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Characteristics of diffusion-tensor imaging for healthy adult rhesus monkey brains

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Research Highlights

(1) This study investigated anisotropy values in major white matter tracts and gray matter nuclei in the brains of healthy adult rhesus monkeys. Results revealed differences in anisotropy values between major white matter and deep gray matter nuclei. Similar to the brains of healthy young people, the anisotropy value of the corpus callosum was highest, followed by the posterior limb of the internal capsule and the semioval center, and lowest in gray matter nuclei (caudate nucleus).
(2) Results from this study are relatively extensive. Not only do they contribute to our knowledge of monkey brain microstructure, but they also provide information regarding the differences and similarities between monkey and human brains.

Abstract

Diffusion-tensor imaging can be used to observe the microstructure of brain tissue. Fractional sotropy reflects the integrity of white matter fibers. Fractional anisotropy of a young adult brain is low in gray matter, high in white matter, and highest in the splenium of the corpus callosum. Thus, we selected the anterior and posterior limbs of the internal capsule, head of the caudate nucleus, semioval center, thalamus, and corpus callosum (splenium and genu) as regions of interest when using diffusion-tensor imaging to observe fractional anisotropy of major white matter fiber tracts and the deep gray matter of healthy rhesus monkeys aged 4–8 years. Results showed no laterality ferences in fractional anisotropy values. Fractional anisotropy values were low in the head of date nucleus and thalamus in gray matter. Fractional anisotropy values were highest in the splenium of corpus callosum in the white matter, followed by genu of the corpus callosum and the posterior limb of the internal capsule. Fractional anisotropy values were lowest in the semioval center and posterior limb of internal capsule. These results suggest that fractional anisotropy values in major white matter fibers and the deep gray matter of 4–8-year-old rhesus monkeys are similar to those of healthy young people.

Key Words

neural regeneration; neuroimaging; rhesus monkey; fractional anisotropy; brain; white matter; gray matter; MRI; diffusion-tensor imaging; grants-supported paper; neuroregeneration

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INTRODUCTION

Dogs and pigs have been selected as models for studying cerebral arteries, but because primate brains are nearest anatomically to the human brain, it is the most ideal experimental model to study the brain[1-4]. However, how to perform non-invasive experiments *in vivo*, and how to evaluate metabolism and microstructure are difficult problems. Rhesus monkeys have a close relationship with humans and have a taxonomic lineage of Mammalia-Primates-Anthropoidea-Cercopithecidae-Macaca. The gross structure of their brains is similar to that of the human brain, including cerebral sulci and gyri[5]. However, the similarity of brain microstructure between rhesus monkeys and humans requires further investigation.

Diffusion-tensor imaging (DTI) can show differences in brain microstructure[6-7]. DTI makes examining the connections between brain structures and nerve function possible. Recent experiments have confirmed the consistency at the cellular level between microstructures obtained *via* DTI and those from histology. One study showed that overlapping sections of cells reached 94–100%, while overlapping sections of fiber bundles reached 84–100%[8]. Therefore, DTI can be used as a near-perfect means to display brain microstructure[8]. Combined with other functional imaging technology such as MRI, diffusion-tensor imaging helps to relate the microstructure of parts of the brain and the functional connectivity between them[9]. DTI has already been applied to the fields of cerebral surgery, brain function, brain aging, traumatic brain injury, and cerebral ischemia[10-14]. DTI can show microstructure damage such as Wallerian degeneration earlier than conventional MRI does[15], and also reveals abnormalities that cannot be found by conventional MRI, such as those found in patients with obsessive-compulsive disorder. Compared with normal subjects, DTI revealed that patients with obsessive-compulsive disorder had lower fractional anisotropy values in the cingulate fasciculus, inferior fronto-occipital fasciculus, and superior longitudinal fasciculus, but higher ones in the left uncinate fasciculus[16]. Therefore, DTI studies of the monkey brain can enhance our understanding of monkey-brain microstructure[17], which contributes to the in-depth assessment and the prediction of long-term results in experimental monkeys. In addition, DTI can be performed *in vivo*, is non-invasive, uses no radiation, and can be repeated many times, all of which make DTI helpful for monitoring experimental data and tracking changes over time. However, research that uses DTI in rhesus monkeys has only recently emerged. While increasing DTI data from rhesus monkeys can help form a standardized framework that will facilitate comparisons between different studies[18], but anisotropy values in major white matter tracts and gray matter nuclei in the brains of healthy adult rhesus monkeys does not yet exist.

