



Basic Neuroscience

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.

ARTICLE INFO

Article history:

Received 20 June 2013

Accepted 24 July 2013

Keywords:

Macaque monkey

Skull implants

Osseous-integration

Chronic brain electrophysiology

Hydroxyapatite

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.

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

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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

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 m³ (15 m³ 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 ABSplus 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

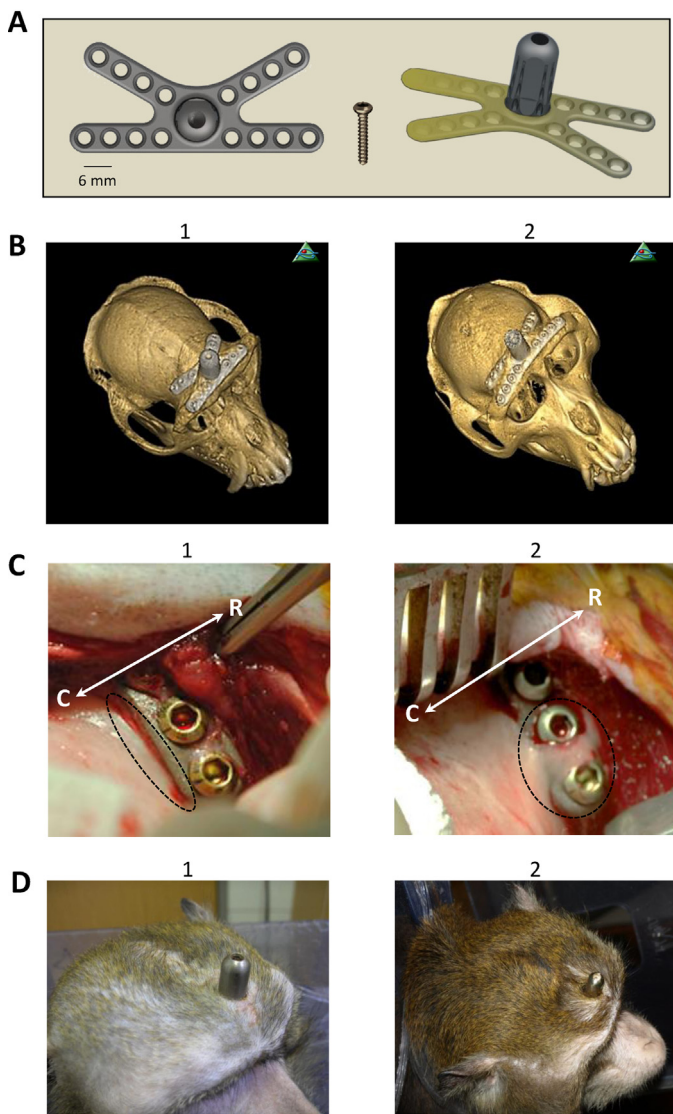


Fig. 1. (A) View of the head fixation device (design derived from Adams et al., 2007). (B) Head fixation device fixed on the monkey's skull (B1: monkey Mk-JZ and B2: monkey Mk-LI). (C) Osseo-integration of the head fixation device was observed during the surgery for chronic recording chamber implantation. The incision made along the skull midline (Rostro-Caudal (R-C)) allowed observation of an osseo-integration along the footplate for MK-JZ (C1) and above the footplate for MK-LI (C2). (D) Head fixation device, after 1 month (D1) and 3 months (D2) after implantation (in Mk-LI).

part after implantation (Fig. 1 D1 & D2), is a vertical cylinder of 10 mm in diameter and 20 mm high, with rounded edges starting at 3 mm from the top. The length of the cylinder was shorter than the height of the wire mesh of transfer cages used to channel the monkeys into the primate chairs (see (Schmidlin et al., 2011)), so as to avoid any accident in the cage. An 8 mm deep hole with an internal thread was designed at the top of the cylinder. This was to allow the device to be fixed to the experimental set-up, thus ensuring a firm head fixation. When the animal was not in the experimental set-up, the opening on top of the cylinder was closed with a headless screw. The head fixation device placed and fixed on the skull of the monkey is shown in Figs. 1B1 and B2 (see also supplementary video sequence #3 <http://www.unifr.ch/neuro/rouiller/research/multi/lanz/l3.html>).

In the present study, the head fixation device (Fig. 1) was implanted on monkeys of about 8 kg. For smaller monkeys, a comparable head fixation device could be used, but modified with shorter footplates accommodating only 12 screws.

2.4. Coating process

In line with recent reports (Adams et al., 2007, 2011; McAndrew et al., 2012), the aim was to develop a stable and solid implant without using dental or orthopedic cement. Important to this study was that the base of the head fixation device (which would be in contact with the bone) was coated with of a naturally occurring mineral form of calcium apatite known as: Hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3(\text{OH})$) (phosphate minerals groups) abbreviated as HA or HAP. This material is widely used to coat implants, to provoke a strong connection to the host bone. The main applications are coatings for orthopedic hip implants for the cementless implantation technique. HA is the preferential material for this application due to its chemical similarity with natural bone, allowing bone to bond directly to HA coated surfaces. The poor mechanical properties of synthetic calcium-phosphates hindered the use of this material for load bearing implants. As a result natural HA-coatings on mechanically stable substrates have become widely used. Vacuum plasma spraying (VPS) has been established as the most suitable technique for industrial coating production. This innovative coating technique in the field of the electrophysiological research allowed a better anchoring of the implant to the skull, as well as faster adherence. The advantages of this coating were demonstrated earlier on a canine model (Cook et al., 1992) and on human patients (Jaffe et al., 2007).

