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Refined methodology for implantation of a head fixation device and chronic recording chambers in non-human primates

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HIGHLIGHTS

- A head fixation device and a chronic recording chamber can be implanted without using dental resin or orthopedic cement.
- Complete osseous-integration of implant can be obtained thanks to a hydroxyapatite coating.
- A perfect matching of the implants with individual skull surface can be ensured with a plastic replicate of the skull (3D printing).
- Implanting surgeries can be greatly facilitated by the use of personalized implants and 3D printing.
- Outstanding longevity of the implants used: 4 years for head fixation device and 1.5 years for chronic recording chamber.

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ABSTRACT

The present study was aimed at developing a new strategy to design and anchor custom-fitted implants, consisting of a head fixation device and a chronic recording chamber, on the skull of adult macaque monkeys. This was done without the use of dental resin or orthopedic cement, as these modes of fixation exert a detrimental effect on the bone. The implants were made of titanium or tekapeek and anchored to the skull with titanium screws. Two adult macaque monkeys were initially implanted with the head fixation device several months previous to electrophysiological investigation, to allow optimal osseous-integration, including growth of the bone above the implant's footplate. In a second step, the chronic recording chamber was implanted above the brain region of interest. The present study proposes two original approaches for both implants. First, based on a CT scan of the monkey, a plastic replicate of the skull was obtained in the form of a 3D print, used to accurately shape and position the two implants. This would ensure a perfect match with the skull surface. Second, the part of the implants in contact with the bone was coated with hydroxyapatite, presenting chemical similarity to natural bone, thus promoting excellent osseous-integration. The longevity of the implants used here was 4 years for the head fixation device and 1.5 years for the chronic chamber. There were no adverse events and daily care was easy. This is clear evidence that the present implanting strategy was successful and provokes less discomfort to the animals.

1. Introduction

In the field of neurosciences, the macaque is a model of choice (scientifically and ethically justified; see Weatherall report, 2006). This monkey is highly adapted for neuronal investigations due to its large similarity to the human brain from an anatomical and a functional point of view. In modern neurosciences, there is a large range of approaches to investigate brain function, also applicable, to some extent, to non-human primates: functional brain imaging (fMRI), electroencephalography (EEG), positron emission tomography (PET), transcranial magnetic stimulation (TMS), single

Abbreviations: HA, hydroxyapatite; CT, computed tomography; MRI, magnetic resonance imaging; VPS, vacuum plasma spraying.

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neuron recording, etc. The quality of the resulting data depends on the level of interferences caused by artifacts, which may be produced, for instance, by muscular contractions related to head and/or eyes movements or mastication. A further challenge in this type of research lies in the fact that the animal has to be awake, and the head must be kept fixed. Indeed, any head movement would create recording artifacts. Furthermore, in the case of electrophysiological recordings, there is a risk that, in a non-head fixed system, the recording electrodes move and cause brain injuries. That is why it is preferable that the animal's head be immobilized when it is performing behavioral tasks. To this aim, until recently, numerous laboratories used a head fixation device anchored to the skull with dental acrylic cement (Fuchs and Luschei, 1970; Lisberger and Westbrook, 1985; Guo and Li, 1997; Kermadi et al., 1997, 1998; Liu and Rouiller, 1999; Churchland and Lisberger, 2000) or orthopedic cement (Durif et al., 2003; Peeters et al., 2009; Kaeser et al., 2010, 2011). Such an approach allowed creation of a firm point of fixation, but the interface between the dental resin or the orthopedic cement and the bone was not optimal. It was observed that the cement adhered to the bone in a superficial way without integration between the two components (bone and cement). This represented a considerable risk of fracture. Although variable from one animal to another, the presence of cement (dental or orthopedic) exerted a detrimental impact on the bone in the mid- and long-term run. In particular, the risk of infection, inflammation, growth of granulation tissue and softening of the bone was increased. These effects were often initiated by the high temperature generated when the cement was applied to the bone surface, and as a consequence increased the risk of head fixation device losses of over time.

In line with recent reports (Adams et al., 2007, 2011; McAndrew et al., 2012), the goal of the present study was to introduce a refined method to anchor a biocompatible head fixation device and chronic recording chambers on macaque's skull, without the use of dental resin or orthopedic cement. This aim was achieved here by taking advantage of newly developed materials and coatings which are used for orthopedic surgery. These are generally assimilated by the bone instead of being rejected by it. However, for a perfect integration between implants and bone, a perfect match of the shape of the implants (head fixation device or recording chamber) with the skull surface of each individual monkey is required. A 3D replicate of the skull of the living monkey was obtained based on CT and MRI data. This replicate was used to accurately guide the positioning of the implants on the skull as well as to derive their shape so that they would perfectly match the contour of the skull at the calculated target position.