DTIs are obtained by applying more than six nonlinear gradient magnetic fields to detect anisotropy, using a process based on diffusion-weighted imaging. The main parameters include the average diffusion coefficient, fractional anisotropy, relative anisotropy, and volume rate. Diagrams of fractional anisotropy, relative anisotropy, and volume rate can be established separately. Diffusion-tensor tractography can be obtained by analyzing DTI data with specialized software. Fractional anisotropy (FA) is the main parameter that describes the anisotropy of cerebral white matter fibers, and its value (between 0 and 1) is related to the integrity of myelin, fiber density, which can reflect whether white matter fibers are complete or not[19]. Higher values indicate stronger nerve conduction[20]. FA is the ratio between the sum of squares of the diffusion-tensor eigenvalues and the sum of squares of their differences. FA can provide high signal-to-noise ratio (SNR) and high contrast of white and gray matter and is more sensitive than relative anisotropy, without being affected by gradient factors (combined as the b value)[21]. Thus, FA is the most widely used technique in research using DTI.

DTI obtains images *via* differences between the diffusion anisotropies of different regions.

Different brain tissues have different diffusion anisotropies. Even within cerebral white matter (neuronal axons), the different fibers have significant differences in anisotropy. Dispersion in the anterograde direction of nerve fibers is primarily affected by the axon, endoplasmic reticulum, mitochondria, and other subcellular neurofilament structures, with few restrictions. Vertical dispersion is restricted by the myelin sheath and the perineurium containing high concentrations of lipids. With the integrity of nerve cell axons and the presence of peripheral interstitial structures, diffusion perpendicular to direction of a nerve fiber direction is more difficult than that in the anterograde direction. FA is the best method to evaluate white matter tracts, and can reflect their integrity. Therefore, the current study primarily analyzed the FA of major cerebral white matter tracts (the anterior and posterior limbs of the internal capsule, semioval center, the genu and splenium of the corpus callosum). In addition, the major basal nuclei have a close relationship with several white matter tracts, and form a functional group with them. Thus, we also analyzed the FA of the head of the caudate nucleus and the thalamus.

Previous studies using DTI on normal human brains showed that FA values are associated with aging[22-27]. Research on 23–76-year-old normal and healthy men showed that while FA values of the semioval center, the genu of corpus callosum, and the peripheral occipital and parietal lobes around the corpus callosum significantly decreased with aging, those of the splenium did not[22]. Similarly, Pal *et al* [23] studied 142 normal subjects aged 10–52 years old; the research focused on the amount of white matter in the occipital cortex, internal capsule and its limbs, lenticular nucleus, caudate, and the corpus callosum (genu and splenium), and they found that FA values decreased with aging. Zhang *et al* [24] showed that FA values in the anterior and posterior limbs of the internal capsule and corpus callosum (genu and body) differ significantly between different age groups: young group > middle-aged group > elderly group. Wu *et al* [25-26] demonstrated that the normal aging process of the human brain was accompanied by a decrease in FA values in cerebral white matter. Although the volume of white matter was relatively constant in older brains, the FA values clearly declined in the brains of middle-aged people[27]. Therefore, research employing FA values should consider the influence of age. Meanwhile, gender also influences the magnitude of FA. A previous study has shown that FA values in the caudate nucleus and white matter regions of men was higher than that in women[23].

DTI study of monkey brains has only arisen in recent years, and is mainly divided into aspects such as technology, brain function, brain growth, and ischemic evaluation. In terms of technology, key themes are the effects of different *b* values and different spatial resolutions on imaging accuracy[28-29], the effects of different pulse-repetition times on DTI quantitative indicators[30], and the application of multi-channel coils[31]. In terms of function, Sridharan *et al* [32] showed that error rate in cognitive tasks was higher in rhesus monkeys with low gray-matter volume and low FA values of white matter tracts. Mars *et al* [33] compared parietal region divisions between monkey and human brains. Shamy *et al* [34] showed that hippocampal damage could result in changes to multiple brain-memory related domain interactions. Another study by Shi *et al* [35] investigated growth and development of rhesus monkey brains, and showed that FA values in white matter regions clearly increased in monkeys 1–6 years old, which provides important insights into the nature of aging, including factors such as demyelination, axonal density changes, fiber channel reconstruction, and synaptic modification processes. In terms of aging, Bendlin *et al* [36] used DTI to investigate the effects of caloric restriction on brain aging. Duce *et al* [37] showed that the changes of gene expression triggered or accelerated aging of cerebral white matter in monkey brains. Makris *et al* [38] reported that the DTI parameters changed during the normal aging process. Guo *et al* [39] evaluated cerebral ischemia and reperfusion injuries in cerebral infarct centers and ischemic penumbrae. Although research concerning DTI in monkeys has increased in recent years, studies focusing on the anisotropic differences of major white and deep gray matter in normal rhesus monkeys are relatively rare. The similarity of FA in rhesus monkeys to that in healthy humans is not known.

