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Age-related changes in fibre composition of the human corpus callosum: sex differences

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WE found positive correlations between the number of myelinated callosal fibres > 1 μm in diameter and age in humans. The relatively abundant axons with diameters between 1 and 3 μm correlated with age only in females, while the scarce fibres > 3 μm in diameter correlated significantly with age only in males. When analysing different callosal segments, it was found that in the midbody (but not in the splenium) of females the number of fibres > 3 μm also increased with age. In males, the relationship between these large diameter fibres and age disappeared after dividing the callosum into distinct segments. There may, therefore, be sex differences in the course of callosal fibre growth and myelination during the normal lifespan.

Key Words: Ageing; Corpus callosum; Myelin; Sex differences

Introduction

We studied the effects of age on the fibre composition of the human corpus callosum. In humans and in non-human primates, the corpus callosum shows both individual and regional differences in terms of the number, size and myelination of its constituent fibres.[1–3] Across subjects there is a more than two-fold variation in fibre numbers, there being no detectable sex differences.[2] In each corpus callosum, regions connecting primary and secondary sensory areas (the midbody and the posterior splenium) have higher proportions of large diameter, highly myelinated fibres, while regions connecting higher order sensory areas (the anterior splenium) and frontal cortices (the genu) tend to have a high density of thin, poorly myelinated fibres. Furthermore, hemispheric lateralization is inversely related to callosal connectivity, as seen in comparisons of callosum size between right-handers and non-consistent right-handers,[4] and in studies relating partial callosal size or fibre numbers in specific callosal segments and anatomical asymmetry in the Sylvian fissure.[5,6].

An additional variable of interest is age. Although studies of callosal fibre development and myelination have been performed in cats[7,8] and non-human primates,[9] few studies have analysed changes in callosal fibre composition over a lifespan. Godlewski[10] found that the degree of myelination and the numbers of large diameter fibres increased with age during the lifespan of rats. This paper reports an age-related increase in the numbers of medium and large callosal fibres in the human which was more pronounced in females than in males.

Materials and Methods

Brains of individuals of known age and sex were obtained from the Institute of Pathology, Hospital San Juan de Dios, University of Chile, Santiago. The corpus callosa of 20 subjects (10 of each sex; ages 25–68 years) were sagittally sectioned and prepared for light microscopy as described previously.[2] The mean age of females was 43.5 ± 14.8 years (s.d.) and that of males was 46.4 ± 10.1 years. The callosal specimens were divided in thirds according to total straight length, and these segments were embedded in paraffin and stained with the Holmes (for neurofibrils) and the Loyez (for myelin) techniques. The anterior and middle thirds (respectively, genu and midbody) were further subdivided in three parts according to straight length (Fig. 1). The posterior third was divided into the posterior fifth (splenium) and the region between the posterior fifth and the beginning of the posterior third (isthmus). The splenium was further subdivided in three more segments. A locus situated in the centre of each of the subsegments comprising the genu, the midbody and the splenium was used to count fibres. In the isthmus, fibres were counted at two sites (see Fig. 1). Three microscopic fields were used to count fibres in each of these sites.

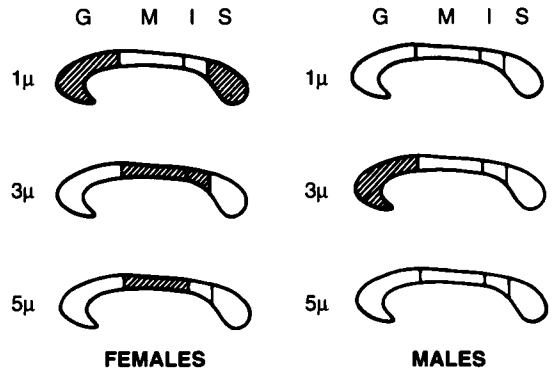

FIG. 1. Dashed regions indicate the callosal segments bearing a significant, positive correlation with age in both males and females, for different fibre types. 1u, fibres > 1 μm in diameter; 3u, fibres > 3 μm; 5u, fibres > 5 μm; G, genu; M, midbody; I, isthmus; S, splenium.

