Journal of Neuroscience Methods 269 (2016) 46–60

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Individually customisable non-invasive head immobilisation system for non-human primates with an option for voluntary engagement

Heather Slater[a,b], Alice E. Milne[a,b], Benjamin Wilson[a,b], Ross S. Muers[a,b], Fabien Balezeau[a], David Hunter[a], Alexander Thiele[a], Timothy D. Griffiths[a], Christopher I. Petkov[a,b,*]

[a] Institute of Neuroscience, Henry Wellcome Building, Newcastle University, Framlington Place, Newcastle upon Tyne NE2 4HH, United Kingdom
[b] Centre for Behaviour and Evolution, Henry Wellcome Building, Newcastle University, Framlington Place, Newcastle upon Tyne NE2 4HH, United Kingdom

HIGHLIGHTS

- Non-invasive head immobilisation for neuroscience experiments in monkeys.
- Individually customised system combining functionality of previous systems.
- Allows access for auditory and visual stimulation.
- Has the option for voluntary engagement to assist habituation.
- Systematically evaluated against scientific and animal welfare needs.

ARTICLE INFO

Article history:
Received 2 March 2016
Received in revised form 6 May 2016
Accepted 6 May 2016
Available online 14 May 2016

Keywords:
Head immobilisation
Non-invasive
Macaque
Monkey
Eye tracking
MRI
Animal welfare

ABSTRACT

Background: Head immobilisation is often necessary for neuroscientific procedures. A number of Non-invasive Head Immobilisation Systems (NHIS) for monkeys are available, but the need remains for a feasible integrated system combining a broad range of essential features.
New method: We developed an individualised macaque NHIS addressing several animal welfare and scientific needs. The system comprises a customised-to-fit facemask that can be used separately or combined with a back piece to form a full-head helmet. The system permits presentation of visual and auditory stimuli during immobilisation and provides mouth access for reward.
Results: The facemask was incorporated into an automated voluntary training system, allowing the animals to engage with it for increasing periods leading to full head immobilisation. We evaluated the system during performance on several auditory or visual behavioural tasks with testing sessions lasting 1.5–2 h, used thermal imaging to monitor for and prevent pressure points, and measured head movement using MRI.
Comparison with existing methods: A comprehensive evaluation of the system is provided in relation to several scientific and animal welfare requirements. Behavioural results were often comparable to those obtained with surgical implants. Cost–benefit analyses were conducted comparing the system with surgical options, highlighting the benefits of implementing the non-invasive option.
Conclusions: The system has a number of potential applications and could be an important tool in neuroscientific research, when direct access to the brain for neuronal recordings is not required, offering the opportunity to conduct non-invasive experiments while improving animal welfare and reducing reliance on surgically implanted head posts.

1. Introduction

The present report describes the development and systematic evaluation of a non-invasive alternative to the use of surgically implanted head posts for use with macaque monkeys, a laboratory

* Corresponding author at: Framlington Place, Institute of Neuroscience, Newcastle University Medical School, Newcastle upon Tyne, Tyne and Wear NE2 4HH, United Kingdom.
E-mail address: chris.petkov@ncl.ac.uk (C.I. Petkov).

http://dx.doi.org/10.1016/j.jneumeth.2016.05.009

animal commonly used as a neurobiological model to advance our understanding of human neurobiology and disorders of the nervous system. Many neuroscientific procedures involving animals require head immobilisation. Typical approaches use an implanted head post, which is attached to the skull of the animal during an aseptic surgical procedure under general anaesthesia (Betelak et al., 2001; Mountcastle et al., 1975). In addition to limiting head movement, the implant can accommodate chambers used for direct neuronal recordings. However, surgical implants carry a risk of infection and can become unstable or fail. If this occurs and the animal cannot be re-implanted, further data collection may not be possible and the animal would need to be replaced. Thus, for approaches that depend on minimal head movement but do not require direct access to the brain, Non-invasive Head Immobilisation Systems (NHIS) could prove beneficial in reducing the reliance on surgical implants. However, if NHIS are to be broadly accepted as viable alternatives they need to address combinations of scientific and animal welfare requirements and show comparable data quality in relation to surgical implant approaches.

