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Chunxia Li, Emory University
James Rilling, Emory University
Xiaoping Hu, Emory University
Todd Preuss, Emory University
Xiaodong Zhang, Emory University
Longchuan Li, Emory University
James Herndon Jr., Emory University
Y Yan, Emory University
G Nair, NINDS
JG Herndon, Emory University

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in-vivo diffusion MRI protocol optimization for the chimpanzee brain and examination of aging effects on the primate optic nerve at 3T

Xiaodong Zhang1,2, Chun-Xia Li1, Yumei Yan1, Govind Nair3, James K. Rilling4, James G Herndon2, Todd M. Preuss2, Xiaoping Hu5, Longchuan Li6

1. Yerkes Imaging Center, Yerkes National Primate Research Center, Emory University Atlanta, GA
2. Division of Neuropharmacology and Neurologic Diseases, Yerkes National Primate Research Center, Emory University, Atlanta, GA
3. NINDS, NIH, Bethesda, MD 20892
4. Department of Anthropology, Emory University, Atlanta, GA
5. Dept of Bioengineering, University of California, Riverside, CA
6. Marcus Autism Center, Children’s Healthcare of Atlanta, Emory University, Atlanta, GA

Abstract

Background—Diffusion MRI (dMRI) data acquisition protocols are well-established on modern high-field clinical scanners for human studies. However, these protocols are not suitable for the chimpanzee (or other large-brained mammals) because of its substantial difference in head geometry and brain volume compared with humans. Therefore, an optimal dMRI data acquisition protocol dedicated to chimpanzee neuroimaging is needed.

Methods—A multi-shot (4 segments) double spin-echo echo-planar imaging (MS-EPI) sequence and a single-shot double spin-echo EPI (SS-EPI) sequence were optimized separately for dMRI data acquisition of chimpanzees using a clinical 3T scanner. Correction for severe susceptibility-induced image distortion and signal drop-off was performed and evaluated using FSL software. DTI indices in different brain regions and probabilistic tractography were compared. A separate DTI data set from n=34 chimpanzees (13 to 56 years old) was collected using the optimal protocol. Age-related changes in diffusivity indices of optic nerve fibers was evaluated.

Results—The SS-EPI sequence acquired dMRI data of the chimpanzee brain with approximately doubled the SNR as the MS-EPI sequence given the same scan time. The quality of white matter...
fiber tracking from the SS-EPI data was much higher than that from MS-EPI data. However, quantitative analysis of DTI indices showed no difference in most ROIs between the SS-EPI and MS-EPI sequences. The progressive evolution of diffusivity indices of optic nerves indicated mild changes in fiber bundles of chimpanzees aged 40 years and above.

**Conclusion**—The single-shot EPI-based acquisition protocol provided better image quality of dMRI for chimpanzee brains and is recommended for *in vivo* dMRI study or clinical diagnosis of chimpanzees (or other large animals). Also, the tendency of FA decrease or diffusivity increase in the optic nerve of aged chimpanzees was seen but did not show significant age-related changes, suggesting aging may have little impact on optic nerve fiber integrity of chimpanzees, in contrast to results for both macaque monkeys and humans.

**Keywords**

DTI; fiber tracking; distortion correction; optic nerve; non-human primate; large animals; aging

**Introduction**

Non-human primates (NHPs) resemble many aspects of human anatomy, physiology, behavior, reproduction, and genetics, and are widely used in biomedical and neuroscience research [1]. There has been increasing interest in using advanced dMRI techniques to examine functional and microstructural abnormalities in various NHP models [2-8], and the relationship of brain aging in humans and other primates [3, 9, 10], as NHPs experience similar aging processes to human [11]. In particular, chimpanzees are our closest relatives and well known for their tool-use skills and complex social and communicative behaviors, making them an invaluable species for comparative studies of behavior and neuroanatomy with humans [12-17]. Moreover, comparative studies of chimpanzees and humans, using archival chimpanzee tissue and MRI scans, are providing unique insights into the biology of human cognitive and neurological disorders [18-21].