Despite the successful application of plasma sprayed coatings in the biomedical field, between 0.5 and 3% of the hip endoprostheses failed due to bacterial infection (Harris and Sledge, 1990). For the present implantation of a head fixation device and a chronic recording chamber the incidence of implant infection was expected to be even higher, as the implants were transcutaneous and it would be more difficult to keep the environment sterile for animal surgery. To generate an antibacterial effect in the HA coating, the integration of silver (Ag) was a promising procedure. Ag is well known for its antibacterial properties against all bacteria strains. As HA has a high exchange rate with metal-ions, an ion exchange process was used to incorporate Ag into the HA spray powder. The obtained powder (HA-Ag) was then used for the plasma spraying process to form a HA-Ag coating on the implants, comparable to the pure HA-coating.

To coat the present implants, this newly developed HA-Ag coating complex was applied for the first time for in vivo applications. Several in vitro experiments showed that the coating releases Ag^+ ions, which was effective against bacterial colonization on the implant surface. As the Ag content in these coatings was very small, the antibacterial effect addressed only the risk of short term implant infection after surgery. Indeed, Ag is also toxic to bone cells, as well as acting against bacteria. However, the bacteria proved to be more sensitive to Ag than the bone cells. In addition, a majority of Ag ions solubilized very fast. They mainly affected the bacteria present on the implant during surgery. Several days later, when the bone cells started growing on the surface of the implant, the dilution of Ag ions was much lower compared with the amount during the first hours. The areas of the implant, which were not meant to be coated, were masked with Polyimide tape and then covered by metal masks. HA-Ag coatings were produced by Medicoat AG (Switzerland) by VPS type MC60. Because the implant fixation must withstand high forces, strong tensile bond strength of the coating bond to the substrate must be guaranteed. Therefore a titanium bond coating was applied before the HA-Ag coating using the same procedure. The Ag concentration in the coating was measured by inductive coupled plasma (ICP) at EMPA, St.Gallen (Switzerland). The Ag content was detected to be 1500 ppm (Fig. 1A, right panel).