Although the cerebral hemispheres are bilaterally symmetric in the normal human brain, laterality differences in anatomy do exist. Greenberg *et al* [40] confirmed that white matter volume was slightly larger on the left hemisphere compared with the right, and that in males, gray matter was slightly larger in the right hemisphere compared with the left, a finding that was opposite in females. DTI of normal human brains showed no laterality differences in fractional anisotropy values for major white matter tracts and deep gray matter[41], but the question remains whether or not this is true in the healthy monkey brain. The recent studies using DTI on normal human brains have demonstrated that FA values vary across structures, being highest in the corpus callosum, high in the posterior limb of the internal capsule and the

semioval center, and lowest in the gray matter nuclei (caudate nucleus)[24, 42-43]. Whether or not these trends are also true of the monkey brain remains unknown. Additionally, whether or not the FA values in the genu and splenium of the corpus callosum are statistically different in normal human brains remains a controversial issue[44-46].

The present study was designed to investigate the following questions: (1) Are the FA features in different regions of the normal monkey brain consistent with those found in the human brain? (2) Are there any laterality differences in FA values in the normal monkey brain? (3) Are there any differences in the genu and splenium of the corpus callosum in the normal monkey brain? (4) Are the FA values of normal young monkey brains similar to those of young human brains?

RESULTS

Quantitative analysis of experimental animals

Fifteen healthy young adult rhesus monkeys aged 4–8 years were enrolled for DTI examination. All 15 were used for final analysis.

MRI and FA images in the normal monkey brain

MRI revealed that monkey brains were normal, with clear structures and clear boundaries between gray and white matter. The gray to white matter signal was the same as that in a normal adult human brain. In our monkeys, the gray matter signal was higher than the white matter signal in T2-weighted imaging (T2WI), but the reverse was true in T1-weighted imaging (T1WI). The caudate nucleus and thalamus can be clearly identified on T1WI and T2WI. Brain volume was obviously less in monkeys compared with humans. In particular, the smaller volume in the monkey brains could be seen in frontal areas on the sagittal view. The proportional volume of the posterior part of the monkey brain was larger than that of the human brain at 24 years (Figure 1). After reconstruction, DTI scans correctly showed that the monkey corticospinal tract originates in the precentral gyrus, extends down through the internal capsule, and continues down through the midbrain and medulla oblongata. Each fiber tract was continuous and uniform in the shape.

