

No Significant Sexual Dimorphism of the Corpus Callosum in Thai Subjects: A Study Using Stained Plastinated Brain Slices

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ABSTRACT The morphological difference between male and female corpus callosum, in terms of total or partial cross sectional areas, have been reported with controversy. While some investigators showed the presence of sexual dimorphism of this structure, others reported none. Most studies suggest that the splenium of the corpus callosum in the female brain is significantly larger and more bulbous than that in the male. In the present study, 32 plastinated brain slices from Thai males and females, 16 of each sex, were used for morphometry of the corpus callosum. Both linear parameters and areas of the corpus callosum were determined. In the sagittal plane, the rostrum of the corpus callosum of the males was slightly larger than that of the females, while the rostral body, the isthmus and the splenium were somewhat larger in the females. In the coronal plane, the area of the male corpus callosum tended to be larger than that of the female. However, the differences were not statistically significant. It is concluded that no significant sexual dimorphism of the corpus callosum was found in the brains of Thai people.

KEYWORDS: Corpus Callosum, sexual dimorphism, Thai, plastination.

INTRODUCTION

The corpus callosum, an arched mass of white matter in the depths of the longitudinal fissure of the brain, is composed of transverse fibers connecting the two cerebral hemispheres. Developmentally, it first appears between 12-22 weeks of gestation¹ and is increased in size to more than triple during post-natal development.² Anatomically, the corpus callosum is divided into four regions³: the rostrum, genu, body and splenium. A narrow area between the body and the splenium is called the isthmus.⁴ There are about 300 million callosal fibers in man.⁵ They arise from large pyramidal cells in deep part of layer III of the cerebral cortex.⁶ With such tremendous number of fibers, the corpus callosum was assumed from the beginning to have important function in correlating activities of the two cerebral hemispheres.

Sexual dimorphism of the human corpus callosum has been widely investigated, with controversial results. The controversy may be due to differences in sex, age, or race of subjects under study or differences

in the method of measurement. Several studies investigating the cross sectional area of the corpus callosum suggested the presence of dimorphism: the female corpus callosum was significantly larger than that of males.⁷⁻²² The width of the anterior part, the body, the isthmus and the splenium was significantly greater in females than in males. Some investigators, however, reported that the corpus callosum area was significantly larger in the males than in the females,²³⁻²⁵ as were the genu²⁴, the body^{24,26}, the isthmus and the splenium.²⁰

Using the advantage of plastinated brain slices with special stains²⁷, the corpus callosum of Thai subjects were compared between males and females in coronal and sagittal sections.

MATERIALS AND METHODS

Brain Preparation, Sectioning, Staining and Plastination.

Human brains were obtained from autopsied cases at Chiang Mai Hospital, Chiang Mai and Ramathibodi Hospital, Bangkok, Thailand. The brains were

from subjects whose causes of death did not include neurological diseases or other debilitating conditions. Sixteen brains, 8 from each sex, were sectioned coronally; and another 16 brains were sectioned sagittally. The male age was 18-70 years (average, 44.4) and the female age, 20-73 years (average, 49.1). The brains were fixed in 4% formaldehyde solution for six months before sectioning.

The fixed brain was rinsed in running tap water overnight. They were serially cut into slices at thickness of 4 mm, in the coronal plane and 6 mm, in the sagittal plane. For symmetric coronal section, an initial coronal cut was made immediately anterior to the temporal poles with a brain knife prior to slicing with a meat slicer. This cut revealed the genu of the corpus callosum. For the sagittal plane sectioning, the brain was cut through the midline, bisecting the optic chiasma, median pontine sulcus and the median sulcus of the medulla oblongata. Symmetrical sagittal sections were then performed with the meat slicer from the medial to lateral direction. All of the brains sections were re-fixed for at least eight hours in 4% formaldehyde solution.^{28,29}

After rinsing by running tap water overnight, the specimens were stained by the Alston's method²⁷.

The slices were immersed in Mulligan's solution²⁸, for 20 min at room temperature, treated with a xylene/polycylens mixture for 20 sec with continuous agitation and immediately transferred to a 2% NaOH solution for 10 sec, again with agitation. For the final staining, the sections were immersed in 2% $K_4Fe(CN)_6$ until the gray matter was stained to the depth, usually for 1 to 2 min. This was followed by washing in running tap water for 5 min. The brain slices showed a marked increase in contrast between the gray and white matter, with brick-red and brilliant white color, respectively.

After staining, the slices were processed through sheet plastination procedure, using BIODUR® S10; a fully liquid silicone rubber of medium viscosity, instead of the usual polyester-copolymer.

