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Short communications

Development of a removable head fixation device for longitudinal behavioral and imaging studies in rats

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HIGHLIGHTS

- We developed a novel head fixation device for behavioral and imaging studies.
- It has an only small influence on the PET image in terms of attenuation of photons.
- The heads of rats can be repositioned on a PET/CT bed at sub-millimeter accuracy.
- The head attachment is removable from the rat's head.
- The device is useful for combined behavioral and imaging studies.

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ABSTRACT

Background: In some behavioral neuroscience studies, an attachment is surgically fixed onto the head of an awake animal to allow the animal to perform learning tasks repeatedly in the same position in a task-training system. A recently developed task-training system enables operant conditioning of head-fixed rats within only a few days, and this system has been rigorously applied to record learning-associated neural activity using electrophysiological techniques. However, the head attachment of this device is made of metal and thus is not suitable for simultaneous brain imaging studies with X-ray computed tomography (CT), magnetic resonance imaging (MRI) or positron emission tomography (PET).
New method: We developed a novel head fixation device with a removable attachment to position the rat head precisely in both imaging and training devices across different sessions. The device consisted of a removable attachment, a clamp and a stage, all of which were made of PET/MRI compatible acrylic resin. We tested the usefulness of the device with ^{18}F-fluorodeoxyglucose (FDG) PET and CT.
Results: The new device did not substantially affect ^{18}F-FDG PET images. Repositioning of the rat's head across sessions and experimenters was at a level of submillimeter accuracy.
Comparison with existing method: The errors of radioactivity concentration of ^{18}F-FDG in the PET image were lower with the present attachment than with the conventional metal attachment. Repositioning accuracy was considerably improved compared with a visual inspection method.
Conclusions: The developed fixation device is useful for longitudinal behavioral and brain imaging studies in rats.

1. Introduction

Behavioral and neurophysiology studies in animals have advanced the understanding of neural mechanisms underlying high-order brain functions (e.g., Shima and Tanji, 1998; Isomura et al., 2003). In many behavioral learning studies, the head of an animal is fixed to an attachment on a task-training system to allow the animal to perform motor tasks repeatedly in a stable position,

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and also to allow researchers to record neuronal activity. Neurophysiology studies on head-fixed monkeys have revealed neural mechanisms underlying cognitive and motor functions (Mushiake et al., 1991; Shima and Tanji, 1998; Isomura et al., 2003). Similar research has also been performed on rodents in head-fixed conditions, where state-of-the-art physiological techniques are available to elucidate the neural circuits related to learning and sensory perception (Stüttgen et al., 2006; Narumi et al., 2007; Isomura et al., 2009; Gerdjikov et al., 2010; Komiyama et al., 2010; Kimura et al., 2012). In parallel, recent imaging technologies for small animals using magnetic resonance imaging (MRI) and/or positron emission tomography (PET) have proved useful in longitudinal studies to evaluate whole-brain structure and/or function (Endepols et al., 2010; Marx et al., 2012; Xi et al., 2013; Sumiyoshi et al., 2014). In behavioral studies, it is ideal to perform longitudinal imaging measurements in parallel with physiological recordings to understand the brain's anatomical and/or functional changes associated with learning in a comprehensive manner.

To fix the head of a rat to a particular behavioral task system, a head-attachment is surgically mounted to the skull (Isomura et al., 2009; Kimura et al., 2012; Hira et al., 2013; Masamizu et al., 2014). However, conventional head attachments are directly and solidly connected with anchor screws and dental resin cement, and thus are non-removable from the head/skull. Also, because the attachment is usually much larger than the size of the rat's head, it is difficult to insert a rat with an attachment into a small space such as the head coil of an MRI scanner. Furthermore, a conventional head attachment is often made of a metal such as aluminum; however, for PET acquisition, metal devices cause errors in image quantification because of attenuation of the photons emitted indirectly from the radiotracer in the device. The development of a head attachment made of low attenuation coefficient materials is important for longitudinal studies combining neurophysiology and imaging technologies.

