the NHIS significantly improves the well-being of the subjects. This non-invasive approach reduces the required surgeries, leading to a faster project kick-off stage and less pain for the monkeys during data collection hours.

The mask system also shows promise in behavioral studies that do not require invasive implants but still demand precise eye-tracking. It can even be employed cage-side, as the back piece is unnecessary for accurate eye-tracking in such contexts. These applications would eliminate the need for surgery. This benefit has yet to be fully utilized, but we are confident that future research will take full advantage of these merits.

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 mask-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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