

# Error Analysis and Some Suggestions on Animal Stereotactic Experiment from Inaccuracy of Rhesus Macaques Atlas

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**Key words:** Animal Experiment; Atlas; Rhesus Monkey; Stereotactic Technique

## INTRODUCTION

The application of stereotactic technique is rapidly growing. For instance, deep brain stimulation (DBS), an important neurostimulation technique in the treatment of Parkinson's disease, dystonia, essential tremor, and other diseases, uses the stereotactic method to implant electrodes.<sup>[1,2]</sup> Extensive animal studies have been conducted to test its efficacy and reveal the mechanisms. The efficacy of this stereotactic technique relies largely on accurate lead placement which renders the electrodes in direct conjunction with the target nuclei.<sup>[3]</sup> In animal studies, *The Rhesus Monkey Brain in Stereotaxic Coordinates* is a widely known stereotactic atlas of rhesus monkeys and commonly used to guide the positioning of DBS leads.<sup>[4]</sup> In this study, we offered some advice regarding the inaccurate placement of DBS leads with this atlas and discussed the possible causes for the inaccurate coordinates. Finally, some suggestions on animal stereotactic experiments were concluded from our experience with the technique.

## ERRORS OF COORDINATES IN OUR EXPERIMENT

We reported a retrospective analysis on the data obtained through our previous study (in accordance with the recommendations from the guidelines for the use and care of experimental animals approved by the Beijing Association on Laboratory Animal Care) which enrolled 12 male rhesus macaques (*Macaca mulatta* provided by the Laboratory Animal Center of the Military Medical Science Academy of China, age  $5.5 \pm 1.2$  years, weight  $6.3 \pm 1.1$  kg). Macaques were randomly assigned to two groups (Group 1,  $n = 6$ ;

Group 2,  $n = 6$ ). Ketamine hydrochloride (10 mg/kg, Hengrui Pharmaceutical, Nanchang, Jiangxi, China) was administered to achieve general anesthesia. Stereotactic apparatuses from two different manufacturers were used (Group 1, David Kopf Instruments, Tujunga, CA, USA. Group 2, Reward Life Science, Shenzhen, Guangdong, China). DBS devices were implanted, targeting anterior thalamic nuclei (ATN, coordinates: 8.5 mm posterior to the bregma, 2.0 mm lateral to the midline, and 20.0 mm from the dura, based on *The Rhesus Monkey Brain in Stereotaxic Coordinates*, which also used *M. mulatta* as the experimental animal).<sup>[4]</sup> About 7.0T magnetic resonance imaging (MRI) was performed to clarify the lead positions. Deviations between the actual lead positions and the ideal ATN positions were calculated. Independent sample *t*-test was performed to determine whether the differences observed in the two groups were significant. A value of  $P < 0.05$  was deemed to be statistically significant.

According to the results, the mediolateral deviations of the actual lead positions to ATN were  $1.1 \pm 0.7$  mm in Group 1 and  $1.0 \pm 0.8$  mm in Group 2. The anteroposterior deviations were  $11.5 \pm 1.6$  mm in Group 1 and  $9.9 \pm 1.2$  mm in Group 2 [Table 1]. The differences between Group 1 and Group 2 were not significant (all  $P$  values  $> 0.05$ ).

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**Table 1: Deviations of the actual DBS lead position and the ideal ATN position**

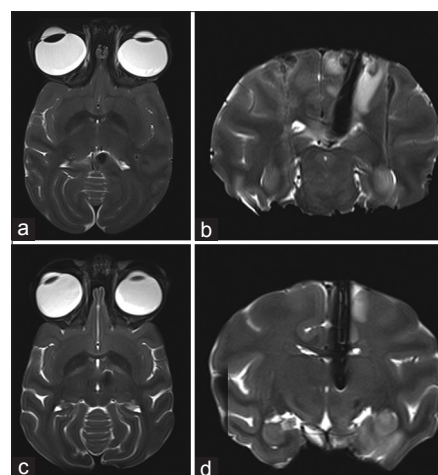
Items	$\Delta x$	$\Delta y$
M1	0.2	10.3
M2	1.2	9.2
M3	0.6	12.2
M4	1.4	8.8
M5	0.7	9.8
M6	0.8	9.3
M7	0.7	10.8
M8	1.5	9.5
M9	2.2	12.8
M10	0.4	11.3
M11	2.4	13.8
M12	0.3	10.7
Mean	1.0	10.7

ATN: Anterior thalamic nuclei; DBS: Deep brain stimulation.