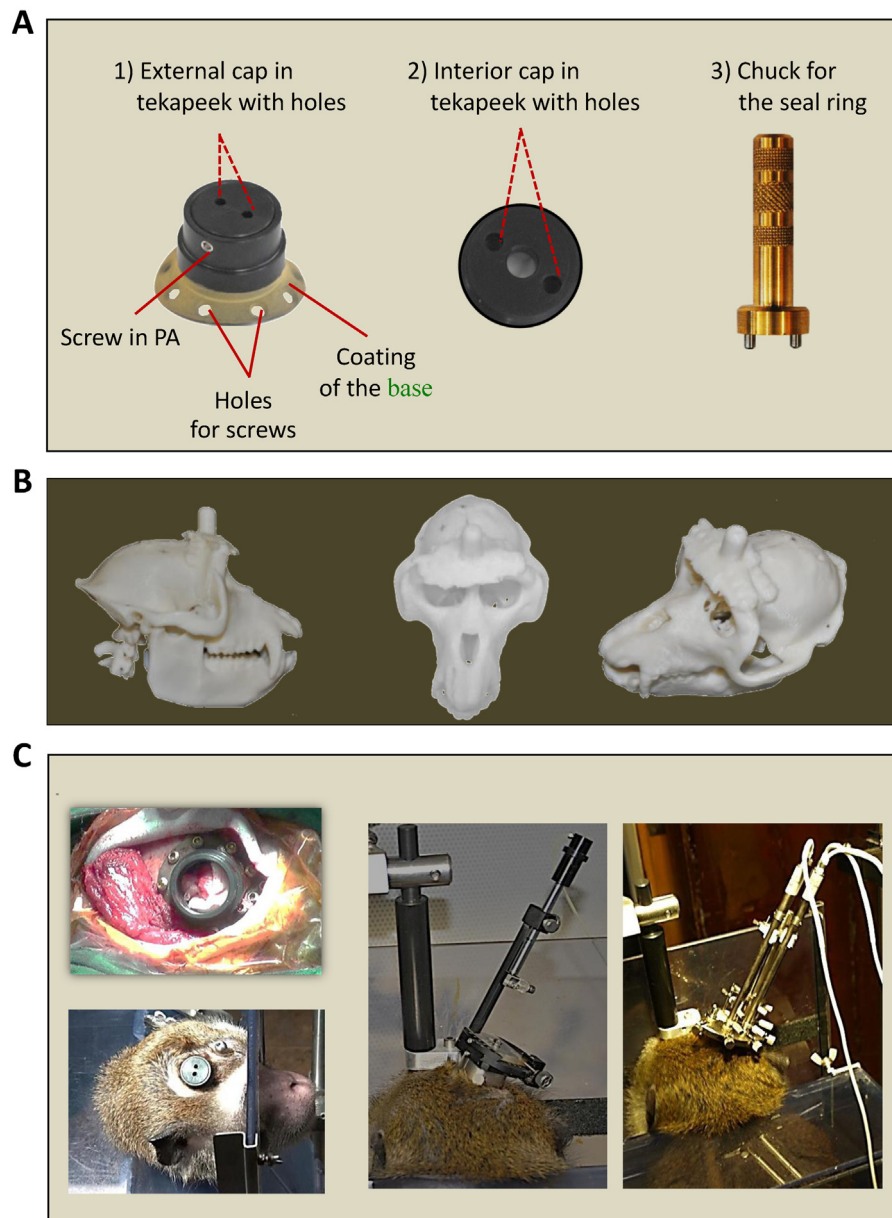


Fig. 2. (A) On the left, general view of the chronic recording chamber built to access the premotor cortex. The bottom edge in yellow corresponds to the coating with hydroxyapatite. In the middle, an interior cap ensured the sealing of the chronic recording chamber and isolated the dura mater, when no electrophysiological recording session takes place. The rightmost panel shows the screwdriver used to manipulate the internal cap inside the chronic recording chamber (see supplementary video sequence #7 <http://www.unifr.ch/neuro/rouiller/research/multi/lanz/l7.html>). (B) View of the 3D print replicate of the monkey's skull in plastic (see text; Mk-LI). (C) On the left, the chronic recording chamber is shown when it was implanted during the surgery (in Mk-LI) and as it appears several months after implantation (in Mk-JZ). In the middle and on the right (Mk-JZ), the pictures show the system used to fix the head of the monkey, together with the adaptation on the chronic recording chamber of a Narishige® single electrode drive system (middle) or a NAN® multiple electrode driving system (right).

2.5. Recording chamber

In parallel to the head fixation device, a similar idea was employed to design new chronic recording chambers. With these new chambers electrophysiological data could be derived from behaving monkeys with their head fixed. The aim here was to increase the animal's comfort (again no dental or orthopedic cement to fix the chronic chamber on the skull, as recently proposed by Adams et al., 2011), while reducing the daily care of the chamber and infection risks. To ensure optimal anchoring of the chronic chamber on the scalp, its shape was adapted to the 3D replicate (print) of the corresponding monkey's skull. This 3D print allowed definition of the exact position and the shape as well as the best fit of the chronic recording chamber on the skull.

In the present study the chronic chamber has a cylindrical shape (Fig. 2 A) and is made of tekapeek- an industrial plastic with high temperature, chemical, electrical and radiation resistance (similar to metals such as titanium). Tekapeek has the advantage of guarding the option to perform subsequent MRI investigations with minimal artifacts. Furthermore, tekapeek is lighter than metal, thus reducing the weight of the chamber placed on the monkey's skull. The milling of the chamber is illustrated in the supplementary video sequence #4 (<http://www.unifr.ch/neuro/rouiller/research/multi/lanz/l4.html>). The chronic chamber is comprised of a base (28 mm in diameter), which adheres to the bone, with a cylinder on top (24 mm in diameter and 9 mm in height) giving access to the dura and offering the possibility to fix an electrode driving system (Fig. 2C). The base

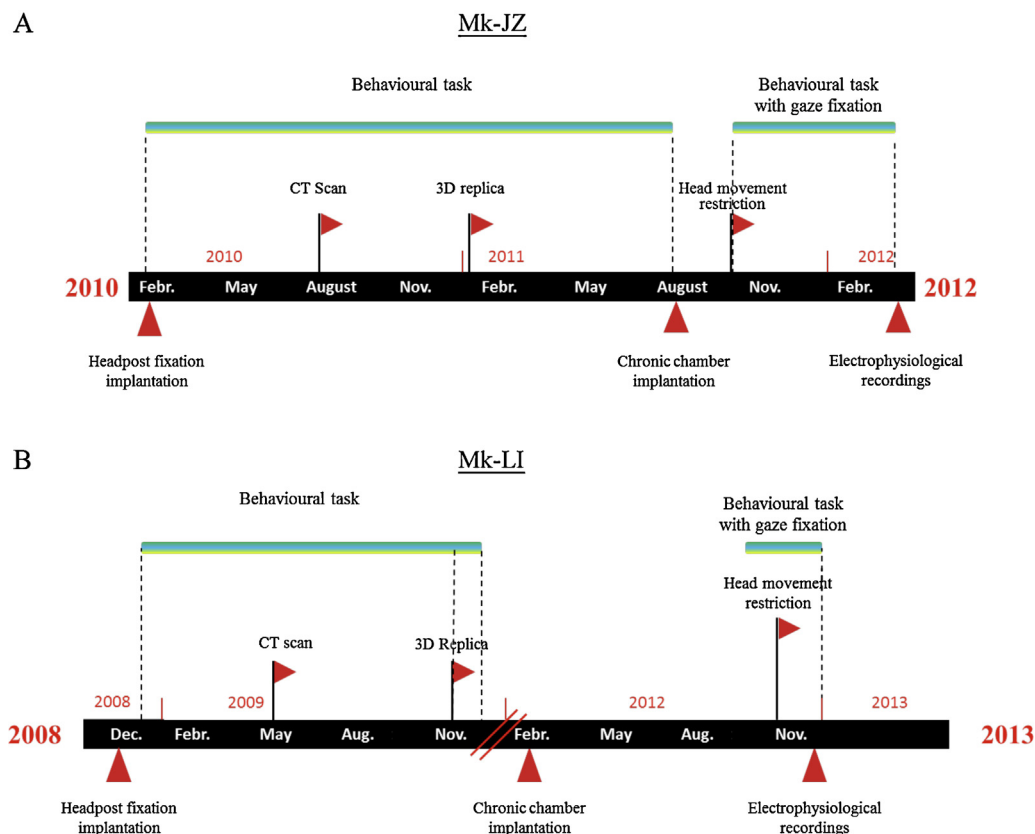


Fig. 3. Timeline of the implantation procedures, behavioral and electrophysiological recordings the two subjects underwent during the overall experimental protocol.

of the recording chamber was coated with HA (Fig. 2A1), as was done for the head fixation device. Briefly, the base of the recording chamber was coated with a titanium bond layer between the substrate and the HA coating. The titanium was very reactive and bonded very well to different materials (for more detail, see “Head fixation device” section above).