With the Holmes stain, fibres > 0.4 μm in diameter were counted in each microscopic field, using a grid that covered 54 × 54 μm at a total magnification of 1850×. Using the Loyez stain for myelin, fibres > 1 μm, > 3 μm and > 5 μm in internal diameter, respectively, were counted in fields of 100 × 100 μm, at a magnification of 1000× (Fig. 2). Fibres > 3 and > 5 μm are referred to as 'gigantic' fibres. Fibre counts were performed using the unbiased method of Gundersen[11–13] to correct for the edge effect. The areas of the embedded and stained callosa were also calculated, and the effects of shrinkage were corrected for. The total number of callosal fibres in each segment was calculated by multiplying the segment area (paraffin-embedded value) by its average fibre density. Pearson product–moment correlations were used to test correspondences between variables, and two-way ANOVAs were used to compare means. Further details of the experimental procedure can be found in our previous report.[2]

Results

In the whole sample, there was no relationship between age and the total number of callosal fibres per subject as determined with the Holmes technique (i.e. fibres > 0.4μm in diameter; r = 0.31; n.s.). However, there were positive correlations between age and total number of fibres >1 μm as determined with the Loyez stain (r = 0.59; $p = 0.006$; these fibres make up about 30% of the fibres counted with Holmes technique); between age and fibres > 3 μm (r = 0.51; $p = 0.02$; which make about 0.1% of the Holmes counts); and between age and fibres > 5 μm (r = 0.54; $p = 0.014$; making up about 0.02% of the Holmes counts). After separating by sex, the correlation between age and number of fibres seen with the Holmes technique (> 0.4 μm) remained non-

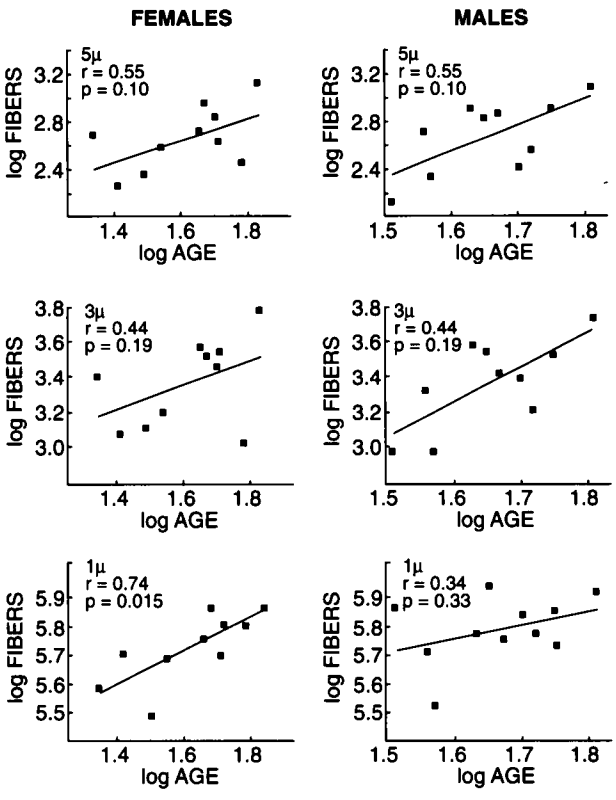

FIG. 2. Log-log graphs showing the correlations between total fibres per subject and age in males and females. 1u, fibres > 1 μm in diameter; 3u, fibres > 3 μm in diameter; 5u, fibres > 5 μm in diameter.

significant in each sex, although a trend was apparent in females (r = 0.58; $p = 0.07$). A correlation between age and number of fibres > 1 μm (Loyez) was seen in females, however, while the correlations between age and number of fibres > 3 μm and > 5 μm in diameter were significant only in males (see Fig. 3). Callosal area showed no age dependency in the whole population or in either sex.

