Aging Clinical and Experimental Research

Brain volumes in healthy adults aged 40 years and over: a voxel-based morphometry study

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ABSTRACT. ***Background and aims:*** *Gender and age effect on brain morphology have been extensively investigated. However, the great variety in methods applied to morphology partly explain the conflicting results of linear patterns of tissue changes and lateral asymmetry in men and women. The aim of the present study was to assess the effect of age, gender and laterality on the volumes of gray matter (GM) and white matter (WM) in a large group of healthy adults by means of voxel-based morphometry. This technique, based on observer-independent algorithms, automatically segments the 3 types of tissue and computes the amount of tissue in each single voxel.* ***Methods:*** *Subjects were 229 healthy subjects of 40 years of age or older, who underwent magnetic resonance (MR) for reasons other than cognitive impairment. MR images were reoriented following the AC-PC line and, after removing the voxels below the cerebellum, were processed by Statistical Parametric Mapping (SPM99). GM and WM volumes were normalized for intracranial volume.* ***Results:*** *Women had more fractional GM and WM volumes than men. Age was negatively correlated with both fractional GM and WM, and a gender × age interaction effect was found for WM, men having greater WM loss with advancing age. Pairwise differences between left and right GM were negative (greater GM in right hemisphere) in men, and positive (greater GM in left hemisphere) in women (–0.56±4.2 vs 0.99±4.8; p=0.019).* ***Conclusions:*** *These results support side-specific accelerated WM loss in men, and may help our better understanding of changes in regional brain structures associated with pathological aging.*

(Aging Clin Exp Res 2005; 17: 329-336)

INTRODUCTION

The normal human brain features a high degree of variability. Some factors are known to affect normal brain morphometry, the best studied of which are age and gender. Several studies have reported a global decline in brain volume with advancing age, together with an increase in sulcal or ventricular cerebrospinal fluid (CSF) (1-5). Instead, studies are in conflict as regards the effect of age on volumes of gray matter (GM) and white matter (WM). Some authors found a more accelerated decline with age in GM volume (6-9) whereas others reported a greater volume loss in WM tissue (5, 10).

The effect of gender on brain volumes has also been extensively addressed. Most studies agree on the finding of greater ventricular CSF volume in men and greater increase in CSF with age in men (6, 11, 12). Again, gender differences in GM and WM volumes are still controversial. Gur et al. (11) and, more recently, Luders et al. (13) found that women have greater GM volumes than men and that age-associated GM loss was more rapid in men. Other studies reported the opposite, men having greater GM and WM volumes than women (9, 14) and similar volume losses for both GM and WM (12). The effect of age is also greater in the left hemisphere in men, but tends to be symmetric in women (1).

Key words: Aging, brain volumes, MRI, normal, voxel-based morphometry.

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Received January 27, 2004; accepted in revised form October 8, 2004.

The aim of the present study was to assess the effect of age, gender and laterality on GM and WM volumes in the right and left hemispheres. A new tool (Ashburner and Friston's voxel-based morphometry – VBM – protocol) (15) allows accurate volumetric estimates through the use of observer-independent automatic algorithms and was applied to a large group of healthy adults aged 40 years and over. By warping the brain into a reference space, the VBM protocol can automatically segment the 3 types of tissue and – based on voxel intensity and its spatial position – can compute the amount of tissue in each single voxel.

METHODS

The subjects of this study were outpatients of the Neuroradiology Units of the Città di Brescia Hospital, Brescia, San Raffaele Hospital, Milan, and Hospital Borgo Trento, Verona, all aged 40 and older, undergoing brain MR scan from March 2001 to August 2003 for reasons other than cognitive impairment. Their scans were negative and satisfied some clinical exclusion criteria, detailed below. The aim of the research was explained to all subjects and their informed consent was collected, in order to use routine sequences for scientific purposes, take additional 3D sequences, and carry out multidimensional assessment.

Scans were deemed negative in the absence of the following findings: brain mass, aneurysm larger than 10 mm, arteriovenous malformation (except for developmental venous anomalies), malformations of the central nervous system, enlarged cisterna magna, meningioma, severe cerebrovascular disease, severe atrophy, large arachnoid cysts, and white matter hyperintensities with signs and symptoms of multiple sclerosis. The prescription of MR for the following conditions was considered as an exclusion criterion: neurodegenerative diseases such as Alzheimer's, Parkinson's, progressive supranuclear palsy, Huntington's disease, multiple system atrophy, amyotrophic lateral sclerosis, cerebrovascular diseases such as stroke and TIA, and head trauma. At the end of the enrolment period, these persons amounted to 307. 3D sequences were not available for 4 subjects, and images were judged of insufficient quality to undergo automated analysis in 39 cases.

Multidimensional assessment, including neuropsychological, neurological and clinical studies and ApoE genotyping, took place in a single session, in some cases right after the scan and in any case within 57 weeks. The mean time between scan and interview was 31±48 days in Brescia, 96±113 days in Milan, and 7±7 days in Verona. This difference was due to the different availability of examination rooms and human resources in the three settings. After the structured interview, 5 patients with alcohol and substance abuse, 1 under corticosteroid or chemotherapy treatment, 1 with obvious cognitive impairment, 2 with a history of severe head trauma, and 5 left-handed subjects were excluded from the analysis, due to the possible effect of these conditions on brain volumes. Twenty-one subjects refused to undergo clinical or neuropsychological testing. The final group was made up of 229 persons (150 from Brescia, 20 from Milan, 59 from Verona).

