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Quantitative analysis of the human corpus callosum under light microscopy

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SUMMARY

In recent years many workers have studied the morphology of the adult corpus callosum and controversy exists regarding gender- and age-associated differences. Callosal size may vary due to differences in the number and size of nerve fibers, glial cells and blood vessels. However, very little is known about the fiber composition of the human corpus callosum and how this might affect the length and thickness. The aim of this study is to investigate the regional differences in the numbers and diameters of several components of the corpus callosum per unit area and to determine age- and sex-related differences in the fiber composition of the corpus callosum. Eight human brains, both male and female, ages ranging from 25 years to 67 years, were studied. Semithin sections taken from different regions of the corpus callosum were stained with toluidine blue, examined under a Leitz DMRHC research microscope at a magnification of 40 x, and viewed at larger magnification on a computer screen after zooming. The number, diameter and area occupied by myelinated nerve fibers, glial cells and blood vessels per $0.01 mm^2$ were noted and analysed statistically. Thinly myelinated (diameter < 1.5 µm) nerve fibers were abundant in the genu and the lowest number was noted in the body per unit area. The area occupied by glial cells was more greater in the body of the corpus callosum. The splenium had a greater number of thick fibers (> 5 µm). The number of myelinated nerve fibers in the body of the corpus callosum was lower than in other regions. This may have been due to the presence of greater number of glial cells and large myelinated nerve fibers occupying a unit area. There was no statistically significant age- or sex-difference in the number of nerve fibers.

Key words: Age – Diameter – Gender – Glial cells – Nerve fibers – Number

INTRODUCTION

The corpus callosum is the largest commissure connecting the two cerebral hemispheres. It consists of three main parts: a genu, body and splenium. The two human cerebral hemispheres are not identical and principles of functional lateralization in the brain are universally accepted. A huge number of commissural fibers in the corpus callosum link the two hemispheres and ensure that they act as a single entity. The corpus callosum thus plays a part in a variety of integrative and cognitive

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functions. In recent years it has become a major area of cerebral research (Berry et al., 1995).

The components of the corpus callosum are nerve fibers, neuroglial cells and a certain number of blood vessels. Of those nerve fibers making up the main mass of the corpus callosum, a certain proportion can be expected to be non-myelinated (Tomasch, 1954).

The size and shape of the adult corpus callosum is thought to vary with sex and age (Witelson, 1989; Going and Dixon, 1990; Allen et al., 1991; Steinmetz et al., 1992; Holloway et al., 1993; Weis et al., 1993; Hopper et al., 1994; Johnson et al., 1994; Parashos et al., 1995; Sahil and Jit, 1996; Suganthy et al., 2002). However, the controversy regarding the sexual dimorphism as well as age related changes in corpus callosum is still unresolved.

Variations in callosal width and area may be due either to the number and size of nerve fibers and the degree of myelination (Habib et al., 1991). A larger callosal area is thought to reflect a larger number of fibers crossing through it and hence a better capacity for interhemispheric transfer. The callosal area and width may also depend on the fiber- packing density. If fiber-packing density is modified, then the callosal area or width may increase or decrease with no actual change in the number of fibers (Berrebi et al., 1988). The amount of glial tissue present may also play a role in callosal width and area. However, very little is known about the fiber composition of the human corpus callosum and how this might affect length and thickness. Tomasch (1954) reported a total of 174 to 193 million axons, both myelinated and unmyelinated with a mean diameter of 1.5 μm. Aboitiz et al. (1992) reported that thin fibers (<1 μm) were denser in the genu in a Western population. Their study also showed no sex difference in the fiber composition of the corpus callosum.

The aim of this study is to estimate the number and size of nerve fibers, glial cells and blood vessels per unit area in the genu, body and splenium of the human corpus callosum and to identify regional difference, if any, and to compare the above parameters in the different parts of the corpus callosum in young and old male and female brains.

Material and Methods

Autopsy specimens of eight normal, fresh South Indian brains from bodies donated to the department of Anatomy, Christian Medical College, Vellore for the purpose of teaching and research were used for the study. The brains from four male and four female, with ages ranging from 25 years to 67 years, were bisected at the midsagittal plane. Tissue from the genu, body and splenium of the corpus callosum were removed, fixed in 3% gluteraldehyde, post fixed in 1% osmium tetraoxide and embedded in resin mixture. Semithin (1 μm) sections were cut using an ultramicrotome with a glass knife. The sections were stained with 0.5% toluidine blue.