Morphometry of the Corpus Callosum.

The corpus callosum in the sagittal plane was measured from four brain slices, two from each half. The two sections were the mid-sagittal and the medial sagittal sections, the latter was 6 mm away from the mid-line. The morphometry for the mid-sagittal plane was the same as described by Choo-wong (1995), which are as follows (Fig. 1(A)):

The linear parameters include:

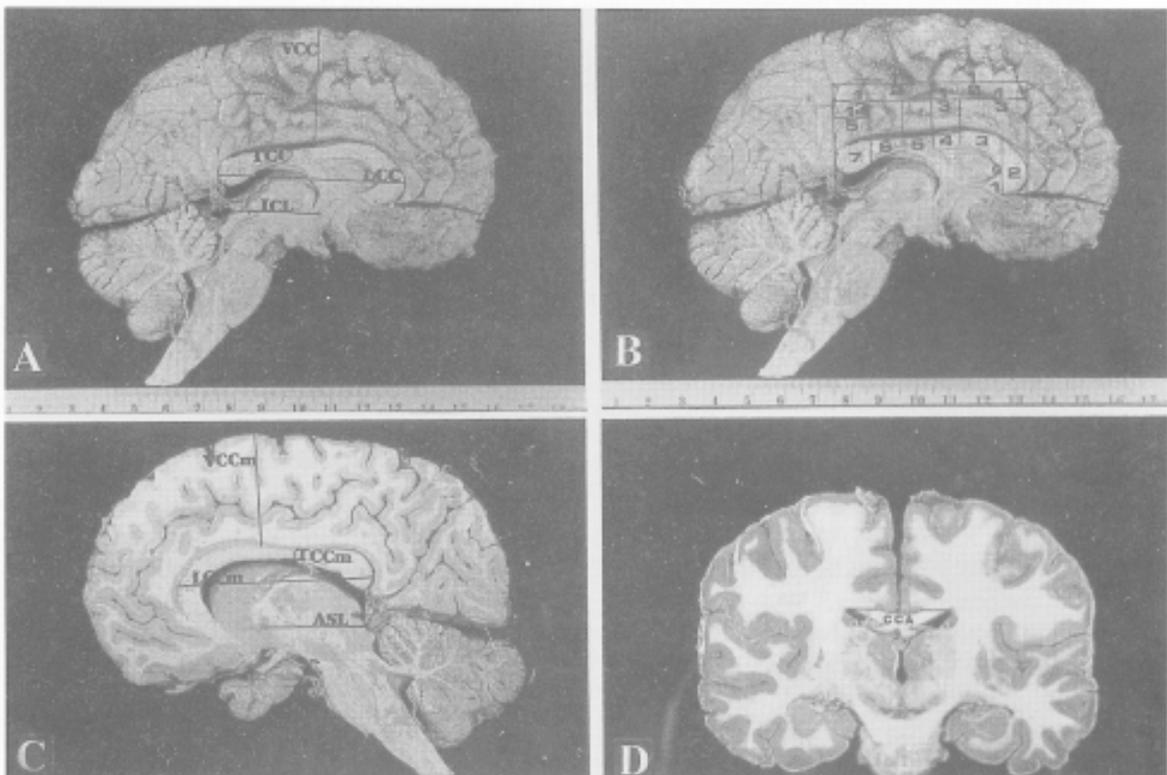


Fig 1. A: Reference lines, in midsagittal section, used in the morphometric analysis. B: The seven subdivisions used for area measurement, no. 1-7. C: Reference lines, in medial sagittal section, used in the morphometric analysis. D: Parameters for the morphometry and corpus callosum area of the coronal plane sections.

1. The distance between the anterior and the posterior commissures or intercommissural line (ICL).
2. The distance from the most rostral to the most caudal ends of the corpus callosum or length of the corpus callosum (LCC). This line was parallel to ICL.
3. The thickness of the thinnest part of the corpus callosum (TCC), this line was perpendicular to the ICL.
4. The distance from the highest point of the cerebral curvature to the cortical surface of the corpus callosum (VCC), this line was perpendicular to the ICL.
5. The ratio of LCC:ICL and TCC:VCC.

For area measurement, the corpus callosum was divided into seven subdivisions as depicted in figure 1(B).

