A new MRI approach for accurately implanting microelectrodes into deep brain structures of the rhesus monkey (Macaca mulatta)

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1. Introduction

Although many brain structures are very small, perhaps only several millimeters in size, finding their accurate location is an important process in neurophysiology and neurosurgery. Incorrectly targeting these structures during experimental or medical procedures can cause significant problems, especially when implanting electrodes deep into the brain where a small misplacement on the surface of the skull may lead to significant errors in reaching the target. The goal of this study was to develop a new method to increase the accuracy of targeting small subcortical brain structures in monkeys. Such an approach will allow for the electrophysiological characteristics and neural networks of these subcortical structures to be addressed efficiently.

The “atlas-guided technique” and the “MRI-guided technique” are the two primary approaches used to target brain structures. The atlas-guided technique, which combines the anatomical atlas and the stereotaxic frame to determine the stereotaxic coordinates of the relevant brain structure, is widely used by neurophysiologists. While this method provides high spatial resolution, it has two serious shortcomings. First, it lacks 3D information for oblique electrode insertion. Second, and more importantly, the atlas-guided method is inaccurate due to the variation in size and shape of brain structures among individuals. While this variation can be minimized for small mammals such as rats by selecting experimental animals with similar size and weight as those used in producing the atlas, it is much more difficult to control for large mammals such as monkeys. This is because the atlas for monkeys is often based on only a few (one to three) individuals, and ordering experimental monkeys of similar size and weight as those used in the atlas is often not possible. For this reason, the variance between the anatomical atlas and experimental animals can be quite significant. Additionally, the individual variation of big mammals is greater than that of the small ones. According to our experience, using the atlas alone to insert an electrode into the superior colliculus (SC) frequently misses the target by several millimeters and requires the repositioning of the electrode until the SC is found. Although this is time consuming, it still does work for traditional electrodes, which can be withdrawn and inserted many times. However, for electrodes that are implanted directly into the brain, such as tetrodes, using the anatomical atlas to precisely target...

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subcortical structures is almost impossible. The atlas-guided technique is therefore not optimal for the study of subcortical structures in monkeys using implanted electrodes.

To increase the accuracy of the targeting technique in primates, many researchers use MRI method instead of the anatomical atlas method to obtain noninvasively a 3D brain atlas for each individual. The development of hardware and software in recent years has improved MRI resolution and subsequently increased its use in targeting brain structures (Asahi et al., 2003; Bjarkam et al., 2004; Subramanian et al., 2005). Although other imaging techniques, such as radiography including X-ray photography and computed tomography are used in targeting (Aggleton and Passingham, 1981), the MRI method is considered more powerful in identifying brain structures due to its ability to detect the contrast in soft tissues (Rebert et al., 1992).

A series of detailed MR brain structure images, however, is not enough for implanting an electrode into the relevant brain area. To use this “individual atlas” as a targeting technique instead of using the anatomical atlas, researchers must solve two important problems.

First, in the atlas-guided technique, a standard stereotaxic procedure, which is used both for establishing the atlas and for experimental surgery, ensures the accuracy of the insertion angle. This means that during stereotaxic surgery when the animal’s head is fixed on the stereotaxic frame, the orientation of the head is the same as the positioning of the head used in the atlas. The horizontal, sagittal, and coronal planes of the head during surgery are respectively parallel to those three planes in the anatomical atlas. Otherwise, a different insertion angle will lead to a different destination despite the same starting point. There is no such “standard procedure” in the MRI-guided technique because the MRI scanner is not a stereotaxic equipment. The first problem with the MRI-guided technique, therefore, is the lack of a corresponding relationship between orientations of the head in the MR image and that of the head during surgery.

Second, even if the head’s orientations in the atlas and in the surgery are matched, researchers still need some form of reference to calculate the implantation coordinates. In the anatomical atlas-guided technique, landmarks on the skull such as the calvarial sagittal suture or the bregma are used to determine that where on the skull an electrode should be inserted so that it can correctly hit the relevant brain structure showing on the anatomical atlas. Since these landmarks are easily recognized on both the atlas and the skull during surgery, they provide good reference points for the stereotaxic coordinate systems of the atlas and the actual monkey head. These bones, however, do not show up well on an MR image. The second problem, therefore, is to establish a reference point that can be identified on both the MR image and the monkey head.

There are two ways to solve those problems respectively: (1) use a MRI-compatible frame to hold the animal’s head in place throughout the entire experiment from the MRI scan to the post-MRI surgery; (2) use external reference markers that are MRI-visible.

For the method presented in this paper, rigid glass tubes filled with a MRI-paramagnetic solution were used as external markers. Due to their slender shape, a precise corresponding relationship was established between the MR image and the head without requiring a MRI-compatible frame. In using slender external markers, this new method solved these two problems and is therefore both convenient and cost effective. Moreover, compared to current MRI-based targeting methods, this novel method is more accurate (errors < 1 mm). Using this new method, microelectrodes were successfully implanted into the SC of the rhesus monkey (Macaca mulatta) and its neuronal discharge signals were recorded.