Micrometry:

One block each from the genu, body and splenium of the corpus callosum was used for this study. From each block, ten randomly selected frames / areas were examined under a Leitz DMRHC research microscope at a magnification of 40 x. A semiautomatic image analyzer Leica Qwin was used to measure the diameter and area, and to count the cells. The images were zoomed on the computer screen. A counting frame of 1000 μm^2 (Fig. 1) was used to count all myelinated fibers resolved under this magnification, glial cells and blood vessels falling within this frame. Ten such frames were counted for each block. In each frame, the cells touching the left and bottom borders of the frames were omitted and the cells touching the top and right borders were included. Summing of counts made in ten frames gave the total number of nerve fibers, glial cells and blood vessels in a 0.01 mm^2area of the corpus callosum. The diameter of the nerve fibers and glial cells and the outer diameter of blood vessels and the area occupied by the glial cells and blood vessels within that area were measured. The measurements were analysed statistically using SPSS version 11.5.

Results

Figure 1 shows the structure of corpus callosum under light microscope. It is composed of nerve fibers of varying sizes, glial cells and blood vessels. Most of the myelinated nerve fibers are small. Unmyelinated nerve fibers could not be resolved under the light micro-

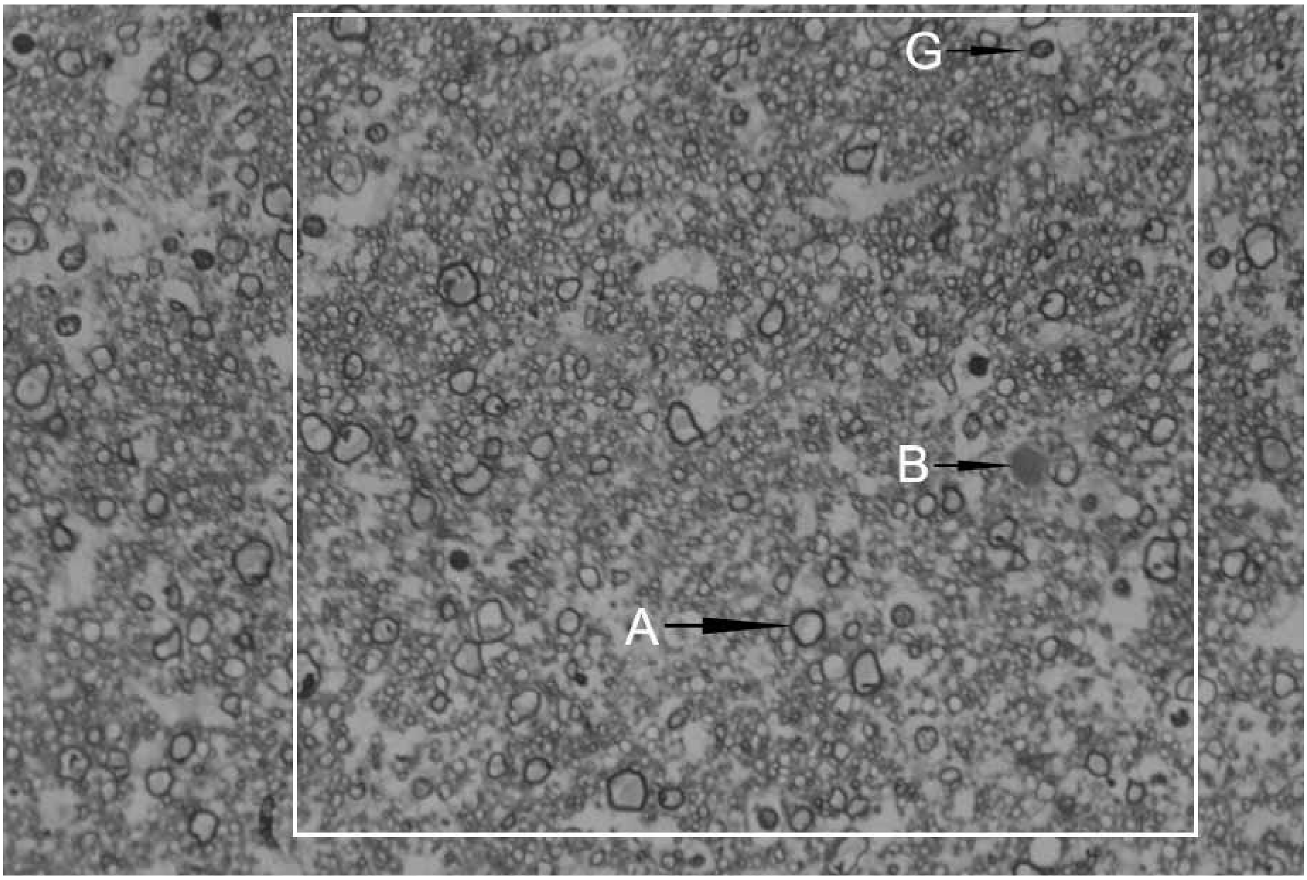

Fig. 1. Toluidine blue-stained section of the body of the corpus callosum of a male showing the various components. A – axon; G – Glial cell; C – Capillary. The square indicates the frame used for counting the various components of corpus callosum. x 120.

scope. Table 1 shows the median, standard deviation, minimum and maximum number of nerve fibers in the various regions of the corpus callosum per 0.01 mm^2. The number of nerve fibers per unit area was higher in the genu and lowest in the body of the corpus callosum (p=0.005). Table 2 shows the number, mean diameter and mean area occupied by the glial cells and blood vessels traced from the ablumi-nal surface in the genu, body and splenium of corpus callosum. Glial cells occupied more area in the body of the corpus callosum. Blood ves-sels occupied larger area in the splenium.