Beside the combination with neurophysiology, one problem with longitudinal PET studies is that the sizes and shapes of brains are often measured variably with PET – even for serial scans of the same animal – because of differences in factors such as radiotracer uptake, statistical noise, and partial volume effects, especially when positioning differs. Thus, it is difficult to co-register the PET images obtained in different sessions. This problem can be at least partially solved if we place the rat precisely in the same position across different scanning sessions.

To address these issues, we developed a head fixation device, which consisted of an attachment, a clamp and a stage, intended for use in longitudinal behavioral and imaging studies. The novel head-attachment was made of acrylic resin and was removable from the rat head. Furthermore, this device was designed to position the rat brain in a PET scanner precisely across different scanning sessions. To test the usefulness of the novel head fixation device, we evaluated the reproducibility of repositioning the rat in the scanner, and the effects of photon attenuation by the attachment devices on the PET images.

2. Materials and methods

2.1. Ethics statement

All animal experiments were performed under the approval of the Animal Care and Use Committee, National Institute of Neuroscience, National Center of Neurology and Psychiatry, and were performed in accordance with guidelines for the care and use of laboratory animals. Experiments were reported according to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.

2.2. Design of head fixation device

The head fixation device consisted of three major components: a head-attachment, a head-attachment clamp, and a stage, all of which were made of acrylic resin (Fig. 1A). The sizes of the attachment and clamp are shown in Fig. 1B. The attachment weighed 4.0 g, and was designed to be compatible with a head-holding device for animal surgery (SR-10AR, Narishige, Tokyo, Japan) and a behavioral task system for rats (Task Forcer R1, O'hara & CO., Tokyo, Japan). The attachment is reusable for experiments.

2.3. Subjects

In this study, we evaluated two characteristics of the device: the reproducibility of repositioning the rats using the head fixation device, and the effects of attenuation of the photons by the device on the PET image. An 8-week-old Long-Evans male rat (232 g) was used to evaluate the reproducibility of repositioning the rat. Under 2.0–2.5% isoflurane anesthesia, the rats had two internal threads surgically attached to the skull with tiny anchor screws (Poly-Ether-Ether-Ketone, M1.2, 2.5-mm-long) and dental cement (Super-Bond C & B, Sun Medical; Unifast II, GC Corporation, Japan). The head attachment was fixed onto the rat skull with two external threads before the experiment. A cylindrical phantom was used to evaluate the effects of attenuation in the device on the PET image. The diameter and height of the phantom were 30 and 115 mm, respectively.

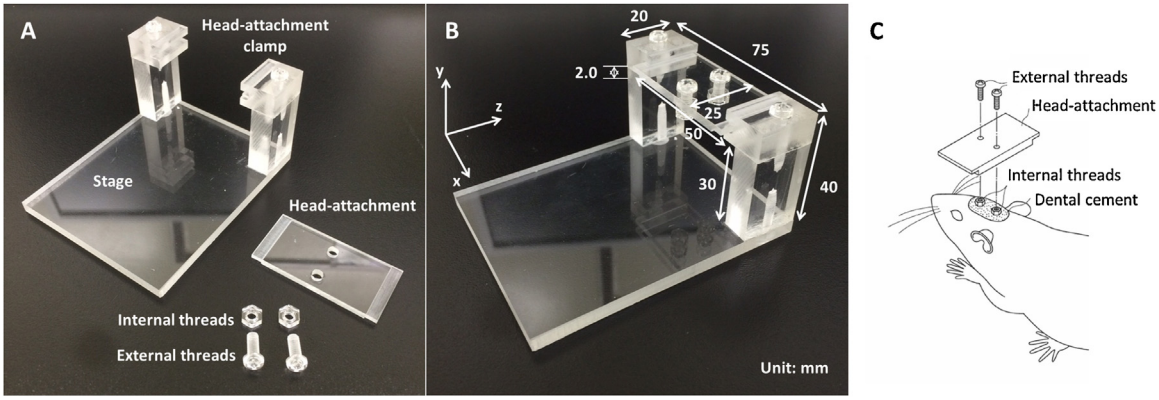

Fig. 1. The head fixation device consisted of three components: a head-attachment, a head-attachment clamp and a stage (A). Each component was made of acrylic resin, and the sizes of the attachment and clamp are shown in (B). The head attachment was fixed onto the rat skull with two external threads (C). Head fixation was completed by just sliding in the holding spot.