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Research report

Gender differences in brain volume and size of corpus callosum and amygdala of rhesus monkey measured from MRI images

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Abstract

While it has been established that the weight of the female rhesus monkey brain is less than that of the male, the sexual dimorphism of specific brain structures has not been well-documented. To further understand potential sex differences, we measured the whole brain volume and the size of the corpus callosum (mid-sagittal) and amygdala (largest coronal section) in MRI images from juvenile to adult male and female rhesus monkeys between 8 months and 7.2 years of age. The mean volume of the male brain was 89.2 ± 1.9 (S.E.M.) compared to the female brain volume of 70.8 ± 0.72 cm^3. The average area of the corpus callosum increased from 8 months to 4.5 years; 0.56 to 0.93 cm^2 in males and 0.45 to 0.66 cm^2 in females. However, the average area of splenium is significantly greater in females (0.280 cm^2), than males (0.184 cm^2). The average area of the amygdala did not change with age; it was 1.07 ± 0.037 (S.E.M.) in males and 1.08 ± 0.022 cm^2 in females. This data suggests that the whole brain volume and the size of the entire corpus callosum of young adult female rhesus monkeys are approximately 20% smaller than those of young adult males. Interestingly, the area of the splenial portion of the corpus callosum is larger in female monkeys. The size of the amygdala showed no sex difference.

Keywords: Sexual dimorphism; MRI; Corpus callosum; Amygdala; Brain volume; Rhesus monkey

1. Introduction

Sexual dimorphism of brain structures has been studied in many species including human and nonhuman primates [13,21]. Anatomical studies in human postmortem brains generally show that men's brains weigh approximately 10% more than women's [20,25]. Similar data have also been reported for rhesus monkey brains [9,18,21]. Gonadal hormones are known to play inductive role in both structural and functional development of the cerebral cortex of the developing rhesus monkey [7,8]. The size of the corpus callosum has been an area of interest in of sexual dimorphism in the human brain. Some studies have reported no size difference [30–32] while others have reported sex differences [20,21]. During the past decade, quantitative magnetic resonance imaging (MRI) has become a common technique for measuring the human brain. The brain volume measured from MRI shows sex differences which are similar to those obtained in anatomical studies of the human brain, finding male brains to be approximately 9% larger than female brains [5,6,16,30]. Similar to the anatomical data, the sex difference in the size of the corpus callosum as measured by MRI has been inconsistent [2,10,12,14,15,32]. Researchers have also found that the human splenium of the corpus callosum is larger and more bulbous in women than in men, but the data differ when obtained from subjects living on different continents [20,24]. Other studies have found that the isthmus region of corpus callosum is larger in females than in males [14]. In addition, the size of the corpus callosum decreases with

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increasing age in human males, while in females, it stays the same size until at least 40 years of age [14,17].

Sexual dimorphism in the size of the amygdala using electron microscopy has been reported in rats. Similar findings have been reported measuring human MRI [15,26]. For example, Caveness et al. reported that the size of the human amygdala was larger in young men than young women. However, other studies demonstrate that upon reaching adulthood, the human male amygdala decreases in size and becomes equivalent to that of the female [5,6].

In nonhuman primates, the average brain weight of male rhesus monkeys has been reported as weighing 90.57 g or 11% heavier than female brains, which weigh 81.50 g [9]. This is consistent with our earlier study, which assessed the whole brain volume from MRI of rhesus monkeys between 2 and 4 years of age. This study showed that the male brains are approximately 10% larger in volume than the female brain [24].

The present study was designed to use MRI to assess whether sex differences exist in the sizes of the brain, the amygdala, the corpus callosum, and the splenium, genu and isthmus during early development of rhesus monkeys.

2. Materials and methods

MRIs were obtained from 38 juvenile to young adult rhesus monkeys (*Macaca mulatta*). All macaques were born and raised at the Wisconsin Regional Primate Research Center and the Harlow Center for Biological Psychology at the University of Wisconsin. These animals were housed in indoor cages and were healthy throughout the study. To minimize discomfort and to immobilize the monkeys during scanning, they were anesthetized with 7.0 mg/kg ketamine and 0.6 mg/kg xylazine given i.m. This dose, was repeated as needed every 20 to 30 min. All of the experimental procedures were conducted in accordance with the National Institute of Health Guide for the care and use of animal subjects and the experimental protocol was approved by the Research Animal Resources Center at the University of Wisconsin.