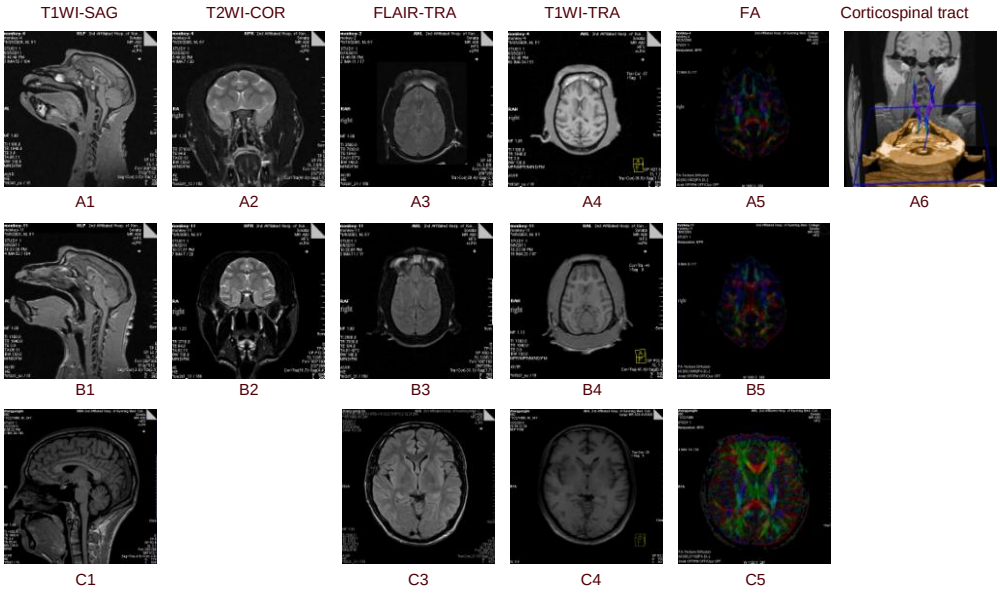

Figure 1 Distinction between a normal monkey brain and a human brain in T1WI, T2WI, FLAIR, and FA images.

(A) Monkey 1, male, 5 years old; (B) monkey 2, male, 8 years old; (C) Normal human, male, 24 years old. Conventional T1WI, T2WI, and FLAIR detections show no difference between the gray matter and white matter signals of healthy adult monkey and human brains. T1WI shows that the white matter signal was higher than that of gray matter one. However, the gray matter signal was higher than the white matter one under T2WI. The volume of the monkey brains was obviously less than that of the human. This was clear on the sagittal view, especially in frontal regions. The volume of the posterior monkey brain was proportionally larger than that in the human brain. The FA image shows that each fiber bundle was bilaterally symmetrical. The different colors represented different distributions. Blue, dorsal-ventral fibers. Green, anterior-posterior fibers. Red, medial-lateral fibers. Corticospinal tract from a monkey brain can be seen to originate in the precentral gyrus, continue down through the internal capsule, and then down to the midbrain and medulla oblongata. Each fiber tract is continuous and uniform in the shape. Block area, region selected for image reconstruction.

T1WI: T1-weighted imaging; T2WI: T2-weighted imaging; FLAIR: fluid attenuated inversion recovery; FA: fractional anisotropy; SAG: sagittal; COR: coronal; TRA: transverse.

Comparison of FA values across hemispheres for white matter tracts and deep gray matter

No laterality differences were found in the average FA values for major white matter tracts and deep gray matter nuclei ($P > 0.05$).

Table 1 Average fractional anisotropy values of major white matter tracts and deep gray matter (bilaterally) of rhesus monkey brains

Region	Left side	Right side	*t*	*P*
Gray matter				
Head of caudate nucleus	0.280±0.022	0.248±0.018	1.115	0.381
Thalamus	0.268±0.011	0.265±0.010	0.173	0.620
White matter				
Anterior limb of internal capsule	0.540±0.025	0.539±0.027	0.018	0.979
Posterior limb of internal capsule	0.632±0.014	0.627±0.015	0.226	0.676
Semiovale area	0.586±0.020	0.590±0.024	–0.014	0.381
Genu of corpus callosum	0.636±0.023	0.644±0.024	–0.242	0.897
Splenium of corpus callosum	0.701±0.022	0.700±0.028	0.037	0.231

DTI software (Neuro 3D MR) was utilized to obtain the fractional anisotropy graph. Each region of interest diagram has a corresponding fractional anisotropy value. A total of 15 monkeys were included. The unilateral fractional anisotropy value of each region and the average unilateral fractional-anisotropy value were calculated, and expressed as mean ± SD. Paired *t*-test was used to compare fractional anisotropy values for the same regions across the two hemispheres. No significant difference in bilateral fractional anisotropy values was detected in any region ($P > 0.05$).

Comparison of the FA value curve diagram between major white matter tracts and deep gray matter nuclei and the FA values of brain regions

As shown in Figure 2, FA values were different between the anterior and posterior limbs of the internal capsule, head of the caudate nucleus, semioval center, thalamus, and the corpus callosum (body and splenium). FA values were low in the gray matter (head of caudate nucleus and thalamus), and high in the white matter. Within the white matter, values were lowest in the anterior limb of the internal capsule, and highest in the splenium of the corpus callosum. One-way analysis of variance (Student-Newman-Keuls) was applied to compare FA values among different regions (posterior limbs of the internal capsule, head of the caudate nucleus, semioval center, thalamus, the genu and splenium the corpus callosum).

Results showed no significant difference between the head of the caudate and thalamus. In white matter, except for no significant difference between the genu of the corpus callosum and the posterior limb of the internal capsule ($P > 0.05$), there were significant differences among other white matter structures ($P < 0.05$). Namely, FA values were highest in the splenium of the corpus callosum, high in the genu and the posterior limb of the internal capsule, and low in the semioval center and the anterior limb of internal capsule.