In females, when the corpus callosum was divided into different segments (Fig. 4), the number of fibres > 1 μm in diameter (Loyez) in the genu and the splenium correlated with age (r > 0.72; $p < 0.02$) and number of gigantic fibres (> 3 and > 5 μm) in the midbody and the isthmus, but not in the genu or the splenium, correlated significantly with age (r > 0.67; $p < 0.04$). In males, however, the significant relationship between gigantic fibres and age was observed only for fibres > 3 μm in the genu (r = 0.72; $p < 0.02$) but not in any other callosal segments.

Discussion

In previous studies[2,14] we found no relationship between age and total number of callosal fibres observed with the Holmes technique. The age correlations reported here do not affect the pattern of fibre

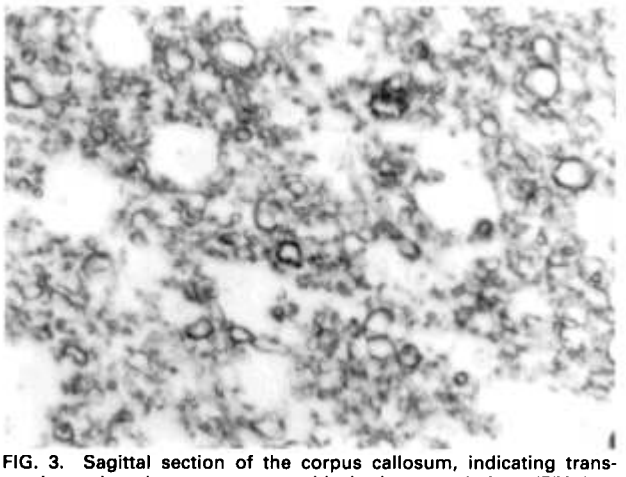

FIG. 3. Sagittal section of the corpus callosum, indicating transversly sectioned axons as seen with the Loyez technique (750×)

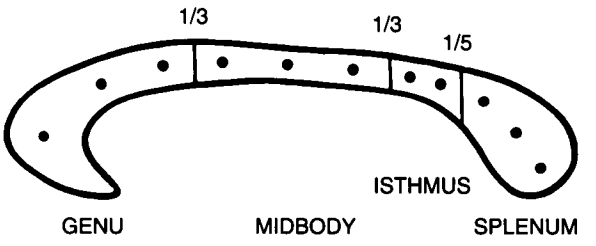

FIG. 4. Partition of the human corpus callosum as performed in this study. Dots indicate the regions where fibre counts were performed.

type distribution along the corpus callosum[2] or the correlation between callosal fibre counts and perisylvian asymmetries that we saw previously.[14]

Age was an unexpected variable affecting the numbers of myelinated callosal fibres. If anything, we would have expected the number of these fibres to tend to decrease with age, by analogy to the Betz cells of the motor cortex. Our results indicate the reverse: an increase with age in the numbers of callosal fibres of specific size ranges. The most likely interpretation of these findings is that with age, callosal fibres grow in size and myelination, resulting in an increase in the numbers of large diameter fibres at the expense of small diameter fibres. The reason why no concomitant negative age correlation was observed in the numbers of small fibres is probably that large calibre fibres account for only a small proportion of the total fibres. Overall, our findings are consistent with results of a previous study in the rat,[10] in which increased age was associated with increased thickness of myelin sheaths and with an increase in the numbers of thick fibres.

In females only, the relatively abundant fibres between 1 and 3 μm in diameter increased with age, while the much scarcer fibres > 3 μm and > 5 μm increased significantly with age only in males. The correlation between the number of fibres >1 μm and age in females was concentrated in the genu and the splenium (Fig. 4), regions through which higher-order frontal/prefrontal and temporoparietal areas are connected, respectively. This implies that the increase in size and myelination of this fibre range mostly corresponds to frontal/prefrontal and higher-order sensory areas.