However, the anteroposterior deviations were significantly greater than the mediolateral deviations (all  $P$  values  $< 0.01$ ). The huge anteroposterior deviations resulted in the inaccurate replacement of DBS leads in all cases. The lead positions were generally acceptable by moving coordinates anteroposterior forward for 10.0 mm [Figure 1].

## DISCUSSION

Our results are surprising because stereotactic surgeries require extreme accuracy. During operations, slight fluctuation of the actual lead positions could be caused by unstable mounting of stereotaxic apparatus, individual differences in scale-reading, variations of head shape, intracranial structures, and other conditions. However, the overall 10 mm deviation is quite high, which is usually caused by mistakes in the procedures. We thoroughly examined each step of the procedures and the apparatuses involved, yet failed to find the negative results. The use of two newly installed stereotactic apparatuses (both within 6 months) reduced the possibility of device corrosion. Moreover, the procedures were performed by three experienced neurosurgeons, which made the procedure-related anthropic factors unlikely. Thus, it is hard to explain where the deviations come from based on the current data. We assume that the deviations may reflect the inaccuracy of the atlas, which cannot be excluded. Besides, our study recruited local rhesus macaques that were originally from the Northeast part of China, which was geographically different from the rhesus macaques involved in the atlas. Considering the variations in the natural habitat of these different rhesus macaques, anatomical variations (i.e., the position of bregma, the brain size and the relative bearings of cerebral nuclei and bregma, etc.) could occur among the rhesus macaques. There is another possibility that the atlas contained error, and we wonder if our modified data can also be used in other rhesus monkey from other environment, the anteroposterior axis should be move forward 10.0 mm. Accordingly, further studies could be conducted regarding this subject.



**Figure 1:** A group of 7.0T magnetic resonance imaging images showing the lead positions before and after moving the entire coordinates 10.0 mm forward. (a) Axial image before adjustment. (b) Coronal image before adjustment. (c) Axial image after adjustment. (d) Coronal image after adjustment.

Pre-operative MRI and microelectrode recording during surgery are necessary for patients who undergo DBS to determine the positions of electrodes.<sup>[5]</sup> However, it could be difficult to follow this protocol for each experimental animal. Hence, the atlas plays an essential role to accomplish the non-MRI-guided or nonmicroelectrode recording operation for laboratory animals. In this study, post-operative 7.0T MRI was employed to verify the accurate location of the leads. Nevertheless, due to the limited accessibility of 7.0T MRI in many research institutes, the atlas seems to play a more essential role in these places. Hence, if the atlas is not accurate, the data may not be effective in those experiments.

A lot of articles did not stress the pathology examination after experiments and few of them provided the proof of the position accuracy, which could be a potential cause for great deviation, so the data may not be authentic.<sup>[6,7]</sup> Considering the current situation, we conclude some factors that may influence the accuracy of the coordinate.

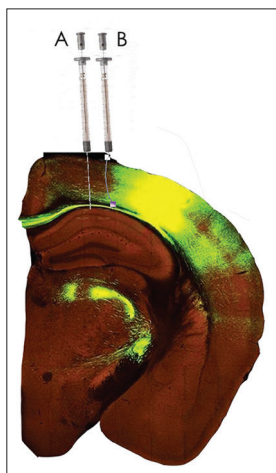
The term brain shift describes the movement and deformation of the brain in terms of its anatomical and physiological position in skull, which could result in a considerable impact during stereotactic surgery. Many factors could be involved in this process, such as cerebrospinal fluid (CSF) loss, pneumocephalus, and intracranial hemorrhage. Coenen *et al.* found a reduction of the CSF loss in patients with DBS surgery can minimize the brain shift and enhance the electrodes accuracy.<sup>[8]</sup> CSF loss mainly occurs in the early phase of surgery. In this study, a simple burr hole was used to minimize the CSF loss, which can be applied to animal experiments, especially mammal experiments. Moreover, CSF loss in the later phase of surgery can be effectively avoided through the closure of burr holes.<sup>[9]</sup> Since the pneumocephalus is usually related to the CSF loss, the reduction of the CSF loss could decrease the volume of the intracranial air.<sup>[8]</sup> The cerebrovascular may get damaged during stereotactic operation. Intracranial hemorrhage may