Diffusion magnetic resonance imaging (dMRI) is a powerful non-invasive technique that is widely used to characterize the property of water diffusion and microstructural integrity of white matter pathways in the brain [22, 23]. Also, it allows for the three-dimensional trajectories of fiber pathways in the white matter to be reconstructed by using fiber tractography algorithms [7, 24] to examine the brain connectivity [25-30]. Diffusion MRI data acquisition and processing protocols have been well-established on modern high-field clinical scanners for human studies. However, these protocols are optimized for scanning the human brain but not the chimpanzee brain because there exist several practical technical challenge and considerations in acquiring dMRI data on chimpanzees *in vivo*.

A sagittal T$_2$-weighted image and coronal T$_1$-weighted image of a whole adult chimpanzee head are shown in Figure 1 (left column). The chimpanzee head is similar in overall size to that of humans, but it has a large extended jaw while its brain is only about one third of human brain volume. When a regular human brain dMRI protocol is applied, each voxel in MR images of chimpanzees covers more brain tissue than in humans, resulting in poorer anatomical resolution and partial voluming. In addition, their prominent sinus cavities
relative to their brain size make susceptibility-induced geometry distortion and signal loss major challenges as well.

Echo-planar imaging (EPI) based sequences are mostly used for dMRI data collection \textit{in vivo}, but the technique is highly susceptible to geometric distortion and signal loss due to its sensitivity to magnetic field inhomogeneity and motion [31]. Moreover, chimpanzees’ protruding jaw anatomy and short neck preclude the use of multi-channel phased-array coils typically designed for human head anatomy, which eliminates the option of parallel imaging to reduce distortion in a conventional 3T setting. Second, as chimpanzees have much smaller brains than do humans, it is necessary to acquire high-resolution dMRI images in order to achieve comparable anatomical resolution. For example, to obtain partial voluming effects similar to that of 2 mm isotropic voxel human data, a smaller isotropic voxel (such as 1.3mm) is needed for chimpanzees. Increased spatial resolution demands a larger acquisition data matrix and can lengthen the echo time (TE) substantially. As a result, the diffusion MR image quality can be degraded because of more severe susceptibility-related distortion, signal drop-off, and motion artifacts.

Single-shot EPI (SS-EPI) sequences are normally used in clinical scanners for dMRI acquisition but their use with chimpanzees is limited by severe distortion and signal drop-off because of their long TE. In contrast, segmented multi-shot EPI (MS-EPI) sequences allow for high-resolution imaging with reduced TE to alleviate the susceptibility-induced distortion and signal drop-off. However, MS-EPI is highly vulnerable to physiology-associated motion artifacts. Gating can be used to reduce the motion artifacts significantly, but it dramatically increases scan duration [32], which may raise concerns about possible adverse effects of anesthesia on the physiology of chimpanzees and recovery from prolonged administration of anesthetics [33]. MRI clinical scanners are generally installed with standard shimming systems and an RF head volume coil appropriate for humans, but without advanced shimming systems or dedicated RF coils optimized for the chimpanzee brain which is three- time smaller than the human brain and housed in a huge skull. In addition, the chimpanzee has a very short neck with a wide shoulder, an extended jaws, and no forehead, making it impossible to place the whole brain in the center of a standard RF volume coil used for the human head. Structural T1 or T2-weighted images are not susceptible to the unique cranial anatomy and large cavities in the chimpanzee skull and can be acquired with decent quality when scanned with a standard clinical setting. However, the EPI image of the brain can be severely distorted with poor signal-to-noise ratio (SNR) and homogeneity. Therefore, it is necessary to develop an optimal \textit{in-vivo} dMRI scanning protocol for chimpanzee brain studies in order for them to be scanned with conventional clinical scanners.

Aging results in the decline of certain brain functional and behavioral capacities in human and animals [34-37] and is associated with anatomical and microstructural alteration in the brain [10, 38-40]. NHPs are excellent animal models of human neurodegenerative disorders and aging [41, 42] as they experience similar aging processes to human [11]. Interestingly, some neurodegenerative diseases, such as Alzheimer disease, that occur in humans are not known to occur in our closest biological relatives, the NHPs [20, 43]. Previous MRI studies demonstrated that there are few aging-related volumetric changes in chimpanzee brains

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compared to human [40, 44]. Also, age-related white matter degeneration has been seen in previous studies of human [45-47] and other mammalian brains, including rhesus monkeys [48, 49] and mice [50, 51]. Interestingly, as reported in a previous DTI study of the brain in human, chimpanzee, and macaque monkey, age-related fractional anisotropy (FA) reduction or diffusivity increase was seen in major white matter bundles of the central nervous system in human, chimpanzee, and monkey brains [10].