The exact position of the chronic chamber on the skull was defined by superimposing a MRI scan of the monkey's brain (providing anatomical position of sulci and cortical gyri for example) with the corresponding CT scan (providing an accurate replica of the skull surface) performed in the same animal. Then the shape and contour of its base was precisely adjusted to the bone surface, as it appeared on the 3D print of the skull. The base of the chronic chamber is comprised of 7 holes used to position fixation screws (Fig. 2A1). The cylinder was larger at the bottom than at the top forming a shoulder on which the electrode driving system rested (Fig. 2C). The internal diameter of the cylinder was 21 mm, which corresponded to the size of the grid guiding the electrodes held by the electrode driving system.

When the animal was not in the experimental set-up, the chronic recording chamber was covered with a cap. Instead of using a standard cap placed on top of the cylinder, a cap was designed which could be placed inside the cylinder. The advantage here is that the size of the implant is reduced. To ensure appropriate sealing, the cylinder is closed first with a sealing ring (Fig. 2A2), which is screwed into the cylinder using an ad-hoc screwdriver (Fig. 2A3). The sealing ring was used mainly to cover a round piece of silastic placed on top of the dura. The sealing ring is 5.2 mm thick with a diameter of 20 mm, and a central opening of 4.7 mm (Fig. 2A2). The central opening prevents a pressure increase on the silastic joint when the sealing ring is screwed/unscrewed. To allow the screwing/unscrewing of the sealing ring two small

3 mm holes were made. Finally, an external cap is positioned on top of the cylinder (Fig. 2A1), ensuring hermetic closure of the chamber. The external cap is a 7.8 mm thick disk with a diameter of 20 mm, comprised of two holes on the top, and a threaded hole on the side. The hole on the side needs to be aligned with a corresponding hole in the side wall of the cylinder, in order to close the chronic chamber by means of a headless polyamide screw (M5 × 3). The cap can be removed by means of a modified crowbar. A comprehensive view of the chronic recording chamber was available in the supplementary video sequence #5 (<http://www.unifr.ch/neuro/rouiller/research/multi/lanz/l5.html>).

2.6. Implantation surgery

First the animal was sedated by an intramuscular injection of a mixture of ketamine (Ketanarkon® 10 mg/kg body weight), benzodiazepine (Midazolam 0.1 mg/kg) and methadone (0.2 mg/kg). This sedation allowed the preparation of the monkey for the surgical intervention, involving shaving the skull and preparation for an intravenous injection of Propofol (diisopropylphénol), coupled with gas anesthesia (Sevoflurane; see below). In this step, the analgesics Carprofen (Rimadyl®, Pfizer, 4 mg/kg) and broad spectrum antibiotics (Albipen®, Intervet, 30 mg/kg) were injected subcutaneously. In addition, to reduce edema, dexamethasone (Decadron 0.3 mg/kg, mixed 1:1 with saline) was injected i.m. Finally, an injection of atropine (0.05 mg/kg i.m.) was given to reduce bronchial secretion.

In the operating room, under sterile conditions, the animal was intubated, allowing ventilation with a 50%/50% mixture of O₂ and air, containing 2.5% Sevoflurane to ensure anesthesia. This was complemented by continuous i.v. perfusion of Propofol (Fresenius 0.1 mg/kg/min). At potentially painful steps of the surgery

(e.g. craniotomy), an i.v. flow of opioid (Fentanyl 0.1 $\mu\text{g}/\text{kg}/\text{min}$) was used. In addition, during the entire surgery, the animal received a continuous i.v. perfusion with lactate-ringer at a rate of 5 ml/kg/h.

During the entire surgical procedure, physiological parameters were continuously monitored (e.g. body temperature, O_2 saturation, heart rate, respiration rate, exhaled CO_2). At the beginning of the procedure, the skin of the head was cleaned and disinfected with an iodized solution (Betadine or Povidone-iodine). An incision of the skin along the skull midline of about 10 cm was made with a scalpel. The muscles were then reclinined from the skull to expose the bone surface. The head fixation device was then positioned on the skull at its foreseen position (as rostral as possible: see Fig. 1B). At this step, if necessary, in order to ensure a perfect fit on the skull surface and taking advantage of the flexibility of the titanium, the footplates of the head fixation device could be slightly adjusted with sterile tools. Before anchoring the head fixation device on the skull, the bone (periosteum) was abraded extensively. The insertion of the screws was done according to the protocol by Adams et al. (2007). Namely the first screw to be implanted was situated on the left-hand side of the leg of the arch; the second was placed on the last hole of the right posterior leg. For insertion of each screw, a power drill was used to make, small pilot holes of a smaller diameter. Furthermore, to avoid bone damage resulting from a temperature increase, a flow of saline (0.9%) from a large syringe was used for cooling during the drilling. An increase in temperature could cause bone softening near the holes. The screws were carefully inserted by hand, without exerting too much pressure, in order to minimize risks of cracking or damaging the bone. The length of screws was chosen according to the CT scan performed a few weeks before the operation and the 3D printing of the skull (see above).

Once the head fixation device was anchored to the skull, first the muscles then skin were stitched to the midline. A small opening was left on the posterior part of the cylinder of the head fixation device to act as a natural catheter, allowing for possible leakage of secretions, and to inject antibiotics, if needed. Several months later, a similar surgical procedure was conducted to anchor the chronic recording chamber on the surface of the skull (see supplementary video sequence #6 <http://www.unifr.ch/neuro/rouiller/research/multi/lanz/16.html> and Fig. 2C- top left panel).

