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Effect of age and calorie restriction on corpus callosal integrity in rhesus macaques: A fiber tractography study

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HIGHLIGHTS

- Genu and splenium white matter integrity declines with aging in rhesus monkeys.
- There is an anterior-to-posterior gradient in white matter integrity in macaques.
- Calorie restriction has no effect on corpus callosal integrity in macaques.
- These age-related findings in monkeys are similar to those demonstrated in humans.

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ABSTRACT

The rhesus macaque exhibits age-related brain changes similar to humans, making an excellent model of normal aging. Calorie restriction is a dietary intervention that reduces age-related comorbidities in short-lived animals, and its effects are still under study in rhesus macaques. Here, using deterministic fiber tracking method, we examined the effects of age and calorie restriction on a diffusion tensor imaging measure of white matter integrity, fractional anisotropy (FA), within white matter tracks traversing the anterior (genu) and posterior (splenium) corpus callosum in rhesus monkeys. Our results show: (1) a significant inverse relationship between age and mean FA of tracks traversing the genu and splenium; (2) higher mean FA of the splenium tracks as compared to that of genu tracks across groups; and (3) no significant diet effect on mean track FA through either location. These results are congruent with the age-related decline in white matter integrity reported in humans and monkeys, and the anterior-to-posterior gradient in white matter vulnerability to normal aging in humans. Further studies are warranted to critically evaluate the effect of calorie restriction on brain aging in this unique cohort of elderly primates.

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1. Introduction

Calorie restriction (CR), in the absence of malnutrition, has been reliably demonstrated to protect against age-related diseases and to prolong healthy lifespan in several shorter-lived animal models [1,2]. Ongoing studies in rhesus macaques (*Macaca mulatta*) indicate that CR is effective in delaying the development of age-related diseases [2–5], although consensus has not been reached on its effect on lifespan. While the precise mechanisms underlying the effects of CR are not fully understood, it appears that CR, at least in part, may act through anti-oxidative and anti-inflammatory mechanisms [6]. Brain white matter (WM) is especially sensitive to inflammatory and oxidative damage due to its high metabolic activity [7,8]. Using diffusion tensor imaging (DTI), a type of magnetic resonance imaging (MRI) that is sensitive to WM fiber integrity by observing water diffusion [9], our group has previously demonstrated that there is an inverse relationship between age and DTI measures related to WM integrity in diverse association and projection fiber tracts [10]. This voxel-based morphometry (VBM) study was specifically suited for a whole brain approach, which compares DTI measures in each voxel between subjects. While this study was a first exploratory step in understanding the effects of CR on WM, additional work is clearly warranted.

In the present study, we examined the effect of age and CR on corpus callosum (CC) fiber integrity in rhesus monkeys using fiber tractography. Tractography is a post-processing method where tensors of cerebral WM water diffusion can be mathematically extrapolated from DTI. These tensors can track the three-dimensional fiber orientation in the brain macroscopically using various deterministic or probabilistic algorithms [11]. In tract-based analyses, an *a priori* region of interest (ROI) is used as seed region from which fibers are traced in the native space of each animal, thus precluding the need to register individual images to a common space and introducing error due to normalization, and fiber characteristics are compared between subjects. Unlike VBM, this method thus does not rely on effective between-subject registration and can be restricted to specific brain regions [12]. The rhesus monkey (*M. mulatta*) provides a valuable animal model for studying human aging because it exhibits several age-related brain and cognitive changes similar to humans [2,4,10,13,14]. In captivity, median life expectancy of macaques is approximately 26 years, 10% survive beyond 35 years, and maximum lifespan is approximately 40 years [15]; overall, macaques age at a rate of two and a half to three times that of humans [2]. In this study, we assessed age and diet effects on fractional anisotropy (FA), a measure of the proportion of diffusion within a voxel that is directional, which reflects tissue integrity and tends to decrease with aging [16,17]. We evaluated the average FA of fiber tracks traversing two distinct spherical ROIs: (1) the genu of the CC, and (2) the splenium of the CC. The CC is one of the largest and most widely studied compact WM regions using imaging methods in human aging and disease [16–20]. Choosing specific ROIs within the CC thus ensured consistent seed placement among subjects. Additionally, the extensive existing literature elucidating the effects of human aging and disease on CC provided a suitable comparison for our current findings in this nonhuman primate model of aging. The genu of the CC primarily contains interhemispheric prefrontal fibers, whereas the splenium contains fibers from the posterior parietal, occipital, and medial temporal cortices [21]. Our aim was to capture WM microstructural measures of fibers that project toward the cortex in native space at the single subject level. We evaluated these ROIs specifically because these regions have been previously reported to show age-related FA decreases in humans [17,22,23]. Additionally, this method also allowed us to assess any anterior to posterior differences in FA within the CC. For instance, FA is greater in posterior CC than anterior CC regions at all ages in humans [24,25]. We hypothesized that there would be a negative relationship between mean track FA and age, that posterior FA would be greater than anterior FA across all subjects, and that CR animals would exhibit higher track FA values than controls.