DTI-derived indices are sensitive to changes due to both white matter microstructural architectures and fiber configurations. Unfortunately, many major brain white matter bundles have complex fiber configurations (i.e., crossing, kissing, fanning and bending), making the interpretation of findings based on DTI-derived indices challenging [52, 53]. The optic nerve, which transmits visual signals from the retina to the brain, exits the retina as a single bundle. Therefore, the system is well suited to understand white matter microstructural changes such as axon loss and myelin degeneration using DTI indices, without the need to considering complex patterns of changes due to different sensitivity of crossing fiber pathways contained within one MR voxel[54, 55]. The total number of optic nerve fibers varies substantially with species and there are over one million (1,186,172) myelinated fibers in the optic nerve of an adult human [56]. Previous studies used light and electron microscopic method to examine the specimens of humans [57, 58], monkeys [59-61], and rats [62], have demonstrated axonal loss and/or demyelination in aging optic nerve. Diffusion tensor MRI has been demonstrated to be a robust approach to examine the optic nerve integrity in human and other animals [54, 63-66].

In the present study, we aimed to optimize dMRI data acquisition for collecting high-quality diffusion MRI in chimpanzees in-vivo using a standard 3T clinical scanner. Also, the optimal dMRI data acquisition and processing approach was used to reveal and characterize the evolution of optic nerve integrity of chimpanzees during aging.

**Methods**

**Subjects and animal care**

Scan were carried out in 34 adult female chimpanzees, ranging in age from 13-56 years. The chimpanzees were scanned between 2008—2011 for a NIH-funded project on brain aging research that yielded data on cerebral white matter changes [10]. Subjects were initially immobilized with ketamine injections (2–6 mg/kg, i.m.) and were subsequently anesthetized with propofol (10 mg/kg/hr) or 1-1.5% isoflurane anesthesia following standard veterinary procedures. The subjects remained sedated for the duration of the scans as well as the time needed for transport between their home cage and the scanner location. During MRI scans, animals were immobilized with cushions and strips around the head. They were placed in the supine position and spontaneously breathing. End-tidal-CO$_2$, inhaled CO$_2$, O$_2$ saturation, blood pressure, heart rate, respiration rate, EKG, and body temperature of animals were monitored continuously. After completing the MRI scan, the chimpanzees were temporarily housed in a single cage for 6 to 12 hours to allow the effects of anesthesia to wear off before being returned to their home cage and cage mates. The veterinary staff and research staff observed the general well-being (i.e., activity, food intake) of the chimpanzees twice daily after the scan for possible distress associated with anesthetic accesses. All procedures were
approved by the Institutional Animal Care and Use Committee (IACUC) of Emory University (YER-2001206).

**Diffusion MRI protocol optimization**

Six adult female chimpanzees (*Pan troglodytes*) (ID: Azeala, Callie, Frannie, Jaimie, Julie, Liza) were scanned for protocol development. MRI scanning was performed on a Siemens 3T Trio scanner with the Siemens HE birdcage coil (Siemens Healthcare, Erlangen, Germany). A diffusion-weighted, 4-shot, double spin-echo EPI sequence (MS-EPI) was used as the first dMRI protocol with the parameters: TR = 5740 ms/TE = 91 ms, FOV = 230×230 mm², matrix size = 128×128, voxel resolution = 1.8×1.8×1.8 mm³, 60 diffusion directions, b-value=0, 1000s/mm². Two averages were acquired with opposite phase-encoding directions (left – right) for distortion correction [67]. Extra 10 images with b = 0 sec/mm² were also obtained with matching imaging parameters. The total scan time for the MS-EPI protocol was 51 minutes.

The same 6 chimpanzees used for MS-EPI protocol development were re-scanned after the 3T Trio scanner was upgraded to the Siemens Trio TIM system (two years later after the first scans) with a single-shot EPI sequence (SS-EPI) and a Siemens HE head coil (similar with the prior Siemens HE head coil in dimension and design) using the parameters: TR = 5900 ms/TE = 86 ms, FOV=230 mm × 230 mm with the percentage of FOV in the phase-encoding direction = 56.30%, matrix size: 72×128, 41 slices covering the whole brain, voxel resolution = 1.8×1.8×1.8 mm³, b-value=0, 1000s/mm², 60 gradient directions, eight averages with opposite phase encoding directions for the susceptibility distortion correction. Partial Fourier (factor = 6/8) was applied to minimize the TE. Extra 10 images with b = 0 sec/mm² were collected. The total scan time for the SS-EPI protocol was 53 minutes. Therefore, the basic parameters such as spatial resolution, b-values, and gradient directions, and scanning duration were same in the two protocols for comparison purpose. The full sample of thirty-four chimpanzees (13 to 56 years old) was scanned using the SS-EPI protocol.