Table 3 shows the mean number of nerve fibers in the different regions of corpus callo-sum in males and females. The Mann-Whit-ney "U" Test did not reveal any significant difference between males and females in the number of nerve fibers occupying the various regions of corpus callosum. Table 4 shows the mean number of nerve fibers per unit area in young and old brains. The Mann-Whitney "U" Test showed no significant difference. Table 5 shows the diameter of nerve fibers in different regions of the corpus callosum. Thin fibers (<1.5 μm) were more abundant in the genu, while the body and splenium had thick fibers (>5 μm) and the fiber diameter reached 12.1 μm in the splenium. Table 6 shows the diameter of the nerve fibers in males and females. Thin fibers were more abundant in females in the genu (p<0.001), body (p=0.004) and splenium (p<0.001), while medium sized fibers (1.6-5 μm) and thick fibers were more abundant in males. Table 7 shows the diameter of nerve fibers in young and old brains. In the genu, thin fibers were more abundant in the older age group than in young brains. In the body and splenium, thin fibers were more abundant in young brains and large diameter fibers were more abundant in the older age group.

Table 1. Comparison of number of nerve fibers / 0.01 mm^2 in the different regions of the corpus callosum (N=8) using the Kruskal Wallis test.

S.No.	Region	Median	Standard Deviation	Minimum	Maximum	p-value
1.	Genu	2420	505	1531	3164	0.005
2.	Body	1564	299	1174	2047	
3.	Splenium	1907	357	1449	2443	

Table 2. Mean number, mean diameter, mean area of glial cells and blood vessels in 0.01 mm² area of eight corpora callosa.

S.No.	Region	No. of glial cells	Mean diameter of glial cells in µm	Area of glial cells in µm²	No. of blood vessels	Mean diameter of blood vessels in µm	Area of blood vessels in µm²
1.	Genu	42	4.2	87.49	20	3.2	43.75
2.	Body	46	4.96	122.42	16	5.13	43.82
3.	Splenium	22	5.07	63.23	24	4.55	64.97

Table 3. Comparison of mean numbers of nerve fibers in male and female corpora callosa using Mann-Whitney «U» test (n = 4 males «M»; n = 4 females «F»).

S.No.	Region		Median	Standard Deviation	Minimum	Maximum	P value
1.	Genu	M	2223	687	1531	3167	0.486
		F	2439	304	2197	2922	
2.	Body	M	1627	391	1174	2047	1.0
		F	1563	235	1384	1907	
3.	Splenium	M	1714	346.9	1449	2118	0.686
		F	1907	293.1	1484	2443	

Table 4. Comparison of mean numbers of nerve fibers in young and old corpora callosa using Mann-Whitney 'U' test (n = 4 young «Y»; n = 4 old «O»).