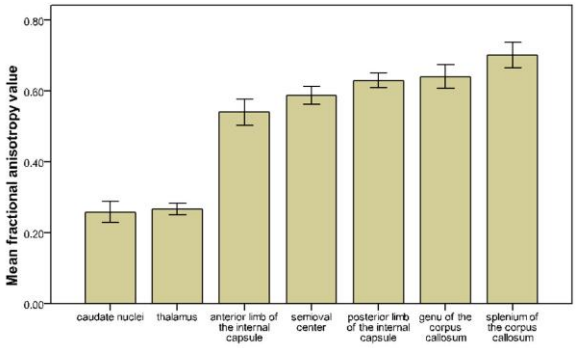

Figure 2 Mean fractional anisotropy value (FA) in the major white matter tracts and gray matter nuclei of the healthy adult rhesus monkey brain.

In white matter, except for no significant difference between the genu of the corpus callosum and the posterior limb of the internal capsule ($P > 0.05$), there were significant differences among other white matter structures ($P < 0.05$). Data are expressed as mean ± SD for each group ($n = 15$; one-way analysis of variance and Student-Newman-Keuls test). The fractional anisotropy values were highest in the splenium of the corpus callosum and lowest in the head of the caudate nucleus.

DISCUSSION

The main characteristics of the methods

We used FA values that could be used to compare results with data from previous DTI studies. FA is the most common parameter for describing cerebral white matter anisotropy[19]. Its magnitude is associated with the integrity of myelin sheaths, fiber density, and parallelism[19]. FA values reflect whether white matter is damaged or not[19]. In addition, FA images exhibit a high signal-to-noise ratio, making the contrast in white and gray matter regions higher than what is seen in relative anisotropy. Additionally, FA is not affected by the b value[21]. The regions of interest included the major white matter tracts (anterior and posterior limb of the internal capsule, the semioval center, and the genu and body of the corpus callosum) and gray matter nuclei (caudate nucleus and thalamus). These sites are easily identifiable in the FA map, which ensured the accuracy and repeatability of the set points for the regions of interest *in vivo*, and reflected

the differences in anisotropy of the major white matter tracts and gray matter regions. The regions of interest consisted of two pixels (2.81 × 2.81 mm^2). Each region was calculated three times, and an average value that avoided the volume effect, and ensured the accuracy and reproducibility of the data, was obtained. The rhesus monkeys were male, so variation due to gender was eliminated. Monkeys were 4–8 years old (the prime of life), reducing the effect of different ages. Monkeys were anesthetized with an intravenous injection of ketamine 10 mg/kg and diazepam 1 mg/kg, thus eliminating motion artifacts.

Conventional MRI of normal adult rhesus monkey brains

Rhesus monkeys are macaque primates that are often used to study human brain physiology and function because of their similarity to humans. Here, conventional MRI confirmed that rhesus-monkey brain structure is similar to that of humans, including the telencephalon, diencephalon, brain stem, and cerebellum. Each brain lobe was similar to that of a human brain, but brain tissue volume was significantly less than what is found in the human brain. The sagittal view showed that monkey-brain volume, especially the forepart, is very small, whereas that of the posterior regions was relatively big. The cerebellum and occipital lobe was relatively well developed. These facts are consistent with a previously published study[5], which demonstrated that brain mass of normal elderly rhesus monkey was about 1 390.9 g (18–19 years old), which is equivalent to a 6- or 7-month-old human brain.

In addition to showing that the general structure of rhesus monkey brains is similar to that of human brains, conventional MRI revealed that the gray and white matter signal performance was also consistent similar across species. The signal of gray matter was higher than that of white matter in the monkey brain under T2WI, but the reverse was true under T1WI. Major white matter (internal capsule, corpus callosum, and semioval center) and gray matter (caudate nucleus, thalamus) structures were similar to those in human brains. .

FA values in cerebral white and gray matter of normal adult rhesus monkeys

FA images of normal adult rhesus monkey brains revealed that fiber bundles were symmetrical across hemispheres. FA values did not show laterality differences in the major white matter (internal capsule, corpus callosum, semioval center) or gray matter structures (caudate nucleus and thalamus). However, significant differences in FA values were found between each different region (anterior and posterior limbs of internal capsule, head of the caudate nucleus, semioval center, and the body and splenium of the corpus callosum). FA was low in gray matter, but high in white matter. No significant difference in FA values was detectable between the head of the caudate and the thalamus. FA values were highest in the splenium of corpus callosum, followed by the genu of the corpus callosum and the posterior limb of the internal capsule, and lowest in the semioval center and anterior limb of internal capsule.