We aimed to contribute to the ongoing effort to develop and refine non-invasive head immobilisation options, identifying several scientific and animal welfare considerations. We summarise recent NHIS against eight criteria shown in Table 1. This shows that most recent systems are individually customisable to better fit the animal's head, however, surprisingly little is known about how the systems impact on levels of distress or discomfort experienced by the animals during habituation to or use of the system. Facemasks have been used to allow an animal to voluntarily engage with an experimental setup for eye tracking and measurement (Fairhall et al., 2006; Kiorpes et al., 2012), see ♦ in Table 1, rows 8–9. It may be useful to implement an automated system to allow the animals to voluntarily engage with the facemask at their leisure, which could help them to habituate to full head immobilisation (Table 1, rows 1–7), but this is currently not available as an option. Moreover, it remains unclear the extent to which pressure points form during immobilisation, or how this is monitored and addressed if pressure points do occur, in order to alleviate pain or prevent sore formation and infection. Also some systems block access to the ears for high-fidelity auditory stimulation and it remains unclear how adaptable the systems are to different types of laboratory settings, since most are often demonstrated within a single setup. Lastly, it is important that any system is robust and shown to work with animals of different sizes.

To address these needs, we designed and evaluated a system that combines the essential features of the available systems while also extending the range of features. This effort resulted in a system that has considerable flexibility in how it is used, which, to our knowledge, for the first time incorporates an option for automated voluntary engagement with the facemask as an initial step towards the animals habituating to immobilisation using the full-head helmet. We comprehensively evaluated the system against the specified criteria within the context of documented behavioural habituation and training steps as several animals were trained to use the system. We further assessed performance on challenging auditory tasks. Some of the results are also directly compared with those from the animal's own surgically implanted head posts. We also provide a cost–benefit analysis to help others assess the desirability of such a system for applications in other laboratories. The findings, in many cases, bode well for this system as a practical comprehensive approach for non-invasive head immobilisation that is not overly time consuming to implement and as a relatively low cost alternative to surgically implanted options where direct access to the brain is not required.

2. Methods

2.1. Subjects

All of the animal procedures performed were approved by the UK Home Office and comply with the Animal Scientific Procedures Act (1986) on the care and use of animals in research and with the European Directive on the protection of animals used in research (2010/63/EU). We support the Animal Research Reporting of In Vivo Experiments (ARRIVE) principles on reporting animal research. All persons involved in this project were Home Office certified and the work was strictly regulated by the U.K. Home Office.

Seven male rhesus macaques (*Macaca mulatta*) from a group of pair housed animals were used for the development and evaluation of the system described here. The pen sizes in our colony range from 130 × 240 cm to 215 × 240 cm. All are 230 cm high, and hatches between neighbouring cages are used to increase the space available to the animals. One monkey (M1, 5 years, 12 kg) was naïve to behavioural and head immobilisation training, not having previously had an implanted head post. Two other monkeys (M2, 11 kg; M3, 16 kg, both 8 years old at the time of testing) did not have implanted head posts at the time of assessment, but had previously had head post implants. These head posts had become unstable and were removed at 7 months and 4 years after implantation, respectively. The other animals (M4, 6 years, 12.5 kg; M5, 6 years, 14 kg; M6, 8 years, 15 kg; M7, 4 years, 6 kg) had existing implants, allowing direct comparison between implanted animals and those using the NHIS. Table 2 summarises the procedures conducted using the head immobilisation device with each animal in this report.

2.2. General design features of the nonhuman primate, non-invasive head immobilisation system

In collaboration with the Freeman Hospital Cancer Radiotherapy Unit at Newcastle upon Tyne, UK, we prototyped and developed a non-invasive head immobilisation system for nonhuman primates, using similar design approaches as those in use in human radiotherapy cancer treatment units. In developing the nonhuman primate NHIS, we combined the experience of the Freeman Hospital Unit in developing and using highly customised whole head or limb immobilisation in human patients with our experience working with nonhuman primates on neuroscientific procedures.

The system was designed to achieve head immobilisation for macaques of different sizes providing a highly customised fit and allowing for visual and auditory stimulation and for the animals to receive fluid rewards as positive reinforcement (Fig. 1). The transparent plastic allows the animals to see through the facemask while it is being placed, which makes placement of the facemask less intimidating or distressing. The plastic can be greatly modified while retaining structural strength; air holes can be created and the plastic can be thinned in problem areas to prevent overheating and to alleviate pressure points. It can be easily modified to incorporate fittings for a wide range of scientific and laboratory attachments, which can readily be integrated into the facemask or full-head helmet system.

2.3. Creating the head model

We used two different methods to create a head model from which the helmet system could be made.

2.3.1. Head impression using plaster bandages and alginate

For one approach, we created an impression of the whole head using plaster bandages and alginate moulding putty (BabyRice Chromatic Alginate Moulding Material mixed with water). The head impression was filled with plaster to create the head model.