S.No.	Region		Median	Standard Deviation	Minimum	Maximum	P value
1.	Genu	Y	2420	195.5	2039	2446	0.886
		O	2559	739.6	1531	3164	
2.	Body	Y	1867	251.9	1457	2047	0.057
		O	1406	203.5	1174	1670	
3.	Splenium	Y	1947	273	1484	2118	0.486
		O	1674.5	469.9	1449	2443	

Table 5. Distribution of number of nerve fibers according to diameter in different regions of the corpus callosum.

	<1.5 µm	1.6-3 µm	3.1-5 µm	> 5 µm
Genu	13673	4727	408	19
Body	7711	4402	696	83
Splenium	9266	4647	717	107

Table 6. Comparison of diameters of nerve fibers in male and female corpora callosa (n = 4 males «M» ; n = 4 females «F»).

		<1.5 µm	1.6-3 µm	3.1-5 µm	> 5 µm	p-value
Genu	Male	6408	2503	220	10	<0.001
	Female	7265	2224	188	9	
Body	Male	3783	2297	360	36	0.004
	Female	3928	2105	336	47	
Splenium	Male	4137	2315	473	71	<0.001
	Female	5129	2332	244	36	

Table 7. Comparison of diameters of nerve fibers in young and old corpora callosa (n = 4 young «Y»; n = 4 old «O»).

		<1.5 µm	1.6-3 µm	3.1-5 µm	> 5 µm	p-value
Genu	Young	6654	2241	197	9	0.5
	Old	7019	2486	211	10	
Body	Young	4600	2335	278	25	<0.001
	Old	3111	2067	418	58	
Splenium	Young	4750	2434	286	26	<0.001
	Old	4516	2213	431	81	

Discussion

The fibers in the corpus callosum provide abundant bi-directional neural connections between most of the corresponding cortical areas of the two hemispheres. The corpus callosum allows information stored in the cortex of one hemisphere to pass to corresponding cortical areas of the opposite hemisphere (Guyton and Hall, 2006). It plays an essential and fundamental role in the integration of lateralized sensory, cognitive and motor operations.

Morphological examination of the corpus callosum may provide important insight into the functional organization of the cerebral cortex (Parashos et al., 1995). The substance of the corpus callosum is composed almost exclusively of nerve fibers. The other components, such as neuroglial cells and capillaries, may play a role in determining the size of the corpus callosum. In the present study, the number and area occupied by the glial cells were greater in the genu and body of the corpus callosum. Blood vessels occupied a greater area in the splenium (Table 2). A certain proportion of nerve fibers making up the main mass of the corpus callosum were unmyelinated. The myelinated fibers of the corpus callosum are very fine (Tomasch, 1954). Most of the very small myelinated and unmyelinated fibers would be missed in light microscopic investigations (Innocenti, 1986). The fibers that could be measured under light microscopy were recorded in this study.

The smaller and more numerous unmyelinated axons in the corpus callosum are involved in allocation and guidance of attention (Peters, 1988). The prefrontal cortex is considered to be a key cortical substrate of the highest-level mental processes. These higher-order processing areas are connected through small diameter, lightly myelinated fibers that pass through the genu of the corpus callosum. In the present study, highest concentration (73%) of thinly myelinated (slow-conducting) (<1.5 µm) fibers was found in the genu (Table 5), in accordance with the report by Tomasch (1954).

Most of the documented functions of the corpus callosum require fairly fast and direct interhemispheric connections (Peters, 1988). Primary and secondary areas are connected through large diameter myelinated nerve fibers (Aboitiz et al., 1992). These larger, more heavily myelinated and faster-conducting fibers are concerned with the transfer of information that serves to integrate sensory input and to coordinate movement across the hemispheres. The body of the corpus callosum connects the motor, somatosensory and auditory areas. In the present study, the body had the lowest number of myelinated nerve fibers per unit area (Table 1), as reported by Aboitiz et al. (1992). However, they were of larger diameter (Table 3), reaching a maximum of 8.9 μm in the South Indian population. The diameter of the nerve fiber in a western population was reported to reach up to 15 μm in the body (Aboitiz et al., 1992). In addition to the large diameter fibers, glial cells occupy more area in the body (Table 2). This increased number of glial cells associated with larger diameter fibers could account for the decreased fiber density in the body. Ng et al. (2005) stated that language and mathematics proficiency may be related to the morphometry of the fibres interconnecting both posterior parietal and temporal lobes. The splenium is known to conduct fibers from the temporal, parietal and occipital cortices. In the present study, the splenium had more large diameter fibers. These had a diameter of up to 12.1 μm (Figure 3). The better myelinated posterior part of the corpus callosum may account for the higher speed of nerve transmission and a more efficient neural network.