Comparison of FA between each region of the normal adult rhesus monkey brain and the human brain

The purpose of medical research is to explore the pathogenesis of human disease. Therefore, experimental animals should have structures, functions, and metabolism that are as similar as possible to those in humans (from http://www.86wiki.com/view/40149.htm).

Monkeys are the most advanced animals in the evolution, so they are best experimental model. Although conventional MRI shows that the anatomy of the monkey brain is similar to that of a human brain, it cannot tell us much about similarities in microstructure. Therefore, we compare our data with previous studies that addressed FA in normal human brains.

Whether there is difference regarding the microstructure in FA remains poorly understood. Shi *et al* [35] investigated the changes in DTI parameters of the normal rhesus monkey brain in a 1–6-year period of growth and showed that patterns of FA, mean diffusivity, axial diffusivity (AD), and radial diffusivity (RD) exhibited significant changes over time. Most of these patterns are similar to those from human studies, yet a few followed unique patterns. Overall, they observed substantial increase in FA and AD and a decrease in RD for white matter along with similar yet smaller changes in gray matter. Determining whether FA values in white and gray matter of monkey brains are similar to those of human brains, or if they have their own unique FA patterns, requires further investigation.

Results from the present study showed that FA values in major white matter and deep gray nuclei of rhesus monkey brains were symmetrical across hemispheres. No significant difference in FA values was found between the same regions across hemispheres. A previous study about brain volume reported laterality differences in volume between two hemispheres[40]. In males, left hemis-

phere white matter volume was slightly larger than that in the right, and right hemisphere gray matter volume was slightly larger than that in the left. The reverse was true in females. The anisotropy of white matter in the normal human brains has also been shown not to differ across hemispheres[41], thus we can say that in this respect, the microstructures are similar across species.

FA values differed depending on the structure. Values for the corpus callosum was largest, followed by the posterior limb of the internal capsule, semioval center, and caudate nucleus, findings that are consistent with previously published studies[24, 42-43].

Whether FA values differ between the genu and splenium of the corpus callosum of normal human brain remains controversial. Studies have reported that FA values of the splenium were slightly higher than those of the genu, but there was no statistical difference[43-44]. However, other studies reported statistically significant differences between the two regions[24, 45], findings identical to what we report here. Shimony *et al* [46] believed that different FA values in different parts of the white matter tracts were not only associated with nerve fiber distribution, but also with diameter and density of neural fibers, number of glial cells in the white matter. Different regions of the corpus callosum connect different lobes of the brain, and the different fiber distributions and densities were evident: bent-forward fibers were visible in the genu of the corpus callosum and transverse fibers were observed in the body of the corpus callosum. Here, the FA values were highest in the splenium of the corpus callosum, similar to what was seen in human brains[47-49], indicating another similarity in microstructure across species.

FA values can quantify anisotropy, but in current studies of normal human brains, fractional anisotropy values were shown to be different in each region. Taking the corpus callosum as an example, Sun *et al* [47] reported that the average FA value of a normal corpus callosum was 0.429. Chepuri *et al* [45] reported that mean FA values were 0.400 and 0.539 in normal genu and splenium of the corpus callosum, respectively. Zhong *et al* [43] reported that FA values of the genu and splenium of the corpus callosum were 0.75 and 0.62, respectively, and suggested that the main reason for the differences was the different ages of the subjects. The average age of subjects in a study by Zhong *et al* was 36.9 years, but was 50 years in a study by Sun *et al*. The results from a study by Zhang *et al* concerning normal youth (aged 20–39 years) were identical to that of Zhong's study, and revealed that the FA values of the genu and splenium of the corpus callosum were 0.706 and 0.741, respectively. Results from our study demonstrated that the mean FA values of the genu and splenium of the corpus callosum were 0.640 and 0.70, respectively. The average age of monkeys in this study was 6.2 years (4–8 years), and the FA values found in their brains were similar to those of young human brains.