2.7. Daily care for the head fixation device

After the implantation of the head fixation device, the wounds and scars were cleansed daily with an antiseptic iodized solution (Betadine[®] 500 ml, Mundipharma Medical Company). The animal was examined for possible inflammation and appearance of infection as well as observation of general behavior. An anti-inflammatory and antibacterial cream (Panalog[®] ad US. Vet., Novartis) was applied around the implant. Furthermore the animal received antibiotics and analgesic for at least 10 days. The big advantage with this type of implant was that after about 1 month, daily care was no longer necessary. However, a bi-weekly cleaning with Betadine was done, as well as a daily routine check of the animal.

2.8. Daily care for the chronic recording chamber

The chronic recording chamber's daily care required more time and was more frequent than that of the head fixation device. Just after surgery the wounds and scars were cleansed daily with an antiseptic iodized solution (Betadine[®] 500 ml). During the following ten days, the animal received injections of antibiotics (Albipen[®]) as well as analgesics (Rimadyl[®]). At the end of every daily care, a cream (Panalog[®] 15 ml, Novartis) was applied around the chronic chamber. Similar to head fixation device, this treatment

was pursued during one month post-implantation. Afterwards cleaning the external part of the chronic recording chamber was no longer performed on a daily basis, but a superficial cleaning with Betadine[®] was required about twice a week.

The inside of the chronic recording chamber required a lot more attention and care (see supplementary video sequence #7 <http://www.unifr.ch/neuro/rouiller/research/multi/lanz/17.html>). The chamber was cleansed at least once every three days using sterilized material (gaze band, surgical forceps, silastic joints, two different caps). During the recording period, cleaning was performed at every recording session. The inside of the chronic chamber was cleaned using Betadine. Before closing the chamber, the silastic joint (Silicone sheeting) (LP 500-9, Manufactured by LPI) was coated with a layer of antibiotic cream (Fucithalamic Vet[®], 3 mg/g; Dechra). Once the daily recording sessions had begun, the dura mater was scraped once a week, in order to limit tissue growth and dura thickening, which would block the penetration of recording electrodes.

2.9. Timeline

To clarify the time course of events the two subjects were to follow during the experimental protocol, a timeline was established and displayed, in Fig. 3. This timeline indicates the starting point of the procedures (headpost fixation implantation, CT scan, 3D replica, chronic chamber implantation) and the behavioral tasks/first electrophysiological recordings accomplished. At the time of the publication the protocols were still on-going.

3. Results

3.1. Head fixation device

To ensure a high and stable bone integration of the head fixation device, the monkeys were implanted very early, well before the behavioral training was started. Mk-LI was implanted on November 12th 2008, whereas Mk-JZ was implanted on February 19th 2010. As shown in Fig. 1D, after respectively 1 month (1 D1) and 3 months (1 D2) post-implantation, only the cylinder of the head fixation device was visible on the rostral part of the monkey's head. The skin retracted and adopted a position fitting the circumference of the cylinder of the head fixation device. There was no infection or inflammation around the implant and therefore only minimal care was necessary at 2 weeks intervals in the form of cleaning around the implant with Betadine[®].

After a period of behavioral training, the monkeys underwent the implantation of a chronic recording chamber. This surgery provided the opportunity to check the integration of the head fixation device to the bone, as it appears after 1 year in Mk-JZ and after 3.5 years in Mk-LI (Fig. 1C). The implant was perfectly adapted to the topography of the skull, as expected after the final adjustment of the footplates during the first surgery. The osseous integration was not the same for both animals, which is in line with the different time intervals. In the first monkey (Mk-JZ; Fig. 1C1), after one year, it was observed that an osseous layer had settled along the footplate edge. In the second animal (Mk-LI; Fig. 1C2), after three and a half years, there was a clear bone growth over the footplate and in between two adjacent titanium screws. From a behavioral point of view, the animal presented no discomfort in relation to the head fixation device during the behavioral training. Note that, during the entire training period, the head of the animal was not fixed in the experimental set-up, to avoid mechanical constraints, which could disrupt bone growth around the footplates. The use of the head fixation device to fix the monkey's head using a rigid arm anchored to the experimental set-up is shown in Fig. 2C.



Fig. 4. Pictures showing the building up of another chronic recording chamber to access a different area (thalamus) after completing the investigations in the premotor cortex (see text). On the left, the picture shows a simulation of the new chronic recording chamber before it was fabricated. On the right the picture shows the real chamber in tekapeek and placed on the 3D replicate printing of the monkey's skull (Mk-LI).