2. Methods

2.1. Subjects

The present experiments were conducted on two adult *Macaca fascicularis*, originating from our own breeding colony. At the time of headpost fixation (see Fig. 3), one animal (Mk-LI) was 9-years-old and weighed about 8.0 kg, whereas the second animal (Mk-JZ) was 7-years-old and weighed about 8.0 kg. The body weight was checked daily. In case of a 10% loss of weight, the experiment would be interrupted until weight was regained (an interruption criterion that was not met in the course of the present study). Between daily experimental sessions the animals shared living quarters with other monkeys (groups of 2 to 5 animals) in an enclosure of 45 m^3 (15 m^3 until 2010; see e.g. (Kaeser et al., 2011)). They could freely move and had free access to water. The experiments were conducted according to the guidelines of the National Institute of Health (Guide for the Care and Use of laboratory Animals, 1996), of the European Community (Guidelines for Animals Protection and Use for Experimentation) and the ARRIVE guidelines (http://www.nc3rs.org.uk) (Animal Research: Reporting In Vivo Experiments), as well as the Swiss veterinary authorities (cantonal and federal) who approved the experimental procedures.

2.2. 3D replicate (print) of the monkey's skull

The first stage was to obtain such a 3D replica of the living monkey's skull. The acquisition of the skull morphology involved using a computed tomography scan (CT scan) (Department of radiology at Hôpital Fribourgeois [HFR]). The obtained CT scan was processed with the Osirix software (64 bits) in order to fabricate a 3D reconstruction of the skull. This model was transferred to the Engineering School of Fribourg for final processing. The final 3D print was performed with the following equipment: 3D printer, 3D uPrint Plus which uses Fused Deposition Modeling (FDM) Technology to build 3D replica with ABS*plus* thermoplastics. The principle of the 3D replica of the skull is illustrated in the supplementary video sequence #1 (http://www.unifr.ch/neuro/rouiller/research/multi/lanz/l1.html). The present 3D replication took approximately 25 h. It was then polished, including removal of unwanted plastic parts by overnight treatment in a chemical bath. Because the 3D replica was based on CT data, the skull surface and the bone thickness was a 1:1 representation of the monkey's skull. Although the thickness of the skull could be determined by the CT images, the 3D model was used instead during surgery and was advantageous.

2.3. Head fixation device

Similar to other recent studies (Adams et al., 2007, 2011; McAndrew et al., 2012) the aim here was to develop a stable and solid implant without using dental or orthopedic cement. In this study the head fixation device initially developed by Adams et al. (2007, 2011) was chosen as a base and was modified according to experimental needs. The material used to elaborate the head fixation device was titanium, which has been used for more than 30 years in the medical industry. Titanium presents the advantage of being, along with gold and platinum, one of the most biocompatible metals, and is resistant to body fluids (Rubo de Rezende and Johansson, 1993). Titanium demonstrates high corrosion resistance and the highest strength-to-weight ratio of any known metal. One of the most important advantages associated with the use of titanium was that bone adheres well to it and yields good osseous-integration (Brånemark et al., 1969; Albrektsson and Albrektsson, 1987; Rubo de Rezende and Johansson, 1993; Augat et al., 1995; Betelak et al., 2001). The head fixation devices were manufactured (Ateliers Clément S.A. CH – 1731 Ependes) from a pure titanium cube (CP, Grade 2) as mono-blocks, allowing excellent osseous-integration (note however that Grade 5 would be recommended if one wanted to reduce artifacts for subsequent MRI). Because the head fixation device needed no welding, a break at the weld line between the post and the footplate was prevented (Adams et al., 2007).

The head fixation device used in the present study is illustrated in Fig. 1A. From a mechanical point of view, it could be divided into two different parts. The base of the implant presented a "K-shaped" footplate designed for attachment to the most rostral part of the skull (Fig. 1B) with 12 or 16 bone-titanium screws (cortex screws Ø 2.7 mm, self-tapping; SYNTHES®; length of 6 or 8 mm), depending on the weight of the animal and the size of the skull. The precise shape of the base of the implant may be refined using the 3D print of the monkey's skull, as explained in the section "recording chamber" (see also supplementary video sequence #2 http://www.unifr.ch/neuro/rouiller/research/multi/lanz/l2.html). The upper part of the head fixation device, which is the only visible