cause hydrocephalus even intracranial hypertension in the acute stage and result in tissue adhesion and atrophy. All of these consequences can result in brain shift.<sup>[10,11]</sup> The atlas is based on the rigid position instead of brain-shift-position, which lead to the inaccurate coordinates.

Besides the brain shift, an inappropriate use of atlas also plays a crucial role in coordinate inaccuracy. Most atlases have strict restrictions, for instance, one atlas is often merely fit for some specific species with a certain limited weight. Furthermore, we had experiences using rodent as an experimental animal in the previous study. The animals were decapitated and brain was processed for pathology examinations after DBS implantation. We concluded that if the weight of rodent is not in the scope of atlas' recommendations, the coordinates are frequently inaccurate. Unlike rodent, the primate brain could have a huge variation, the error due to the use of atlas should not be ignored during data analysis.

The inaccuracy causing by the apparatus is also analyzed. In rodent experiments, the electrode is often made by experimenter using affixing dental acrylic and the syringe tube, which might disintegrate when bearing external force. The rats are implanted with guider cannula before electrode implantation. The cannulas are fixed by dental acrylic to two or three stainless steel screws which are fastened on the skull. The experimenters often drill a hole in skull, whose diameter is often larger than the cannula. Therefore, the position could change when dental acrylic solidifies. The informal use of microinjector could also cause inaccuracy. The experimenters may not carefully examine pinhead before injection and a minor bend of the pinhead could alter the puncture route [Figure 2].

Concerning the problem, we offer some suggestions for the procedures of stereotactic experiments. Before conducting the experiment, a careful examination of the apparatus including microinjector, electrode, and stereotactic apparatus



**Figure 2:** A sketch map showing how the bend of the pinhead influence the target (A) the straight pinhead reach the target exactly (B) the bend pinhead diverge when puncture encounters the resistance from the brain.

could be helpful. Then, the optimal puncture angle should be chosen to avoid hemorrhage. Furthermore, the big hole can be closed with bone wax to avoid the position change when dental acrylic solidifies. We suggest pre-operative MRI and microelectrode recording are better in experiments involved primates. Subramanian *et al.* conducted an experiment to measure the accuracy of different stereotactic method with rhesus monkeys, and the results showed that MRI-guided stereotaxy was completely accurate in 80% as compared to 38.5% in atlas-guided stereotaxis. In addition, if we change the target after post-operative MRI and deliver a second operation, unlike the microelectrode, the DBS electrode might cause a great damage around the target and lead to deviation in the following operation.<sup>[12]</sup> Only the accuracy of coordinate confirmed by pathology examination or post-operative MRI could the subsequent experiments be conducted.

In conclusion, the advice was as follows: (1) researchers who would like to target ATN in Chinese rhesus macaques should adjust the anteroposterior axis forward 10.0 mm. *The Rhesus Monkey Brain in Stereotaxic Coordinates* might not be suitable for Chinese rhesus macaques. (2) Future studies should verify the coordinates before conducting any specific experiments and normalize the procedure in one experiment. (3) The target confirmation through MRI-scan or pathology examination after the premier experiment could be an essential instruction on adjusting the coordinate.

Although the growth of applications for stereotactic techniques is optimistic, little attention has been paid in terms of the coordinate accuracy, which would probably impede its future developments. Various error sources are analyzed in this article. Compared with clinic, the normalization of animal stereotactic experiments need to be improved, which would guarantee the results from animal experiments.

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### Conflicts of interest

There are no conflicts of interest.

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