**Image distortion correction and SNR evaluation**

The dMRI data were pre-processed for eddy-current and distortion correction using the FSL package (http://www.fmrib.ox.ac.uk/fsl/). Comparison was conducted for the six animals scanned using both MS-EPI and SS-EPI protocols. The SNRs of b0 image (single average) were calculated and compared between the two scan protocols, conducted by manually drawing ROIs at the central semiovale bilaterally and at the background of the images. The SNR was computed in terms of contrast ratio: SNR = μ / σ, where μ represents the mean of the signal in the central semiovale and σ represents the standard deviation of the noise at the background. As the SNR of b0 image is spatially varying (due to the sensitivity and RF homogeneity of the coil) and region dependent, the SNR calculations here were only approximated.

**ROI evaluation of DTI indices in the chimpanzee brain between two scan protocols**

After dMRI data were preprocessed, the FA maps acquired using two different protocols were aligned through a rigid-body transformation with six degrees of freedom (dof) using
FSL. Four ROIs at the splenium, the genu of the corpus callosum, the left and right posterior limb of internal capsule (PLIC) and the cerebral peduncle (CP) were drawn in each subject’s original FA images.

**Probabilistic Tractography**

Probabilistic tractography was performed to track the precentral corticospinal tracts (pCST), the callosal fibers passing through the genu of the corpus callosum (Genu), and cingulum bundles (CB), on the data acquired using the two protocols respectively. As shown in Figure 1C, the seed mask (yellow), waypoint mask (cyan) and cortical target mask (red) were used for tractography. The position and voxel numbers for each mask were identical for the two protocols.

The pCST was tracked separately in each hemisphere, with a cortical target mask at the white matter/grey matter boundary mask of the precentral gyrus, a waypoint mask at the posterior limb of internal capsule and a seed mask placed at the basilar pons (Figure 1C1, i–iii). The callosal fibers passing through the genu of the corpus callosum were tracked with a seed mask placed at the mid-sagittal slice of the genu of the corpus callosum and a waypoint mask place in the forebrain (Fig 1C2, i, ii). The cingulum bundles were tracked using two seed masks in each hemisphere placed at the anterior and posterior ends of the cingulum bundles above the corpus callosum and a waypoint mask between them (Fig 1C3, i–iii). The background images were the color map of the diffusion tensors overlapped on the corresponding T1-weighted images.

**ROI analysis of the chimpanzee optic nerve**

In order to perform ROI analysis on optic nerves of chimpanzees, the dMRI parameter maps were interpolated with home-built Matlab codes, and then resliced with the software Analyze 9.0 (Mayo Clinic). Region of interests (ROIs) were selected manually using the software MRlco on the coronal slices located within the 1/2 section of the optic nerve starting from the disc. Only the central voxel in the cross-section of the optic nerve was selected on each slice, as shown in Figure 2. Mean values of MD, AD($\lambda_\parallel$), RD($\lambda_\perp$), and FA in the ROIs were calculated for each optic nerve. In order to evaluate aging effects, these data were further divided into 3 age groups: 13-20 (n=17), 22-38 (n=10), 40-56 (n=7) years old. One-way ANOVA and Pearson correlation were used for statistical analyses.

**Results**

**Susceptibility-induced distortion correction**

An example of b0 images acquired using the MS-EPI and SS-EPI protocols before and after the corrections for the susceptibility-induced distortions are shown in Figure 3. The original image data acquired by the MS-EPI protocol had less distortion compared to that with the SS-EPI protocol (Figure 3A vs. Figure 3B). Segmentation-related ghost artifacts could be seen in the diffusion weighted images by MS-EPI, which were absent in those acquired by the SS-EPI protocol. In addition, the susceptibility-induced distortions were severe for the SS-EPI protocol, but they were reasonably corrected in brain images by using the reversal
phase-encoding method [67]. No significant mismatch could be seen between the corrected b0 images from both SS-EPI and MS-EPI protocols.