3.2. Recording chamber

The chronic recording chamber, illustrated in Fig. 2, was implanted in both monkeys (August 23rd 2011 in Mk-JZ and February 9th 2012 in Mk-LI), with the aim to access the premotor cortex in the right hemisphere (Fig. 2C). As the shape of the chronic chamber was well adapted to the 3D print of the corresponding monkey's skull (Fig. 4) the implantation during surgery was straightforward and the final position easy to achieve. Indeed, only the targeted position on the skull provided a perfect match between the base of the chronic chamber and the contour of the skull. A preliminary positioning of the chronic chamber was performed to determine the region of the bone to be removed. The bone was then marked with a sterile pencil around the circumference of the inside of the chronic chamber. The contour of the cylindrical piece of bone was cautiously removed using a drill, exposing the dura mater. This was immediately photographed in order to register the position of blood vessels to be avoided in subsequent electrode penetrations. The positioning of the chronic recording chamber on the skull immediately after screw fixation is shown for MK-LI in Fig. 2C -top left panel. Depending on the monkey's size and the position of the chronic chamber, a part of the temporal muscle had to be removed, especially if the chronic chamber was located laterally. First the muscles were sutured and then the skin, all around the chronic recording chamber. Complete scarring took place over a few weeks (Fig. 2C). The skin around the chronic chamber (see supplementary video sequence #7 <http://www.unifr.ch/neuro/rouiller/research/multi/lanz/l7.html>) required only minimal, bi-weekly, cleansing with Betadine®, as was the case for the head fixation device. As illustrated in Fig. 2C, the chronic recording chamber allowed fixation of different electrode driving systems like the Narishige® system (Narishige International limited, Japan, Fig. 2C) and the NAN® system (NAN Instrument, LTD, Israel, Fig. 2C).

The present approach eliminates the use of dental acrylic resin or orthopedic cement, and gives the option to remove a chronic chamber at a later time point when electrophysiological investigations in the corresponding brain region are complete. In this fashion another brain region could be targeted for a subsequent step. It is true that a chamber fixed with acrylic cement could possibly be removed, but the underlying bone might be in bad condition and certainly more traumatized (soft bone, presence of infection, bone thickness not suitable for re-implantation, etc.), as compared to the present approach. Such a strategy would allow the use of the same trained monkey to extend the investigations to additional brain regions. In the present case, once the electrophysiological recordings are completed in the premotor cortex, the first chronic

recording chamber implanted will be removed and replaced by a second one, designed to reach the thalamus (Fig. 4). The second chamber is basically the same as the first one: cylindrical shaped with a bottom edge large enough to ensure a perfect seal with the bone. In addition, the edge will be extended laterally and rostrally in order to cover the skull area where the first chamber had been implanted (Fig. 4). A round protrusion of the tekapeek will be made to fit perfectly into the bone hole drilled for the first chamber. This second chamber will be positioned more caudally and near the midline to allow vertical penetrations to reach the thalamus.

4. Discussion

The present report provides evidence that both the head fixation device and the chronic recording chamber can be implanted on a monkey's skull for a long period (4 years for the head fixation device), without the adverse events observed in the past with dental resin or orthopedic cement, in terms of inflammation, infection or rejection. To demonstrate the benefit of the present approach, a complementary retrospective analysis (Table 1) was made from data collected in our laboratory on subjects with head post fixation fixed with different cements (dental resin or orthopedic cement). These data demonstrate that the use of cement is associated with a much shorter duration of implants and an increase in infections and/or loss of the device, when compared with the two monkeys included in the present study. Therefore this new protocol guarantees an excellent osseous-integration of the implants. This is due to: (1) a coating of the implants with HA (Figs. 1 and 2), thus potentiating the integration offered by the titanium itself and (2) a 3D print replicate of the skull of the living animal for precise design and adjustments to individual skull shape. The 3D print step represents a crucial improvement in allowing production of truly custom-fitted implants that perfectly fit the monkey's skull. The longevity of the implants demonstrated here on two monkeys (up to 4 years for the head fixation device) is substantial progress and should be compatible with various behavioral and electrophysiological protocols conducted in non-human primates. As illustrated in the supplementary video material, the two implants are well tolerated by the monkeys, even during the cleaning procedures. Due to the elimination of dental acrylic resin or orthopedic cement, the implants have a limited mass above the monkey's head, reducing the probability that the animal hits its head against obstacles in its environment (e.g. enclosure). Another advantage of avoiding dental resin or orthopedic cement is a considerable time gain, about one hour, during surgical implantation of the head fixation device or the chronic recording chamber. In addition, surgery undertaken to anchor the implants is much easier, as the difficult process of

Table 1

Retrospective survey of head post implants fixed to the skull with dental resin or orthopedic cement in 11 monkeys, involved in electrophysiological protocols (1994–2006). The table lists for each monkey the duration of implantation (before the device had to be removed or was lost), the occurrence of infections and the devices losses.

Subject ($n = 11$)	Implantation duration (months)	Infections	Loss of device
MK-1	10		
MK-2	12		
MK-3	7		X (2x)
MK-4	6		
MK-5	11		X
MK-6	3		
MK-7	12	X	X (2x)
MK-8	6		
MK-9	8		
MK-10	6		X (2x)
MK-11	9	X	X
Average	8	18%	45%

skillful application, in successive small amounts, of dental resin or orthopedic cement, is skipped. Thus the present work supports the idea of elimination of dental acrylic resin or orthopedic cement, as previously suggested by Adams et al. (2007, 2011) or McAndrew et al. (2012).

Another valuable contribution of the present study consisted in confirming the suitability of the “K-shape” base of the head fixation device, which allowed the elimination of dental acrylic resin or orthopedic cement, as initially proposed by Adams et al. (2007). The longevity of the implanted device was observed to be at least 17 months by these aforementioned authors. This was extended to 3 years and 8 months in a more recent report (Adams et al., 2011). In the present study, the follow up is more than 4 years. No incidents were seen, despite the fact that it was used to fix the head of two strong adult male macaque monkeys (8 kg body weight) in behavioral and electrophysiological experiments. The clean and discrete appearance of the head fixation on the monkey’s head is also confirmed in the present study (see Fig. 6 of Adams et al., 2007; Fig. 1D in the present report). Experience shows that it is crucial to implant the head fixation device early enough in the protocol, so that the osseous-integration can occur during several months, before the monkey’s head is fixed in the set-up. At least 3 months is recommended, but longer may be safer. This relatively long delay can be anticipated to some extent, as the head fixation device can be successfully implanted in juvenile monkeys (Adams et al., 2007). In the present study, in contrast to the work of Adams et al. (2007), a further improvement was introduced in the form of coating the head fixation device with HA in order to enhance the osseous-integration. Although titanium is compatible with MRI investigations in spite of some artifacts, one could also envisage, in the future, building the head fixation device from tekapeek, as the chronic recording chamber, to minimize artifacts. The “K-shape” head fixation device (Adams et al., 2007, 2011; present study) is clearly less bulky and less uncomfortable for the monkeys than the head ring approach. (Isoda et al., 2005).