Pal *et al* [23] studied 142 subjects aged 10–52 years, and showed that FA values reached a peak at about age 30, and decreased with further aging. In contrast, aging was associated with rising FA values in the deep gray nuclei. Westlye *et al* [50] studied 430 8–85-year-old healthy people and found that FA values tended to be stable at the age of 40, decreased slowly in late adulthood, and declined rapidly in old age. Recent studies[51-52] have confirmed that FA values declined with aging. High FA values in pre-adults was associated with increased myelin and dendritic growth of axons[53]. Schulte *et al* [54] considered that the decrease in FA values related to age was associated with changes in microstructural integrity. Although the ratio of major myelin protein did not change with age, the total amount of pure myelin decreased with age. In addition, the total volume and the total length of the myelinated fibers decreased with aging. The increase in FA values in the gray matter is probably associated with iron deposition.

Wisco *et al* [55] confirmed that the average volume of each region of the rhesus monkey brain decreased with age in a young group (5–12 years), middle-aged group (16–19 years old), and old group (aged 24–30). The mean volumes found an overall decrease from young to old age in forebrain white matter (11.53%), forebrain gray matter (2.08%), caudate nucleus (11.79%) and globus pallidus (18.26%).

This study concerning FA in normal white matter tracts and gray matter nuclei of young rhesus monkeys can provides new data that help characterize the microstructure of monkey brains and further understanding about their similarities to human brains. Adluru *et al* [18] believes that more DTI data from rhesus monkeys that contributes to forming a standard framework will be beneficial for comparing results between different studies.

In addition to FA value analysis, white matter fiber tracts can be visualized on diffusion tractography by special software. In this study, the corticospinal tract was reconstructed and was clearly shown.

Limitations

This study has several limitations. First, we only considered the major white matter structures (internal capsule, corpus callosum, semioval center) and large gray nuclei (caudate nucleus, thalamus), and did not address other brain structures. The main reason lies in that while monkey brain anatomy is similar to humans, the capacity is far less. Because tiny structures cannot be identified on FA maps, guaranteeing the accuracy and repeatability of results from regions of interest is difficult. In addition, partial volume effects and non-Gaussian diffusion are major drawbacks of DTI[56]. Tiny structures are more prone to artifacts resulting from partial volume effects. A 1.5 T or 3.0 T MRI can improve the spatial resolution and signal-to-noise ratio of a FA maps. Therefore, the next step is to study the smaller structures in a 3.0 T MRI machine.

Second, DTI has many parameters, with average diffusion coefficient, fractional anisotropy, relative anisotropy, and volume ratio being the most common. This study only analyzed the FA values. Multiple parameters were simultaneously analyzed in the same animal, which could provide much information. For example, the combined application of average diffusion coefficient and FA can be used to divide cerebral ischemia and necrosis into different stages. An average diffusion coefficient decrease and an FA increase indicates that water molecules have entered intercellular space from the extracellular space and diffusion is restricted. Cytotoxic edema induces cell swelling and results in a narrowing of the gap between the myelinated nerve fiber bundles in white matter. Deformation of the extracellular space structure limits water-molecule flow across the nerve-fiber direction, indicating that the tissue structure is still intact, and that the damage at this stage is reversible. An average diffusion coefficient increase and FA decrease suggests the occurrence of irreversible damage, structural damage, nerve-fiber demyelination, and loss of normal tissue microstructure. The reason why this study only analyzed the FA is that FA maps provided high signal-to-noise ratio, had gray-white matter contrasts that were more sensitive than that found in relative anisotropy, were not affected by the *b* value, and were easily compared with results from other studies. Further experiments will seek to utilize multi-parameter analysis.

Third, this study only used male rhesus monkeys, and therefore could not explore differences between sexes. Abe *et al* [41] confirmed that no gender differences exist in FA values in bilateral brain tissues. However, Pal *et al* [23] showed that FA values in the caudate nucleus and white matter were higher in males than in females. Thus, differences in FA values between male and female monkeys will be investigated in future projects.

Fourth, different devices might obtain different FA values[57]. Ciccarelli *et al* [42] revealed that the coefficient of variation for FA values ranged from 1.7% to 7.1%, with values smallest in the corpus callosum and followed by the optic radiation. This indicates that the coefficient of variation of FA values was also associated with the fiber structures of each region. This is the reason for choosing the major white matter tracts and large gray matter nuclei.