Calculations of SNR

The mean and standard deviation of the averaged SNR across the six subjects for a single b0 image was 11.4±1.04 in the MS-EPI protocol and 11.3±1.24 in the SS-EPI protocol. As the scan time was the same for the two protocols in the present study, the estimated SNR ratio for the total diffusion MRI data between the SS-EPI and MS-EPI is 2:1, assuming all repetitions are averaged for analysis.

ROI analyses of FA in the chimpanzee brain

The mean and standard deviations (SD) of FA from the six chimpanzees scanned by the two protocols are presented in Figure 4. Paired t-tests were conducted, with each subject’s FA (acquired by the two protocols in each ROI) as pairs. No significant differences in FA were detected between the two protocols, except for lower FA detected at the right PLIC by the MS-EPI protocol ($F(1,4)=-2.912, p<0.033$), compared to that by the SS-EPI protocol. The inter-protocol % coefficient of variation (CV) ranged from 4.73 to 6.55, with the minimal variability at the splenium and the maximal variability at the right PLIC. For each ROI, the mean and (inter-protocol) standard deviation for mean FA were used to calculate the sample size required to detect inter-protocol effect size of 1 ~ 5% (with two-tailed significance level of 0.05 and a power of 0.8). Overall, the ROI analysis of quantitative examination showed no significant difference between the two protocols in most brain regions.

Probabilistic Tractography

The tractography results for each of the protocol-development subjects are shown in Figure 5. Generally, more tracts survived in the data acquired by the SS-EPI protocol compared to the MS-EPI protocol, even though the number of voxels in the masks, the total number of samples sent in each voxel and the thresholds for display were strictly identical for each fiber pathway system. When the waytotal numbers (i.e., a count on the total streamlines survived the tracking criteria) for each fiber system were compared, the results confirmed the evident differences by the visual comparison in Figure 5: There were differences in the waytotal number between the two protocols at the genu ($F(1,4)=-2.85, p<0.036$), the left pCST ($F(1,4)=-3.593, p<0.016$), the right CB ($F(1,4)=-4.676, p<0.005$), and a trend toward significance at the right pCST ($F(1,4)=-2.458, p<0.057$) and left CB ($F(1,4)=-2.343, p<0.066$). The data acquired by the SS-EPI protocol showed significantly higher waytotal numbers than those by the MS-EPI protocol.

Optic nerve diffusivity changes in chimpanzees during aging

The mean diffusivity (MD), axial diffusivity ($\lambda_\parallel$), radial diffusivity ($\lambda_\perp$) and fractional anisotropy (FA) of the optic nerve of 34 chimpanzees ranging in age from 13 to 56 years old are illustrated in Figure 6. No significant age-related change was detected with any parameter by one-way ANOVA or Pearson correlation. A further age-group analysis of DTI indices in the chimpanzees optic nerve using ANOVA did not reveal any significant change.
in DTI indices between any age groups except a near-significant MD increase (13%, p<0.07) was seen between the 22-38 age group and 40-56 age group (Figure 7).

**Discussion**

In the present study, we compared separately optimized multi-shot and single-shot EPI protocols for acquiring diffusion MRI data on chimpanzee brains *in vivo*, using a 3T clinical scanner with a standard birdcage volume coil. The multi-shot-EPI protocol shows the advantages of acquiring data with minimal susceptibility-induced distortion while the single-shot EPI protocol presents with advantages of absence of segmentation-related artifacts and allowing more averages to improve image SNR with the same scan time. Both protocols were able to obtain high-resolution dMRI images with comparable FA values in most white matter bundles of the brain. However, the single-shot EPI protocol showed higher sampling efficiency (therefore higher SNR) and significantly improved fiber tracking results in the projection, commissural and association pathways, and therefore is recommended for acquisition of chimpanzees’ diffusion MRI data *in vivo* using a clinical high field MRI setting. As other large mammal animals (like pigs) have similar cranial anatomy as the chimpanzee, the present dMRI data acquisition/processing strategy can be used in the dMRI studies of the large animals on a clinical setting. In addition, the evolution pattern during optic nerve aging was revealed by DTI and showed very mild changes of diffusivity in the optic nerve of elderly chimpanzees compared to young/middle-aged adults, indicating a lack of relationship between DTI indices of the optic nerve and the aging process in chimpanzees, different from what has been reported in macaque monkeys and humans.