1. LCC was used as the linear axis to subdivide the corpus callosum into anterior and posterior halves; anterior; middle and posterior thirds and the posterior one-fifth of the region or region 7, which is roughly congruent with the splenium.
2. The perpendicular to the axis at point G was used to define the anterior most division of the corpus callosum, roughly congruent with the genu or region 2 and the rostrum or region 1.
3. Region 3 or the rostral body was defined as the anterior one-third minus regions 1 and 2.
4. Region 4 or the anterior midbody was defined as the anterior one-half minus the anterior one-third.
5. Region 5 or the posterior midbody was defined as the posterior one-half minus the posterior one-third.
6. Region 6 or the isthmus was defined as the posterior one-third minus the posterior one-fifth.

The area of each region was determined by using a scanner and the software program "Adobe Photoshop". Each region was then calculated as a percent of the total area of the corpus callosum (CC). The area of the total sagittal surface (TSS) is outlined by the border of the brain section, excluding the cerebellum and the lower part of the brainstem. A line is drawn from the upper border of the corpus callosum toward the optic chiasm. The brainstem below this line is not included in the TSS.

For the medial-sagittal section, the linear parameters depicted in figure 1(C) include:

1. The distance between the anterior commissure and the superior colliculus (ASL).
2. The distance from the most rostral to the most caudal ends of the corpus callosum (LCCm, "m" stands for medial, this makes the distinction between the mid- and medial sections' abbreviations). This line was parallel to the ASL.
3. The thickness of the thinnest part of the corpus callosum (TCCm), this line was perpendicular to the ASL.
4. The distance from the highest point of the cerebral curvature to the cortical surface of the corpus callosum (VCCm), this line was perpendicular to the ASL.
5. The ratio of LCCm:ASL and TCCm:VCCm. The area measurement, with LCCm used as LCC, was identical to that of the mid-sagittal brain slices.

For the coronal planes, the area of the corpus callosum as seen in the sections was sequentially determined, using a scanner and "Adobe Photoshop," as for the sagittal sections. The corpus callosum area (CCA) was that between the superior and inferior lines of the corpus callosum depicted in figure 1(D). The sections for this determination, each of the ten sections, began from the posterior part of the genu toward the splenium. The area of the total coronal surface (TCS) of the section was also determined. The ratio of the corpus callosum area to the whole coronal brain surface area was calculated as a percentage: $(CCA:TCS) \times 100$.

Statistical Analysis

Numerical data, expressed as means \pm SE, were analyzed by SPSS/PC Student T-Test software program.

RESULTS

In the sagittally sectioned brains, the age of the male subjects was 41.4 ± 7.3 and that of the female was 45.6 ± 5.3 ; in the coronally sectioned brains, the age of the male subjects was 47.1 ± 6.5 and that of the female was 52.6 ± 5.9 . Statistically, there is no difference in the age group of any pairs of the subjects. After staining and plastinating, the surface of the gray matter was intensely brick-red. The degree of shrinkage was $12.9 \pm 1.03\%$ in the sagittal sections and $9.2 \pm 0.45\%$ in the coronal sections.

Table 1 reveals the linear parameters in mid-sagittal and medial sagittal sections of the male and

female brains. No statistically significant difference was detected in any parameters determined, although there tends to be a longer length of the corpus callosum (LCC) in the female (5.89 cm in male vs 6.29 cm in female, in mid-sagittal sections; 6.32 cm in male vs 6.47 cm in female, in medial sagittal section). When these parameters were standardized (LCC:ICL) and (LCCm:ASL), the male values seemed to be higher than the female values.

The absolute and relative areas of the total corpus callosum (CC), in both mid- and medial sagittal sections, are shown in Table 2. The CC of female brains seemed to be larger when measured either in the mid- or medial-sagittal sections. The difference, however, is not statistically significant. The total hemispheric surface areas were slightly larger in the females, and this accounts for a closer ratio of CC: TSS in both sexes.

The absolute areas and percentage of region 1 to 7, in the mid- and medial- sagittal sections, are shown in Table 3. Although, the rostrum region (region 1) of the mid-sagittal section of the males seemed to be larger than those of the female counterparts, the difference was not statistically significant. Likewise, the rostral body (region 3) and the splenium (region

7), though somewhat larger in the females, were not significantly larger than those of the males. When the percentage of the regions was compared, a non-significant difference was again found at the rostrum (region 1) and the splenium (region 7), the male seemed to have a larger rostrum but a smaller splenium. The same tendency was also detected in absolute areas of the medial sagittal sections.

Morphometry of the Coronal Brain Slices.

The areas of the corpus callosum in the coronal brain sections were depicted in Table 4. At all levels of sectioning, from the rostral to the caudal end, no significant difference was observed between the male and the female brains. The areas of the male corpus callosum were non-significantly larger than those of their female counterparts.