2. Materials and methods

2.1. Animals

Four rhesus monkeys (Macaca mulatta) weighing 5.0–7.0 kg and designated as M1 (female, 9 years old), M2 (female, 12 years old), M3 (male, 7 years old), and M4 (male, 8 years old) were used in this study. All were obtained from the primate center of the Kunming Institute of Zoology, Chinese Academy of Sciences. All experimental procedures were conducted in accordance with the National Institute’s Health Guidelines for the Care and Use of Laboratory Animals (NIH Guidelines).

2.2. The external marker

Rigid glass tubes with an internal diameter of 0.5 mm, external diameter of 0.9 mm and length of 3–4 cm were used as the external markers and were anchored on the skull of each monkey. They were filled with vitamin AD to provide a bright signal on the MR image, and thereby functioned as a reference point in both the MR image and the animal’s skull to determine the implantation coordinates.

2.3. Pre-MRI surgery to anchor the markers

Surgery was performed on the monkey anesthetized with hydrochloric acuclated ketamine (10 mg/kg, i.m.) and maintained with sodium pentobarbital (20 mg/kg, i.m.). After correctly fixing the monkey’s head on the stereotaxic apparatus, the scalp was incised and retracted along with the muscles overlying the skull. The surface of the skull was cleaned and dried thoroughly. Then, three pores, designated as a, b, and c (Fig. 1) were drilled with the purpose of anchoring the markers. The pores were a little larger than the aforementioned glass tubes in diameter, and were 1–2 mm in depth. Three markers, corresponding to these pores designated as a, b, and c, were then carried by a standard electrode carrier and vertically fixed into the pores. Then the markers were anchored on the skull by the dental acrylic. Markers a and b were placed on the calvarial sagittal suture (Fig. 1), 10 mm in front of and 20 mm behind the bregma, respectively. When the MRI was performed, the scanning angle was adjusted so that the image scanned through

![Fig. 1. Top view of the locations of three pores on the skull, designated as a, b, and c. The gray circles show the pores drilled on the skull for anchoring the markers. The dashed circle shows the location of the SC, estimated from the anatomical atlas. The middle line shows the calvarial sagittal suture. The X shows the bregma.](image)
both markers. Consequently, the orientation of the MR image corresponded to the monkey’s head under the stereotaxic frame. Marker c was placed near the estimated target area (Fig. 1), the superior colliculus (SC). The target area was estimated according to the anatomical atlas. The sagittal and coronal images scanning through both the SC and marker C were used to calculate the implantation coordinates. The dorsoventral coordinates of these three pores were recorded, and the differences between each (a–b, b–c, and a–c) were calculated. In the post-MRI surgery, the monkey’s head was adjusted so that these differences remained unchanged and misplacement error was eliminated (described later).

2.4. The MRI and electrode coordinates

After anchoring the markers on the calvaria, each monkey underwent MR-imaging of the brain (GE, Signa Excite Twinspeed 1.5 T) with the following parameters: slice thickness = 1 mm; spacing between slices = 1.3 mm; repetition time (TR) = 4000 ms; echo time (TE) = 99.96 ms; inversion time = 0; number of averages = 4; and acquisition matrix = 0, 320, 256, 0.

Markers a and b were used to determine the median sagittal section of the brain during the MRI of the monkey’s head.
The scanning orientation was adjusted so that the image scanned right through the two slender markers. Consequently, the stereotaxic orientation of the head in the MR image was the same as when the head was fixed on the stereotaxic frame during the surgery. This approach functioned as the “standard stereotaxic procedure” used in the anatomical atlas-guided technique.

The function of marker C was to adjust the individual variation of the brain from the anatomical atlas. Estimating from the atlas, marker C was anchored on the top of the target area. After scanning, basing on the coronal and sagittal planes of the MR images crossing marker C and the SC, standard MR-image software was used to calculate the coordinates required for the electrode to reach the SC (Fig. 2). First, the area of the SC was determined from the MR
image and the center of this area was identified as the target point. Second, a straight white line, which mimicked the electrode hitting the target, was drawn parallel to the marker. Third, the distance between the drawn white line and the marker and then the distance from the skull surface to the target point were obtained by using the software. Finally, the coordinates for implanting the electrode were determined by combining these distances with the location of pore C on the skull.

2.5. Post-MRI surgery electrode implantation

During surgery, each monkey was anesthetized with hydrochloric acidulated ketamine (10 mg/kg, i.m.) and maintained with sodium pentobarbital (20 mg/kg, i.m.). All of the tube markers were removed. Together with the calculated target coordinates, the drilled pore C was taken as the reference to determine the inserting point on the skull.