2.1. MRI scans

Using a 1.5 Tesla GE Signa® Scanner, an MRI was created using the following parameters for measurement of the whole brain volume in 60 serial coronal sections. A 1.5 mm thick series of T-1 weighted images were obtained using a pulse sequence-3D SPGR with a TR (repetition rate) of 33 ms, and a TE (echo time) of 15 ms. To measure the corpus callosum and the amygdala, 18 serial coronal, 3 mm thick, T-1 weighted images (prefrontal to mid-occipital lobe) and sagittal images were obtained using a TR of 600 ms and a TE of 20 ms. The monkey's head was positioned in a styrofoam headrest inside a human knee coil. The proper position of the brain was verified by obtaining a mid-sagittal image.

2.1.1. Measurement of brain volume (one-dimensional method)

For each subject, the contour of the cerebrum, cerebellum, and spinal cord at the level below the pyramid, above the decussation and just below the olivary nucleus, was traced on a computer screen for all 60 coronal images using a greylevel tracking program. The voxels of all consecutive images, from the frontal tip to the occipital edge, were added to obtain a whole brain volume. We compared whole brain volumes from 11 male and nine female subjects between 1.5 and 7.0 years of age.

2.1.2. Measurement of the area of corpus callosum and amygdala

Using the mid-sagittal image, the contour of the corpus callosum was manually traced on a computer screen using NIH image 1.55 software (Fig. 1). The number of pixels in the area of the whole corpus callosum was calibrated to square centimeters. MRIs from 15 monkeys, seven males and eight females between 8 months and 4.5 years of age, were used to measure the corpus callosum. Six of these males and six of these females were used to measure the splenium of the corpus callosum. After defining the subsection of splenium, the area was measured using the above method.

For measurement of the amygdala, the coronal image showing the greatest area of the amygdala was selected for each case. The contour was traced on a computer screen and the number of pixels in the area was counted. The size of the amygdala was measured from 21 MRI images of 11 male and 10 female monkeys between 8 months and 7.2

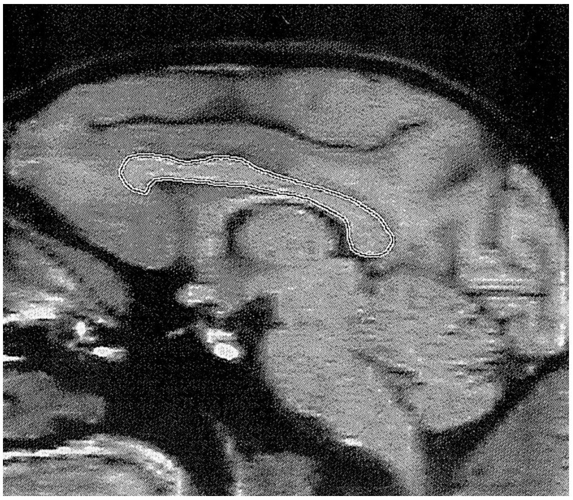

Fig. 1. MRI of a mid-sagittal section of an adult rhesus monkey brain and showing the contour of corpus callosum.

years of age. Unpaired Student's t-tests were used to test for statistical significance.

3. Results

3.1. Whole brain volume

The brain volume of male rhesus monkeys between 1.5 and 7 years of age ranged from 80.5 to 97.6 cm^3. After 1.5 years of age, the male brain volume appeared to be unchanged, averaging 89.2 ± 1.9 cm^3. The female monkey brain volume was also unchanged between the ages of 1.5 and 7 years; the range was 70.9 to 74.8 cm^3, $x = 70.8 \pm 0.7$ cm^3. The results indicate that the volume of the female rhesus monkey brain is approximately 20% less than that of male monkeys between the juvenile stage and early adulthood (Fig. 2) ($t = 8.336$, $df = 18$, $p < 0.0001$).