2. Materials and methods

2.1. Subjects

Thirty-three rhesus macaques (controls = 15, CR = 18), aged 19–29 years, used in this study are part of the longitudinal "Dietary Restriction and Aging Study" at the Wisconsin National Primate Research Center. Four of these animals (controls = 2, CR = 2) were below the age of 20 (middle-aged), whereas the remaining animals were old (20 years or older) at the time of image acquisition. Complete details of the dietary manipulation and experimental setup have been described extensively elsewhere [26,27]; the reader is referred to these prior publications for further information. The study protocol was approved by the Institutional Animal Care and Use Committee of the University of Wisconsin-Madison. Animals were anesthetized using ketamine and xylazine prior to imaging, and all efforts were made to minimize suffering.

2.2. Image acquisition

Image acquisition parameters have been reported previously [10]; the reader is referred to this prior publication for a complete description of image acquisition methods. Briefly, MRI scans were acquired on a General Electric 3.0 T Signa MR Unit (GE Medical Systems, Milwaukee, WI, USA) with a quadrature transmit/receive volume coil (18 cm diameter). Animals were anesthetized and scanned in the morning. DTI was performed in the axial plane using a single-shot, spin-echo, diffusion-weighted echo-planar imaging sequence with diffusion gradients in 12 optimal directions. Imaging parameters include: $b = 816\ s/mm^2$, TR = 10,000 ms, TE = 77.2 ms, NEX = 6, FOV = 160 mm, matrix = 120 × 120, section thickness = 2.5 mm, no gap. A higher-order shimming protocol was run before the DTI scan to minimize image distortion.

2.3. Image processing and tractography

DTI processing was done by personnel blind to the diet status of all the animals. Eddy current correction was completed using tools available in the FMRIB Software Library (FSL) Diffusion Toolbox [28]. We used the DTI software program Diffusion Toolkit (version 0.6.2.1) for calculating diffusion tensors and for mathematically reconstructing fiber tracks. Fiber tracking was then visualized using TrackVis (version 0.5.2.1, Ruopeng Wang, Van J. Wedeen, TrackVis.org, Martinos Center for Biomedical Imaging, Massachusetts General Hospital). Fiber tracks were generated from spherical seed regions of 5 mm diameter placed in the genu and splenium of the CC. The genu was defined as the most anterior coronal slice containing crossing CC fibers, and the splenium as the most posterior coronal slice of the CC. Streamline tractography was performed by following the principal eigenvector (FACT method) [11], with the termination angle threshold set at 35° and FA threshold at 0.15. Average FA of all fibers traversing through these respective seed regions was then obtained. To evaluate the inter-rater reliability of the seed placement method, another experimenter (blind to the animals' diet status) repeated seed region placement in 6 cases ($n = 3$ controls, 3 CR) using the same criteria.

2.4. Statistics

All statistical analyses were conducted using SPSS 21.0 software (IBM Software, Chicago, IL). Difference in age distribution