**Protocol optimization of dMRI data collection and processing of chimpanzee brains**

Susceptibility-induced artifacts would result in severe distortion and signal drop-off in dMRI images, and the adverse effects could become worse when high (or ultra-high) magnetic field is applied. It would be ideal if the image artifact could be corrected during data acquisition to minimize the possible error/bias in post data processing and analysis. The B0 inhomogeneity can be improved to reduce the susceptibility artifact by using a gradient system with high-order shimming. However, this is not usually available on a conventional clinical scanner. A novel and automatic 3D shimming approach was shown to be effective in improving B0 inhomogeneity in macaque brains, but it may not be sufficient and available on every clinical scanner. The multi-shot EPI technique is an effective approach to decrease image distortion and an optimal approach in high-resolution 3D diffusion tensor MRI of macaque brains, but at the expense of increased scan time and susceptible to segmentation-related motion artifacts due to physiological motion. Even though the chimpanzees were sedated during scanning and tight padding was applied to immobilize head motion, we still found physiology-related motion artifacts in dMRI images and significantly fewer tracts in all selected white matter bundles of the brain when the MS-EPI sequence was applied, compared with those with the SS-EPI sequence. This issue could at least partly be due to the segmentation-related artifacts and lower image SNR, causing less “trackability” in the diffusion MRI data.
In comparison, the SS-EPI protocol induced much less motion artifact at the expense of more severe susceptibility induced image distortion than the multi-shot EPI (Figure 3). The phase-reversal distortion correction method employed here has proven very effective in human and macaque monkey DTI studies, although we noticed that the method could fail if signal stacking and/or severe signal drop-off occurred [31, 67]. As the chimpanzee has an irregular, large head but a much smaller brain compared to humans, using full FOV to cover the entire chimpanzee head would substantially increase phase-encoding steps and TE, resulting in severe susceptibility artifacts. Therefore, minimizing TE by applying reduced FOV in the phase-encoding direction and the partial Fourier technique together was very helpful for alleviating the susceptibility-induced distortion in the SS-EPI sequence. Note that the final partial Fourier factor was set at 6/8 instead of 5/8 (the lowest allowed in our diffusion MRI sequence) in our SS-EPI protocol. This was because the current reconstruction software in the scanner usually does not employ an advanced partial Fourier reconstruction algorithm for partial Fourier imaging, such as iterative reconstruction [72, 73], so further reduction of the partial Fourier factor would result in excessive blurring in the image.

Several post-processing algorithms have been proposed to correct the susceptibility-induced distortion in the data acquired using EPI-based sequences [31, 67, 74]. The field-map algorithm proposed by Jezzard et al. is an effective approach for correcting image distortion of regular functional or diffusion-weighted EPI images, but requires an additional scan to acquire the field map. Also, this correction is only approximate due to the limited resolution of the field map and could fail to correct images with severe distortion.

The phase-reversal approach was proposed by Bowtell et al [74] and further improved by Andersson et al [67]. This algorithm requires at least two identical acquisitions with opposing phase-encoding polarities. A nonlinear registration with motion parameters between the two acquisitions (6 degrees of freedom) is used to estimate a three-dimensional warping field, approximating the field inhomogeneities for susceptibility distortion correction. As demonstrated in our in vivo diffusion MRI data on chimpanzee brains, the phase-reversal approach worked very well in the image distortion correction of the two protocols. In particular, the SS-EPI images with severe susceptibility distortion could be corrected properly (Figure 3, bottom row).

Therefore, the use of reduced FOV, partial Fourier technique, and phase-reversal data collection could minimize the susceptibility induced artifacts seen in dMRI images of chimpanzee brains acquired using the SS-EPI protocol. Our results demonstrate the reported data acquisition and preprocessing strategy work properly for dMRI scans of adult chimpanzees on a clinical 3T scanner. In addition, multi-shell dMRI allows for using multi-compartment diffusion models to characterize the abnormality of brain tissues with more specific measures than traditional DTI and has been increasingly used in clinical and preclinical studies [75-78]. Increased b-values in multi-shell dMRI can result in more severe susceptibility artifacts due to increase of TE. The proposed data collection strategy could also be used for multi-shell dMRI studies of chimpanzees. Also, the gradient strength of modern 3T clinical scanners has been increased substantially in recent years (for example, 80mT/m with the gradient rise time of 200T/m/s on all three gradient axes simultaneously).
on Magnetom Prisma vs. 45mT/m on Magnetom Trio by Siemens). The upgrade on the gradient system of high-field MRI scanners will further facilitate the single or multi-shell dMRI data collection of human and large animal subjects (including chimpanzees) for biomedical and neuroscience research and disease diagnosis.