DISCUSSION

The average percentage of shrinkage at 9.2%, 12.9% for coronal and sagittal brain slices after stained-plastination, respectively, were considered lower than that of the brain processed by normal fixation and paraffin embedding, which was found

Table 1. Linear parameters of male (M) and female (F) corpus callosum. N = 8 for each brain section. In mid-sagittal sections: ICL, intercommissural line; LCC, length of the corpus callosum; TCC, thickness of the thinnest segment of the corpus callosum; VCC, line between the upper most point of the median hemispheric border to the cortical surface of the corpus callosum. In medial sagittal sections: ASL, anterior commissure-superior colliculus line; LCCm, length of the corpus callosum; TCCm, thickness of thinnest segment of the corpus callosum; VCCm, line between the upper most point of the medial hemispheric border to the cortical surface of the corpus callosum.

Mid-Sagittal Section

| Sex | ICL (cm) | LCC (cm) | TCC (cm) | VCC (cm) | LCC : ICL | TCC : VCC x 100 |
|-----|-----------|-----------|-----------|-----------|-----------|-----------------|
| M | 2.34±0.08 | 5.89±0.19 | 0.30±0.03 | 3.52±0.11 | 2.54±0.13 | 8.67±1.11 |
| F | 2.51±0.11 | 6.29±0.24 | 0.26±0.04 | 3.66±0.18 | 2.51±0.04 | 7.02±0.89 |

Medial Sagittal Section

| Sex | ASL (cm) | LCCm (cm) | TCCm (cm) | VCCm (cm) | LCCm : ASL | TCCm : VCCm x 100 |
|-----|-----------|-----------|-----------|-----------|------------|-------------------|
| M | 3.19±0.09 | 6.32±0.17 | 0.32±0.03 | 3.54±0.08 | 1.99±0.06 | 9.01±0.83 |
| F | 3.14±0.10 | 6.47±0.30 | 0.30±0.04 | 3.53±0.12 | 2.05±0.05 | 8.54±1.03 |

Table 2. Total corpus callosum (CC) and total sagittal surface (TSS) areas and ratio of (CC : TSS) x 100 in the male (M) and female (F). N = 8.

| | Sex | CC (mm ²) | TSS (mm ²) | CC : TSS x 100 |
|-------------------------|-----|-----------------------|------------------------|----------------|
| Mid-Sagittal Section | M | 465±35 | 6215±205 | 7.51±0.53 |
| | F | 478±40 | 6834±370 | 7.01±0.43 |
| Medial Sagittal Section | M | 542±28 | 7146±140 | 7.58±0.34 |
| | F | 558±51 | 7250±340 | 7.64±0.43 |

Table 3. Areas (mm²) of 7 regions of the male (M) and female (F) corpus callosum. N = 8.

Mid-sagittal sections

| Sex | Value | Region 1 | Region 2 | Region 3 | Region 4 | Region 5 | Region 6 | Region 7 |
|-----|-------|----------|----------|----------|----------|----------|----------|----------|
| M | Abs. | 31±4 | 109±8 | 69±4 | 55±5 | 43±5 | 41±3 | 118±14 |
| | % | 6.8±1.1 | 23.5±1.2 | 15.2±0.9 | 11.8±0.5 | 9.0±0.5 | 8.8±0.4 | 24.9±1.5 |
| F | Abs. | 23±3 | 112±12 | 74±4 | 54±6 | 40±4 | 42±4 | 133±11 |
| | % | 4.7±0.5 | 23.1±1.1 | 16.0±1.1 | 11.2±0.4 | 8.4±0.4 | 8.7±0.3 | 28.0±1.1 |

Medial sagittal sections

| Sex | Value | Region 1 | Region 2 | Region 3 | Region 4 | Region 5 | Region 6 | Region 7 |
|-----|-------|----------|----------|----------|----------|----------|----------|----------|
| M | Abs. | 28±3 | 128±11 | 84±5 | 66±4 | 49±5 | 49±3 | 141±9 |
| | % | 5.2±0.6 | 23.5±1.3 | 15.7±0.8 | 11.4±0.4 | 8.9±0.6 | 9.1±0.4 | 26.2±1.0 |
| F | Abs. | 23±4 | 127±17 | 86±4 | 61±6 | 51±6 | 54±7 | 156±13 |
| | % | 4.2±0.7 | 22.5±1.0 | 15.9±0.8 | 10.9±0.2 | 9.0±0.5 | 9.5±0.5 | 28.1±0.7 |

Table 4. Corpus callosum area (CCA) and total coronal surface (TCS) areas in the male (M) and in the female (F). N = 8 for each brain section.