Although in the whole callosum of females, the number of gigantic fibres (> 3 μm and > 5 μm) had no significant correlation with age, significant relationships with age for these fibre classes were found in the midbody and in the isthmus, but not in the splenium (not even in the posterior third of the splenium, where large diameter fibres connecting primary and secondary visual areas are concentrated[1,2,15,16]; Fig. 4). This suggests that in females, very large or gigantic fibres in the midbody and isthmus, connecting motor, somatosensory and auditory areas,[2,5] increase in size with age. However, the number of large fibres in the splenium that connect visual areas does not increase with age. This may explain the lack of a significant correlation between age and the total gigantic fibres per subject in females (Fig. 3). On the other hand, in males the situation is reversed. The total number of fibres > 3 and > 5 μm increased with age, but we did not find a significant age correlation in either the midbody or the splenium, where these fibres are more abundant. Perhaps in females the age-dependency of gigantic fibres is restricted to motor, somatosensory and auditory areas, while in males the relationship is less region-specific and more dispersed along the corpus callosum. (There was a significant relationship between gigantic fibres and age in the genu of males, but since this segment is characterized by a very high density of small, weakly myelinated fibres and a very low density of medium and large fibres,[2] we consider that this correlation does not involve a functionally significant fibre population.)

In females, a trend was observed in the correlation between age and the numbers of total callosal fibres (> 0.4 μm) seen with the Holmes technique. If true, this weak correlation may result from the fact that a significant number of callosal axons < 0.4 μm in diameter remain undetected by our technique.[2,3,9] With age, many of these small, mostly unmyelinated axons may myelinate and increase in thickness, thus becoming visible under the light microscope.

The increase in myelination and fibre size may serve as a compensatory mechanism for a decrease in the efficiency of information transfer in the older brain, making it necessary to recruit more fast-conducting fibres in order to transmit the same information. An alternative explanation is that the corpus callosum continues myelinating until relatively

advanced age. This would imply that at least some functions increase their interhemispheric transfer velocity with age, and that these functions may be different for males and females. The mechanisms underlying the observed sex differences may depend on humoral factors. Recent reports in the rat indicate sex differences in callosal fibre composition and in callosal plasticity.[17,18] Furthermore, it is known that thyroid hormones affect the connectional topography and fibre composition of the rat corpus callosum,[19,20] indicating that sex differences in lifespan myelination patterns may perhaps result from hormonal influences.

Although fibre sizes increased with age in different callosal segments and in the whole corpus callosum, no age effects were observed in either partial or total callosal area.[5] Our finding differs from an MRI study indicating a reduction in callosal area with age.[21] Using postmortem material, another report[22] indicates a decrease in normalized splenial area with advanced age, a result that could not be confirmed in our sample since we did not have very old subjects (more than 80 years old).

Conclusion

Our findings indicate that in females but not in males, fibres between 1 and 3 μm in diameter, which connect higher-order areas of frontal and temporoparietal regions, tend to increase in numbers with age. Gigantic fibres > 3 μm in diameter tend to increase with age in the whole callosum of males, and in the midbody and isthmus of females. The increase in fast-conducting fibres in the adult may indicate either a compensatory mechanism, or a continued development of these fibres throughout the lifespan. One functional consequence of these findings is that perhaps in females interhemispheric transfer time of certain higher brain functions (mediated by average diameter fibres, > 1 μm) either remains relatively constant (compensatory situation) or decreases (continued development) with age, while in males there would be either an increase or a maintenance of the interhemispheric transfer time with age. Gigantic fibres that tend to connect primary and secondary sensors and motor areas increase with age in both sexes, but the detailed pattern of these relationships differs between males and females.

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General Summary
The present study indicates that in the human, the numbers of relatively large and very large myelinated callosal fibres increase with age. This implies that some myelinated fibres grow in diameter during adulthood. In the whole callosum of females, only fibres between 1 and 3 μm in diameter showed a significant relationship with age. On the other hand, in males only fibres > 3 μm in diameter showed an age correlation. Larger diameter fibres have a higher conduction velocity, which may indicate that at least some interhemispheric functions increase transmission velocity with age. Alternatively, the growth of callosal fibres with age may serve as a compensatory mechanism for an age-related tendency to decrease conduction velocity or signal quality across the hemispheres. In any case, these results indicate that age does not equally affect interhemispheric transfer in the two sexes.