**Evaluation of aging effects on optic nerves of Chimpanzees**

Decrease in FA is usually seen in white matter of aged human brains [79, 80], and the most severe FA decreases are seen in anterior corpus callosum, deep frontal, medial orbital, posterior periventricular, and posterior internal capsule of the aging human brain [81]. The optic nerve is also part of the central nervous system of the brain. The fiber loss of optic nerve due to aging has been reported in previous studies of the specimens of human [57, 58, 82] and monkeys [59, 60]. Also, the substantial loss of total fiber numbers and fiber density in optic nerves of elder macaque monkeys was observed in a previous study of light and electron microscopy[60]. A previous DTI study demonstrated significant and substantial reduction of FA and increase of radial diffusivity (RD) in the optic nerves of aged macaque monkeys (21-27 years old) compared to younger adults (9-13 years old) [49]. Increased radial diffusivity (RD) in the optic nerve could be associated with the decline in vision after optic neuritis in human [83]. FA describes the degree of anisotropy of water molecule diffusion in tissue and is a widely used measure of fiber integrity, while the radial diffusivity is an indicator of myelin damage and axial diffusivity is associated with axonal integrity [84, 85]. The FA reduction and RD increase indicated the fiber loss in optic nerves fiber bundles of aged monkeys, in great agreement with the results of the light and electron microscopy study. In particular, the temporal FA and RD changes in optic nerves of aged monkeys are similar with the previous findings in other white matter bundles of the central nervous system in the same colony of monkeys [10]. In addition, previous longitudinal DTI study demonstrated that the optic nerve in macaque monkeys has similar developmental patterns with the major white matter bundles (like corpus callosum, internal capsule) in the human central nervous system during brain development [86-88]. The DTI findings in developmental and aging brains of monkey and human suggest the white matter maturation and alteration in the optic nerve evolve simultaneously with other major fibers in their central nervous system during development and/or aging.

The tendency of FA decrease and diffusivity increase was seen but no significant progressive change was observed in the optic nerve of chimpanzee during aging (Fig 6). In order to compare the DTI results with previous findings in macaque monkeys, we divided the chimpanzees into three age groups (young adult, middle-aged, and elder). No significant FA, MD, AD, and RD changes were seen between any age groups except a ~13% and near-significant MD increase (p<0.07) between the middle-aged and elder groups (Figure 7). The DTI results suggest there is no significant alteration of integrity in the optic nerve bundles in elderly chimpanzees, different from previous findings in monkey and human studies. A previous study in chimpanzees reported global nonlinear age-related changes in white matter integrity involving late life decreases, however these decreases occurred relatively later in the lifespan of chimpanzees compared with humans [89]. These data suggest that the optic nerve may follow a different age-related trajectory from other white matter pathways.
The life span of chimpanzees in captivity is 50-60 years old, much shorter than humans (~80 years). As MD may have better sensitivity to detect the early alteration in white matter bundles [90], the present findings of near-significant MD increase in the optic nerve of aged chimpanzees may suggest early fiber degeneration, and the significance might be improved with larger sample size for elderly chimpanzees. Such unique evolution pattern in chimpanzee brain aging may grant further investigations to unveil the neural substrate mechanism of the human brain aging by using advanced diffusion MRI techniques.

Also, the great apes including the bonobo, orangutan, and gorilla have similar head anatomy as chimpanzees and could also be examined with the proposed dMRI protocol. In addition, large animals (like pigs) have irregular head geometry and have been increasingly used for studies in stroke [91-93] and traumatic brain injury [94, 95]. As white matter injury and remodeling may play a major role in functional recovery after stroke brain injury [96, 97], the proposed dMRI protocol can be useful for examination of the brain injury in large animal models using a high (or ultra-high) field clinical scanner. In particular, abnormal brain lesions (e.g., stroke) have been seen in captive chimpanzees [98, 99]. The dMRI protocol can be used for routine clinical diagnosis of the large primates as well.