3.2. Area of the corpus callosum

The data from tracings of the contour of the corpus callosum in a mid-sagittal section revealed this area continuously increases between 8 months and 4.5 years of age in both male and female brains. Across this age range, age was significantly correlated with area ($r = 0.612$, $p < 0.02$) (Fig. 3). In the seven males measured, there was a highly significant linear increase ($r = 0.975$, $p < 0.001$) between 8 months and 3.3 years. In females, a significant linear relationship was not found. There was a statistically significant difference in the average area of the mid-sagittal section of the corpus callosum between juvenile and adult males 0.697 ± 0.046 cm^2 and females 0.586 ± 0.037 cm^2 ($t = 1.9$ $p < 0.04$, $df = 13$) (Fig. 4). Qualitatively, the splenial portion of the corpus callosum appears to be larger and more bulbous in female than in male monkeys (Fig. 5). Quantitative measurements of the splenial portion showed significant difference between male and female ($t = 2.62$, $df = 10$, $p < 0.03$). The average male splenium

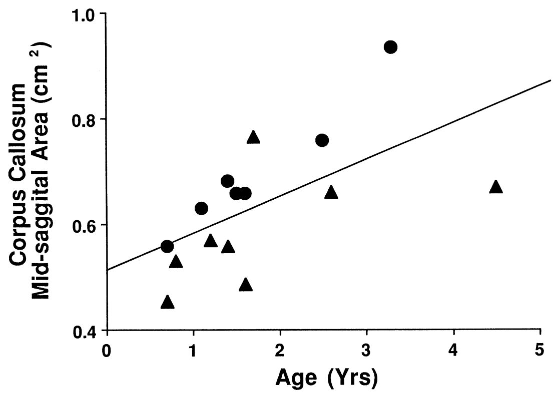

Fig. 3. A significant positive correlation of the mid-sagittal area (cm^2) of the rhesus monkey corpus callosum with age in seven male (circle) and eight female (triangle) rhesus monkeys ($r = 0.612$, $p < 0.02$).

was 0.184 cm^2, while the average size of the female splenium is 0.280 cm^2, or 52% larger.

3.3. Area of amygdala

In both male and female rhesus monkeys, the average area of the largest slice of the amygdala after the age of 8 remained unchanged; 1.08 ± 0.03 S.E.M. cm^2. We found no significant difference in amygdala area between male and female monkeys ($t = 0.252$, $df = 21$, $p < 0.40$). To detect a minimum one-tailed, $p < 0.05$ significant difference in the area of the amygdala of 0.1 cm^2, the approximate difference detected for the corpus callosum, we estimated that we would need nine subjects per group. This is based on the standard deviations of 0.083 that were determined for the amygdala areas, using a power of 0.8 and a beta of 0.2. Although marginal, we should have been able to detect a difference in the amygdalas of the 11 males and 10 females we measured. However, because the coronal image of the amygdala dramatically changes shape over a very small anteroposterior distance, a three-dimensional

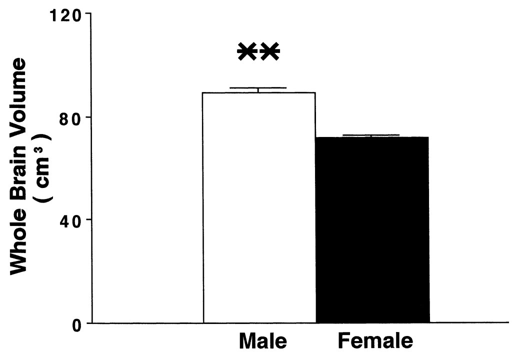

Fig. 2. A comparison showing male ($n = 11$) rhesus monkeys whole brain volume (cm^3) is significantly larger ($p < 0.0001$) than females ($n = 9$).

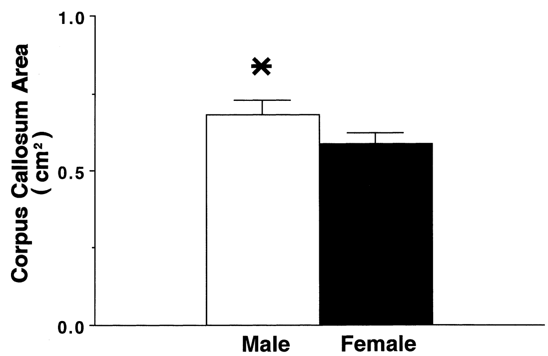

Fig. 4. A comparison showing male rhesus monkeys ($n = 7$) mid-sagittal area of the corpus callosum is significantly larger ($p < 0.04$) than females ($n = 8$).