| Section No. | Sex | CCA (mm ²) | TCS (mm ²) | (CCA : TCS) x 100 |
|-------------|-----|------------------------|------------------------|-------------------|
| 1 | M | 127±9 | 6879±197 | 1.84±0.10 |
| | F | 118±5 | 6199±262 | 1.92±0.10 |
| 2 | M | 125±6 | 7460±156 | 1.68±0.07 |
| | F | 108±9 | 6746±339 | 1.60±0.11 |
| 3 | M | 125±5 | 7780±151 | 1.60±0.06 |
| | F | 112±6 | 7222±385 | 1.57±0.07 |
| 4 | M | 118±5 | 7928±190 | 1.49±0.05 |
| | F | 112±6 | 7529±225 | 1.48±0.06 |
| 5 | M | 125±2 | 8403±255 | 1.50±0.06 |
| | F | 114±7 | 7721±293 | 1.48±0.08 |
| 6 | M | 120±6 | 8388±193 | 1.44±0.08 |
| | F | 112±8 | 7914±337 | 1.41±0.09 |
| 7 | M | 118±7 | 8454±241 | 1.42±0.11 |
| | F | 109±9 | 7820±326 | 1.43±0.15 |
| 8 | M | 113±10 | 8605±185 | 1.33±0.13 |
| | F | 106±9 | 8286±205 | 1.27±0.10 |
| 9 | M | 133±12 | 8324±144 | 1.60±0.15 |
| | F | 112±12 | 8121±279 | 1.37±0.15 |
| 10 | M | 143±22 | 8089±337 | 1.80±0.29 |
| | F | 125±13 | 7945±260 | 1.58±0.18 |

to be 25%.³⁰ This low amount of shrinkage was an advantage of the plastination technique over the conventional paraffin techniques, especially in the freeze substitution step using acetone instead of ethanol at room temperature. This low degree of shrinkage also makes the comparison study of the absolute values more reliable. The degree of shrinkage in the sagittal sections is higher than that

in the coronal sections. This is probably due to the difference in thickness of the sections. The sagittal sections are cut at 6 mm thick and the coronal sections at 4 mm thick. The thicker the section, the more difficult the penetration of plastic substances into the tissue; and, thus, resulted in more shrinkage in the thicker sections.

The statistics of this study did not reveal any significant difference either in the size or shape of Thai male corpus callosum and the female counterparts. Several studies reported similar findings.^{12, 31-45}

In this study, the male rostrum tends to be greater than that of the female, the finding is similar to that of Choowong (1995). This may reflect a sex-specific difference in the interhemispheric connectivity and functional organization of the inferior premotor areas as well as the medial and caudal/orbital prefrontal region. The larger size of the rostral body in the female found in this study was similar finding of Choowong (1995). This region contains fibers from the premotor and supplementary motor areas.

The similar finding that the female isthmus is significantly larger than the male was reported by Witelson (1989), Steinmetz et al (1992) and Clarke et al (1994). The isthmus probably includes interhemispheric fibers from the posterior parietal and superior temporal cortex which involves in language function.

Several investigators reported that the splenial width was significantly greater in the female than the male.^{7-11, 13-16, 22, 46} The difference was not observed in this study, suggesting that this difference may not exist in Thai subjects.

The finding that the corpus callosum area in the male coronal brain tends to be larger than the female counterparts is interesting. Corpus callosum area in the coronal brain may be correlated most with the anterior midbody of the corpus callosum. Thus, the finding that the anterior midbody seems to be larger in the male than the female may be consistent with the finding of Witelson (1989). Interest in anatomic evaluation of the corpus callosum is based upon the expectation that its function will be influenced by its structure. The rostrum connects between the inferior premotor as well as medial and caudal/orbital prefrontal regions of the two hemispheres. The rostral body interconnects the premotor and supplementary motor regions of the cerebral hemispheres. The anterior midbody carries fibers corresponding to regions of the motor cortical of the two hemispheres. The isthmus region appears to be the primary locus for fibers from posterior language areas. The splenium contains fibers relating corresponding regions of the peristriate, parietal, temporal, and occipital lobes of the two cerebral hemispheres. The male rostrum and the anterior midbody morphometry tended to be larger than the female, which may indicate a difference in hemispheric motor organization. The difference in the female

rostral body, the isthmus and the splenium tended to be larger than the male counterparts, may also be indicative of a difference in coordinating motor, language and visual organization.

Although the difference was not statistically significant, the size/shape of the difference, if reliable, may be biologically significant. Due to the small sample size in this study, the data was insufficient to draw any strong conclusions, and much more data is obviously needed.

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