Sex-related changes

Sexual dimorphism has been reported in different parts of human brain. Brain weight (Holloway and De Lacoste, 1986), the size of anterior commissure (Allan and Gorski, 1986), volume of pre optic – anterior hypothalamic area (Swaab and Fliers, 1985) are known to differ between the two sexes. Sexual dimorphism is also apparent in the functioning of the human brain. Sexual differences in the corpus callosum were first reported by De Lacoste-Utamsing and Holloway (1982). Those authors reported greater splenial width and area in females and argued that a large splenium would correlate with a large number of interhemispheric fibers and less lateralization of function. Nevertheless, many other studies have failed to show sex-related differences in the morphology of the corpus callosum both in postmortem specimens and MR images. In the present study no statistically significant difference in the number of nerve fibers was observed in males and females (Table 3). However, a difference exists in the thickness of the nerve fibers. Females have more thin fibers while males have more large-diameter fibers (Table 6). Aboitiz et al. (1992) observed no sex difference in the number of nerve fibers in the corpus callosum. They suggested that the robust difference in brain weight of male and female brains could be due to the difference in the size of the nerve fibers rather than the number of nerve fibers. Our findings also support the hypothesis of Weis et al. (1989) that the female corpus callosum contains proportionally larger number of small diameter fibers, which would result in a lower average conduction velocity across the female callosum.

There are differences in the social behaviour of males and females. Females devote more energy to social communication, involving the inferior parietal and inferior temporal lobes, and thus use both cortices to a greater degree than males (Holloway et al., 1993). The predominance of thinly myelinated fibers (Table 6) observed in the female corpus callosum may be involved in this function. Males are believed to have better spacial skills for visualization and orientation (Weis et al., 1989) due to greater hemispheric specialization (Weis et al., 1989). It is possible that the greater number of large diameter fibers (Table 6) observed in this study may be responsible for this functional difference.

Age changes

As the brain ages, its weight decreases, the gyri shrink, and the sulci and ventricles become larger. This atrophy proceeds slowly to the age of 60 and more rapidly thereafter. The loss of cortical neurons is likely to cause secondary loss of callosal fibers (Going and Dixon, 1990). Studies addressing callosal structure and interhemispheric function suggest that the efficiency of the interhemispheric transfer of information decreases with increasing age and this may be one factor contributing to the wide range of congnitive, motor, visuoperceptual and communication —related difficulties experienced by aging adults (Bellis and Wilber, 2001). Reuter-Lorenz and Stanczak (2000) reported that any age-related decline in callosal size is relatively small and may be restricted to the most anterior regions of the corpus callosum. In the present study, the body and splenium had more small-diameter nerve fibers in the younger age group, but in general there were no age-related changes in the number of nerve fibers (Table 4). Pfefferbaum et al. (2000) stated that much of the regression of the brain occurs at the expense of cortical grey matter and that once brain maturation is complete, the cortical white matter volume remains relatively stable, at least through the 7th decade.

In this pilot study we attempted to measure the number and size of the various components of the human corpus callosum. The sample size is small due to the difficulty in obtaining unautolysed fresh human brains. Our results show that there are more nerve fibers present in the genu of the corpus callosum and the fewest in the body of corpus callosum per unit area. The presence of large-diameter nerve fibers and an increased glial population could account for the decreased fiber density in the body. There is no sex- or age-related difference in the number of nerve fibers. However, differences exist in the thickness of the nerve fibers. Females have more small-diameter fibers while males have a greater number of large diameter fibers. Thin fibers are more abundant in the younger age group and large-diameter fibers are more numerous in the older age group. A study of the human corpus callosum with a larger sample size might validate the above findings.

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