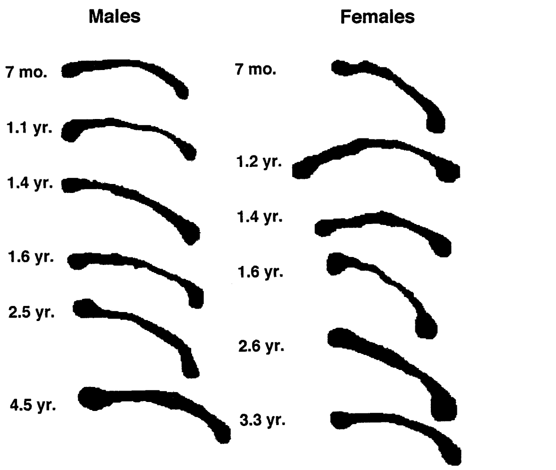

Fig. 5. Representative tracings of the rhesus monkey corpus callosum at ages ranging from 7 months to 4.5 years showing the differences in shape between males ($n=6$) and females ($n=6$). The splenium (right side) is significantly larger ($p<0.03$) and more bulbous in females than in males.

approach to the measurement of the amygdala would likely be more conclusive.

4. Discussion

The present MRI study demonstrates that the average brain volume of male rhesus monkeys is approximately 20% larger than the volume of the female brain. The sex difference assessed from MRI images is approximately two times greater than indicated in the average brain weight of rhesus monkeys from postmortem data [9,18]. The brain volume and weight of rhesus monkeys reaches a plateau after 2 years of age, and the data from MRIs are consistent with these anatomical studies [9,23]. Other studies that compared weight of adult rhesus monkey brains show a wide range of individual variation; however, a smaller range was found in our MRI data [9]. There are numerous reasons for the wide range of variation observed in postmortem brain weight, such as lack of consistent dissection of the brain from the spinal cord, environmental influences including diet and movement restriction as a result of different housing conditions, and genetic background. As in this study, brain volume measured from MRIs from monkeys born and maintained in a controlled environment can rule out the effects of environmental factors and anatomical inconsistencies caused by inconsistent dissection. Results obtained from human studies measuring brain volumes from MRI and postmortem brain weights were in agreement. These studies showed that male brains were approximately 10% larger than female brains in both juvenile and healthy adults [5,6,14,20,21,25,27]. The rhesus monkey brain develops much more rapidly during neonatal to juvenile age than the human brain in similar bio-age group [23]. The synaptogenesis in the prefrontal cortex of rhesus monkeys rapidly increases from 2 months to 3 years of age and thereafter, the synaptic density remained relatively constant [4]. Furthermore, gonadal hormones and their metabolites influence the emergence of cortical function in late neonatal age of rhesus monkeys; sexual differences in the rates of androgen binding, aromatization, and 5 alpha-reduction, apparently cause different developmental sequences of the brain during early development of rhesus monkeys [7,8]. Nonetheless, in both human and nonhuman primates, sex differences of volume and brain weight are similar in these two species.

Sex-related differences in the size and shape of the human corpus callosum is still controversial primarily due to utilization of different measurement techniques [3,10–12,14,15,19,20,22]. We found that the size of the mid-sagittal section of the corpus callosum was different between male and female rhesus monkeys. From juvenile to early adult age, the size of corpus callosum in males was approximately 20% larger than in females. However, the size of the splenium of the corpus callosum was larger and more bulbous in females. In both anatomical and MRI studies, the size of the corpus callosum and splenium in the human brain showed no definitive gender differences in either size or shape [1,3,22,30–32]. Interestingly, the splenial dorsoventral width of the corpus callosum in the human brain was higher in Australian females than males but this difference was not found in American brain samples [20]. Perhaps, the size of the rhesus monkey amygdala showed no sex differences in this study due to the methods employed. Since the amygdala is a relatively large structure, more than one slice should be measured. In human studies, the size and segmental volume of the amygdala and hippocampus were measured using MRI morphometry technique [15,26]. However, because of the difficulty of tracing the complete structures from MRIs, most data are reported as segmental volume [27,29]. These human studies reporting segmental volume are, however, largely interested in development, lateralization, or the effects of pathological conditions on the size of the amygdala and hippocampus [15,27–29]. Although MRI morphometry seems to be more accurate than anatomical measurements, the controversy on sex differences of human brain structures remains unresolved.

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