To ensure accuracy, the stereotaxic orientation of the head in the post-MRI surgery must correspond to that in the pre-MRI surgery. This was achieved by adjusting the angle of the head, so that the differences in the dorsoventral coordinates of pores a–b, b–c, and a–c were the same as they were in the pre-MRI surgery. The difference between a–b is shown in Fig. 3 as an example. After the head was fixed in the stereotaxic frame, the dorsoventral coordinates of pores a, b, and c were measured and their differences were calculated. The eye bar and ear bar were adjusted if necessary to change the angle of the head in the anteroposterior and mediolateral planes until these differences were identical to the previous ones.

Instead of implanting electrodes, glass tubes filled with vitamin AD were inserted into the SC of M1, M2, and M3 to test the accuracy of this method. These monkeys then underwent MR-imaging again to determine if these tubes had been correctly implanted into the target. In M4, tetrodes guided by a silicon tube were implanted into the SC, and the neuronal discharge signals were recorded by the National Instruments™ recording system (PXI-1033).

2.6. Post-mortem histology

To check whether the tetrodes implanted in M4 hit the SC precisely, M4 was euthanized with an overdose pentobarbital and perfused transcardially with 1 L saline and, then, 0.5 L phosphate buffered 4% paraformaldehyde. After the perfusion, the brain was removed and post-fixed in 4% paraformaldehyde for 1 week, and dehydrated in a 25% sucrose solution for 2 weeks. The brain was then cut into 25 μm slices in the coronal plane of the left hemisphere and the sagittal plane of the right hemisphere.

3. Results

3.1. Accuracy checked by MRI

The MR images showed the vitamin-filled tubes were implanted accurately into the target area of M1, M2, and M3 (Fig. 4). The estimated errors were less than 1 mm (average). The tube implanted at an oblique angle in M3 (Fig. 4E) indicated that an electrode can be implanted in any angle using this method.

3.2. Microelectrodes recording

In M4, three tetrodes guided by silicon tubes were implanted into the SC on both sides, one in the left and two in the right. The neuronal discharge signals of the SC were recorded for several days by the tetrodes (Fig. 5).

3.3. Accuracy checked by post-mortem histological analysis

By post mortem of the tetrodes' traces, the brain slices showed that all three electrodes were implanted in the SC (Fig. 6). Although the electrodes did not hit the center of target point in the right hemisphere (Fig. 6C), the error measured from three brain slices was less than 1 mm (average).
4. Discussion

With an error of less than 1 mm, this method enabled micro-electrodes to be implanted into the SC of the monkeys to record neuronal discharge signals.

To establish a “standard stereotaxic procedure” for the MRI-guided technique in both the MRI-atlas and surgery, some researchers use a MRI-compatible frame and fix the monkey’s head on the frame for the entire experiment (Bjarkam et al., 2004; Subramanian et al., 2005). This procedure is inconvenient, however, as animals are placed in unnatural positions during the MR-imaging and the post-MRI surgery has to be performed immediately after the MRI scan. Additionally, according to our experience, these plastic-made MRI-compatible frames can create considerable distortions of the MR image. The novel approach used in the current study has no such confinement and is more flexible. Moreover, for the same MRI scanner, the smaller coil generally offers higher resolution than the larger coil. Without the frame, using the smaller
kneel coil to perform the MRI of the monkey’s head made it possible to obtain a higher quality MR image under the same conditions. Additionally, without the cost of a MRI-compatible frame, our method is less expensive.

Alvarez-Royo et al. (1991) fixed small glass beads onto the monkey’s skull as external markers to determine lesion location of the hippocampus using MRI. With an internal diameter of 2–3 mm, the glass beads were filled with a paramagnetic solution to provide a bright signal on MR images. This method has also been used in several other studies (Nahm et al., 1994; Zola et al., 2000; Manning et al., 2001). In our experience, however, it is difficult to perform MR-imaging directly through the marker’s center, which can create errors when the researcher takes the white dot’s center on the MR image as the real marker’s center. Although this method is accurate enough when the target is of considerable size or shallow (such as hippocampus), this tiny error is problematic when the target is small or located deeply within the brain. The markers used in the present study overcome this disadvantage as their internal diameter is less than the scanning slice thickness (1 mm). Furthermore, due to the slender shape of these markers, the implanting angle can be calculated easily when an obliquely oriented electrode implantation is necessary.

The results from this study have significant implications for medical research. Although the deep brain structure used in this study (SC) is visible by MRI, many subcortical structures are invisible, including the subthalamic nucleus (STN). In clinical surgery, a well-developed method has been used to identify the STN in our study, it is possible to determine the exact location of the MRI visible structure, and indirectly determine the location of the invisible structure.

The new method, using slender glass tubes as external markers, as reported in this paper, is accurate, convenient, economical, and universally available in most laboratories. In addition, the enhanced image quality by using the smaller kneel coil enables researchers to more accurately target the small subcortical structures of monkeys’ brains and better study their electrophysiological characteristics and neural networks.

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