The design of a custom-fitted chronic recording chamber to a living monkey is new, as such an attempt was only recently reported (McAndrew et al., 2012). An important difference however is that these authors imported the CT reconstruction of the monkey’s skull into a 3D CAD (computer-aided design) program where the implant was designed at the target location on the skull. The present study went one step further. The skull derived from the CT scan was printed out in 3D (Fig. 2B and Fig. 4) in order to obtain a true replicate of the living monkey’s skull, similar to what would have been obtained if the monkey had been sacrificed. The replicate of the monkey’s skull can be used to test and determine, with high precision, several options concerning size and position of chronic recording chambers, to reach a given brain region. It is also possible to easily check for possible conflicts between several chronic chambers to be implanted either simultaneously or one after the

other. When the approach and the final position have been chosen, the chronic recording chamber can be fabricated by the machinist using the contour which was determined by means of the 3D skull replicate print. In their report (McAndrew et al., 2012), the authors mentioned that the longevity of the device remained to be seen, especially after reporting that the implant became loose after 6 months, requiring re-implantation. Furthermore, due to a gap between the chronic chamber’s edge and the skull, there was skin and hair growth into the implant (McAndrew et al., 2012). Such undesired event did not occur in the present study, even after a year following implantation of the chronic recording chamber (Mk-JZ). It is very likely that a perfect seal was obtained due to the 3D print replicate of the skull. Using this 3D print, the relatively large and flexible edge at the bottom of the chamber could be formed to adhere very tightly to the skull with the use of titanium screws. As a result, pressure exerted by the screws all around the implant produced a perfect seal (Fig. 2). As evidenced by the cleaning procedure, the inside of the chronic recording chamber is totally impermeable to fluid with respect to the outside. This is an important requirement to minimize risks of infection inside the chronic chamber. The present study differs in several aspects from the recent report of Adams et al. (2011). First, in their report, Adams et al. (2011) used an acrylic free titanium chronic recording chamber whereas here the chamber was machined from Tekapeek. The latter presenting the advantage to be lighter and to reduce artifacts in MRI investigations. Furthermore, Adams et al. (2011) used HA as a paste to seal the chamber to the skull during the surgery; by contrast, in the present study, HA was coated onto the implant itself before implantation. Another important difference is that the titanium chamber used by Adams et al. (2011) was comprised of 5–6 feet, each with one hole to insert a screw to anchor the chamber to the skull. In our case (Fig. 2), the feet were replaced by a continuous thin edge all around the base of the chamber (Fig. 2) which included the holes to insert the screws ($n = 7$). When tightened, the screw pressure ensured a perfect match of the chamber’s base onto the skull surface due to of the relative flexibility of the Tekapeek and its thin continuous edge. The present study demonstrates that Tekapeek can replace titanium to fabricate implants (at least the chronic recording chamber, but possibly also the head fixation device) and exhibits good osseous-integration properties due to the hydroxyapatite coating. The use of Tekapeek is especially favorable for MRI investigation, as artifacts are minimized. Along this line, a further improvement may consist in replacing titanium screws by ceramic screws.

The steps newly introduced in the present study, such as coating the implants with HA and the 3D print replicate of the skull, increases the cost of the experiments, but remains a worthwhile investment considering the longevity of the implants, the ethical and commercial values of the non-human primates, as well as the long time period invested in this type of experiments – mainly

training the monkeys to perform difficult behavioral tasks. Indeed, the rejection of an implant (head fixation device and/or chronic recording chamber) by the bone is dramatic in any case, as it can ruin a long and expensive behavioral and electrophysiological experiment. It is extremely difficult to re-implant a second time at the same location.

In summary, the present study contributes to refining recent proposed techniques (Adams et al., 2007, 2011; McAndrew et al., 2012) to optimizing the anchoring of a head fixation device and a chronic recording chamber on the skull of macaque monkeys. In our opinion it should include a coating with HA and a 3D print replicate of the skull. As such, the present study is well in line with the 3Rs initiative to improve the conditions of laboratory animals. Indeed, the longevity of the implants as well as the minimal discomfort offered by custom-fitted implants contribute to substantially reducing the stress for the monkeys, decreasing the risks of infection and reducing the number of animals used in this type of experiment. The possibility to remove and replace implants is a very positive aspect because the same animal can be used over a longer time period and allows investigation of remote brain regions (e.g. premotor cortex and thalamus).

Conflict of interest

HA is provided by Medicoat AG (Switzerland).

Acknowledgments

The authors thank Joseph Corpataux, Laurent Bossy, Jacques Maillard for their highly valuable daily collaboration for the care of the monkeys in the animal facility, especially before, during and after the various interventions. Our thanks go also to the “Ateliers Clément S.A.” for the fabrication of the head fixation device and to the Fribourg’s Engineering school for the 3D print replicate of the monkey’s skull. The authors thank Jane Favre for proofreading.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jneumeth.2013.07.015>.