In summary, a DTI study of monkey brains is conducive to deep experimental evaluation. Anisotropic patterns of the major white matter tracts and gray matter nuclei of monkey brains was similar to those of healthy young people, presenting with bilateral symmetry, no laterality differences, low gray matter values, and high white matter values. Among white matter, the values were highest in the splenium of the corpus callosum, followed by the genu of the corpus callosum and the posterior limb of the internal capsule. Values were lowest in the semioval center and the posterior limb of internal capsule. This is helpful for understanding microstructure changes and long-term results of prediction. DTI is conducted in vivo, non-invasively, without radiation, can be repeated many times, and is beneficial to monitoring, tracking, and following-up on experimental data .

MATERIALS AND METHODS

Design

A randomized, controlled animal experiment.

Time and setting

Experiments were conducted in the Animal Experimental Center of Kunming Medical University and the Magnetic Resonance Room of the Second Affiliated Hospital of Kunming Medical University in China from June 2011 to March 2012.

Materials

Experimental animals

A total of 15 healthy adult male rhesus monkeys (macaca mulatta) aged 4–8 years old (mean, 6.2 years), weighing 8.2–11.5 kg (mean, 9.15 kg), were provided by Kunming Animal Research Institute, Chinese Academy of Sciences (license No. SYXK (Yun) 2005-0004). They had sensitive reactions and normal nerve function. The monkeys were anesthetized with an intravenous injection

of ketamine 10 mg/kg and diazepam 1 mg/kg, thus eliminating motion artifacts. The protocols were conducted in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, formulated by the Ministry of Science and Technology of China[58].

Methods

MRI and DTI examinations of rhesus monkey brains

MRI was conducted with a Siemens Sonata 1.5 TMR (Siemens, Munich, Germany) superconducting magnetic resonance (gradient magnetic field of 40T, switching rate of 200 mT/ms). MRI examination was performed in the supine position, followed by DTI. Briefly, coronal T2WI and transverse FLAIR were done using turbo spin-fast spin echo. T2WI imaging parameters: repetition time, 3 710 ms; echo time, 94 ms; slice thickness, 3 mm; number, 20; field-of-view, 180 × 180; interval, 0.3 mm; matrix, 256 × 256; bandwidth, 130 Hz/Px. FLAIR imaging parameters: repetition time, 7 830 ms; echo time, 104 ms; inversion time, 2 500 ms; layer number, 20; field of view, 180 × 180; thickness, 3 mm; interval, 0.3 mm; matrix, 256 × 256; bandwidth, 130 Hz/Px. T1WI magnetization was prepared rapid by 3DT1-mpr-ns scan to reconstruct in axial and coronal T1WI, and was used for DTI 3D positioning. Imaging parameters: repetition time, 1 940 ms; echo time, 3.93 ms; inversion time, 1 100 ms; slice thickness, 1 mm; slice per lab, 104; field of view, 200 × 200; matrix, 256 × 256; bandwidth, 130 Hz/Px.

DTI used axial scanning with a single-shot, spin-echo, echo-planar sequence. Imaging parameters: repetition time, 3 200 ms; echo time, 100 ms; field of view, 180 mm × 180 mm; matrix, 128 × 128; layer thickness, 3 mm; layer spacing, 0.3 mm; incentive, 3 times; bandwidth, 1 562 Hz/Px; diffusion sensitive gradient direction (diffusion directions) for 12, diffusion sensitive coefficient *b* value of 0 and 1 000 s/mm^2.

MR data post-processing

T1-mpr-ns and DTI scanning data were input into DTI software (Neuro.3D.MR, Siemens, Bonn, Germany) for FA measurement. Selection of measurement point: bilateral anterior and posterior limbs of the internal capsule, head of caudate nucleus, semioval center, thalamus, and body and splenium of the corpus callosum.

To avoid the volume effect, FA-value calculation of each region of interest employed two pixels (2.81 × 2.81 mm^2). The calculation was done three times for each region of interest, and an average value was obtained for use in analysis.

Statistical analysis

Data were analyzed using SPSS 11.0 software (SPSS, Chicago, IL, USA). Measurements are expressed as mean ± SD. Laterality differences of FA values in bilaterally same regions were compared using paired *t*-test. If no differences were found, FA values from the same region in both hemispheres were merged into a single mean. One-way analysis of variance was employed to compare FA values in each group (seven sites: the anterior and posterior limbs of the internal capsule, head of the caudate nucleus, semioval center, thalamus, and body and splenium of the corpus callosum). The pairwise comparison of intergroup difference was done using Student-Newman-Keuls test (α = 0.05).

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