Conclusions

We have established an optimal dMRI data acquisition protocol and pre-processing strategy for in-vivo diffusion MRI study of the chimpanzee brain using a conventional clinical 3T scanner. Our results demonstrate that the proposed data collection and processing approach can be useful for traditional and advanced diffusion MRI studies or clinical diagnosis on chimpanzees or other large primates (like gorillas, orangutans, bonobos) on a modern high-field MRI scanner. In addition, this approach may be used in the other large animals like pigs (a popular model for stroke and TBI research). Also, no significant age-related changes of DTI indices in the optic nerve were observed in aged chimpanzees, suggesting aging may have little impact on optic nerve fiber integrity of chimpanzees, in contrast to results for both macaque monkeys and humans. Further comparative investigations of different primate species are warranted to reveal the common and unique neural substrates of the human brain aging.

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Highlights

- High quality diffusion MRI images of chimpanzee brains were obtained at a clinical 3T
- Fiber tracking showed higher dependence on image quality than diffusivity indices
- Aging has little effects on optic nerve fiber bundles of chimpanzee
Fig 1.
A) A sagittal T2-weighted image and B) a coronal T1-weighted image demonstrates the anatomical structure of a chimpanzee head and the location of the brain in the head. C1). The seed mask (yellow), waypoint mask (cyan) and cortical target mask (red) placements for tractography of chimpanzee brains. The pCST has a cortical target mask at the white matter/grey matter boundary mask of the precentral gyrus, a waypoint mask at the posterior limb of internal capsule; PLIC) and a seed mask placed at the basilar pons (C1, i-iii). C2). The callosal fibers passing through the genu were tracked with a seed mask placed at the mid-sagittal slice of the genu and a waypoint mask place in the forebrain (C2, i-ii). C3). The cingulum bundles were tracked using two seed masks in each hemisphere placed at the anterior and posterior ends of the cingulum bundles above the corpus callosum and a waypoint mask between them (C3, i-iii). The background images were the color map of the diffusion tensors overlapped on T1-weighted images.
Fig. 2.
Illustration of optic nerve ROI analysis of chimpanzees. Region of interests (ROIs) were defined manually on the axial b0 diffusion-weighted image (left) and a coronal b0 diffusion-weighted image (right) located within the 1/8 and 3/8 section (shaded area) of the optic nerve between optic disc (the beginning of optic nerve) and chiasm. The central pixel in each coronal image of optic nerve was selected (right).
Fig 3.
Illustration of diffusion-weighted b0 images of a chimpanzee brain (Julie) before and after the susceptibility-induced distortion correction. L-R/R-L: left-right or right-left phase-encoding direction. A) Original b0 images (top row). The red contour lines on the corrected were derived on the undistorted corresponding T1-weighted image and imposed on the corrected image. B) Original b0 images (bottom row), acquired with SS-EPI are corrected using phase-reversal method. The red contour lines on B6 were derived from the T1-weighted image and imposed on the corrected image.
Fig 4.
Comparisons of fractional anisotropy (FA) obtained by the MS-EPI and SS-EPI sequences at four ROIs (Splenium, Genu, PLIC (L, R), CP (L, R) in the chimpanzee brains (n=6). PLIC, posterior limb of internal capsule; CP, the cerebral peduncle. Error bar: standard deviation.
Fig 5. Demonstration of the probabilistic tractography results from two chimpanzees scanned with both MS-EPI and SS-EPI protocols respectively. Three white matter fiber systems: 1) precentral corticospinal tracts (pCST), 2) callosal fibers passing through the genu (Genu) and 3) the cingulum bundles (CB). Three white matter fiber systems: 1) precentral corticospinal tracts (pCST), 2) callosal fibers passing through the genu (Genu) and 3) the cingulum bundles (CB).
Fig 6.
Demonstration of progressive changes of DTI indices (FA, MD, AD, RD) in chimpanzee optic nerves.
Fig 7.
Progressive evolution of DTI indices in optic nerves of chimpanzees with the ages of 13-20 (n=13), 22-38 (n=10), and 40-56 (n=7) years old. *, p<0.07; #, p<0.1, compared the 22-38y group by using one-way ANOVA. Error bar: standard deviation.