References

- Adams DL, Economides JR, Jocson CM, Horton JC. A biocompatible titanium headpost for stabilizing behaving monkeys. *Journal of Neurophysiology* 2007;98:993–1001.
- Adams DL, Economides JR, Jocson CM, Parker JM, Horton JC. A watertight acrylic-free titanium recording chamber for electrophysiology in behaving monkeys. *Journal of Neurophysiology* 2011;106:1581–90.
- Albrektsson T, Albrektsson B. Osseointegration of bone implants: A review of an alternative mode of fixation. *Acta Orthopaedica Scandinavica* 1987;58:567–77.

- Augat P, Claes L, Hanselmann K-F, Suger G, Fleischmann W. Increase of stability in external fracture fixation by hydroxyapatite-coated bone screws. *Journal of Biomaterials* 1995;6:99–104.
- Betelak KF, Margiotti EA, Wohlford ME, Suzuki DA. The use of titanium implants and prosthodontic techniques in the preparation of non-human primates for long-term neuronal recording studies. *Journal of Neuroscience Methods* 2001;112:9–20.
- Brånemark PI, Adell R, Breine U, Hansson BO, Lindström J, Ohlsson A. Intra-osseous anchorage of dental prostheses. I. Experimental studies. *Scandinavian Journal of Plastic and Reconstructive* 1969;3:81–100.
- Churchland MM, Lisberger SG. Apparent motion produces multiple deficits in visually guided smooth pursuit eye movements of monkeys. *Journal of Neurophysiology* 2000;84:216–35.
- Cook SD, Thomas KA, Delton JE, Volkman TK, Whitecloud TS, Key JF. Hydroxylapatite coating of porous implants improves bone ingrowth and interface attachment strength. *Journal of Biomedical Materials Research* 1992;26:989–1001.
- Durif C, Jouffrais C, Rouiller EM. Single-unit responses in the auditory cortex of monkeys performing a conditional acousticomotor task. *Experimental Brain Research* 2003;153:614–27.
- Fuchs AF, Luschei ES. Firing patterns of abducens neurons of alert monkeys in relationship to horizontal eye movement. *Journal of Neurophysiology* 1970;33:382–92.
- Guo K, Li CY. Eye position-dependent activation of neurones in striate cortex of macaque. *Neuroreport* 1997;8:1405–9.
- Harris WH, Sledge CB. Total hip and total knee replacement. *New England Journal of Medicine* 1990;323:801–7.
- Ioda M, Tsutsui K-I, Katsuyama N, Naganuma T, Saito N, Furusawa Y, et al. Design of a head fixation device for experiments in behaving monkeys. *Journal of Neuroscience Methods* 2005;141:277–82.
- Jaffe WL, Morris HB, Nessler JP, Naughton M, Shen J. Hydroxylapatite-coated acetabular shells with arc deposited titanium surface roughening and dual radius design. *Bulletin of the NYU Hospital for Joint Diseases* 2007;65:257–62.
- Kaesler M, Wyss AF, Bashir S, Hamadjida A, Liu Y, Bloch J, et al. Effects of unilateral motor cortex lesion on ipsilesional hand’s reach and grasp performance in monkeys: relationship with recovery in the contralesional hand. *Journal of Neurophysiology* 2010;103:1630–45.
- Kaesler M, Brunet J-F, Wyss A, Belhaj-Saif A, Liu Y, Hamadjida A, et al. Autologous adult cortical cell transplantation enhances functional recovery following unilateral lesion of motor cortex in primates: a pilot study. *Neurosurgery* 2011;68:1405–16, Discussion 1416–7.
- Kermadi I, Liu Y, Tempini A, Rouiller EM. Effects of reversible inactivation of the supplementary motor area (SMA) on unimanual grasp and bimanual pull and grasp performance in monkeys. *Somatosensory and Motor Research* 1997;14:268–80.
- Kermadi I, Liu Y, Tempini A, Calciati E, Rouiller EM. Neuronal activity in the primate supplementary motor area and the primary motor cortex in relation to spatio-temporal bimanual coordination. *Somatosensory and Motor Research* 1998;15:287–308.
- Lisberger SG, Westbrook LE. Properties of visual inputs that initiate horizontal smooth pursuit eye movements in monkeys. *Journal of Neuroscience* 1985;5:1662–73.
- Liu Y, Rouiller EM. Mechanisms of recovery of dexterity following unilateral lesion of the sensorimotor cortex in adult monkeys. *Experimental Brain Research* 1999;128:149–59.
- McAndrew RM, VanGilder JLL, Naufel SN, Tillery SIH. Individualized recording chambers for non-human primate neurophysiology. *Journal of Neuroscience Methods* 2012;207:86–90.
- Peeters R, Simone L, Nelissen K, Fabbri-Destro M, Vanduffel W, Rizzolatti G, et al. The representation of tool use in humans and monkeys: common and uniquely human features. *Journal of Neuroscience* 2009;29:11523–39.
- Rubo de Rezende ML, Johansson CB. Quantitative bone tissue response to commercially pure titanium implants. *Journal of Materials Science: Materials in Medicine* 1993;4:233–9.
- Schmidlin E, Kaesler M, Gindrat AD, Savidan J, Chatagny P, Badoud S, et al. Behavioral assessment of manual dexterity in non-human primates. *Journal of Visualized Experiments* 2011.
- Weatherall report. The use of non-human primates in research; 2006, <http://royalsocietypublishing.org/The-Weatherall-report-on-the-use-of-non-human-primates-in-research/>