The three groups were different in age (56.1±10.9 in Brescia, 62.7±6.1 in Milan, and 60.2±10.6 years in Verona; p=0.003 on ANOVA), total intracranial volume (1336±131, 1422±133, and 1400±160 cc; p=0.002) and MMSE score (28.0±1.9, 29.7±0.6, and 28.6±1.9; p=0.001), but were similar for educational level (9.4±4.3, 8.0±5.3, and 10.5±5.5 years; p=0.109) and gender (females: 70%, 65% and 68%; p=0.878 on chi-square test).

MR was performed on a 1.0 Tesla Philips Gyroscan (PG) in Brescia, 1.0 Tesla Siemens Impact (SI) in Verona, and 1.5 Tesla Siemens Vision (SV) in Milan. MR images were acquired with the gradient echo 3D technique as follows: TR= 20 ms, TE= 5 ms, flip angle= 30°, field of view= 220 mm, acquisition matrix 256 × 256, slice thickness 1.3 mm in Brescia; TR= 9.7 ms, TE= 4.0 ms, flip angle= 12°, field of view= 230 mm, acquisition matrix 175 × 256, slice thickness 1.0 mm in Milan; TR= 11.4 ms, TE= 4.4 ms, flip angle= 8°, field of view= 250 mm, acquisition matrix 180 × 256, slice thickness 1.3 mm in Verona.

Voxel-Based Morphometry (VBM) processing

Images acquired with different scanners were processed separately. MR images were reoriented following the AC-PC line and, after removing the voxels below the cerebellum with MRIcro (www.psychology.nottingham.ac.uk/staff/cr1/mricro.html) (16), all images (including brain, cerebellum, and brainstem) were processed by Statistical Parametric Mapping (SPM99) (www.fil.ion.ucl.ac.uk/spm).

Before processing, the anterior commissure was manually set in each image as the origin of the spatial coordinates for the normalization algorithm (17). MR images were processed following a VBM protocol (9) which includes:

1) <u>generation of customized template:</u> after removing the voxels below the cerebellum, images of 40 subjects (in alphabetic order) among those enrolled in Brescia and Verona and all subjects enrolled in Milan were normalized to the Montreal Neurological Institute (MNI) template (18) of SPM99, using a 12-parameter affine transformation, smoothed with an 8-mm isotropic Gaussian kernel and averaged, thus yielding customized templates.

2) <u>generation of customized prior probability maps:</u> these were computed by segmenting the normalized images previously used for the creation of customized templates, smoothing the segmented images with an 8-mm isotropic Gaussian kernel, and averaging the smoothed images. Those voxels whose probability of being brain was greater than 0.5 were smoothed with

an 8-mm isotropic Gaussian kernel, in order to create customized brain masks, and they were smoothed with a 2-mm isotropic Gaussian kernel, in order to create masks for sulcal CSF.

3) main processing: the original images were normalized to the customized template through affine and non-linear transformations, medium regularization, reslicing 2 × 2 × 2 mm, and no masking (19). The normalized images were segmented into GM, WM and CSF using the customized prior probability maps and brain masks. The next step was cleaning, which made use of the Xbrain routine to remove voxels of non-brain tissue from the GM and WM images (e-mail John Ashburner, http://www.jismail.ac.uk/cgi-bin/wa.exe?A2=ind0202&L=spm&P=R6588&I=-3) and of the sulcal CSF masks to remove voxels of non-brain tissue from the CSF images. Voxel values of the cleaned GM, WM, and CSF images were multiplied by the measure of relative volumes of warped and unwarped structures derived from the non-linear step of spatial normalization (Jacobian determinant) (20).

GM, WM, and CSF volumes

A customized program (http://www.jiscmail.ac.uk/cgi-bin/wa.exe?A2=ind0203&L=spm&P=R2321&I=-1) was applied to modulated GM, WM, and CSF images, which were 3D matrices in which the intensity of each voxel was proportionate to GM, WM and CSF volumes within each voxel. The program calculates volumes by summing the voxels of the modulated images by the voxel volumes. Total intracranial volume (TIV) was computed as the sum of GM, WM and CSF volumes. Right and left GM, WM, and CSF volumes were then computed with the above program on half-image matrices (positive x coordinates in the stereotactic space, indicating right and negative coordinates indicating the left hemisphere).

DATA MANAGEMENT AND STATISTICAL ANALYSIS

The TIV was different among the three MR scanners, being greater in those subjects scanned in Milan and Verona than in Brescia. TIV differences may be due to differences in anthropometric features or scanner technical features. The main determinants of anthropometric features affecting TIV are height and gender. The similar gender distribution in the three groups led us to exclude that gender played a major role in TIV differences. Also, height cannot explain TIV differences, since subjects enrolled in Milan were on average 149±5.6 cm tall, whereas those from Verona and Brescia were respectively 166±8.7 cm and 162±24.7 cm tall. As regards scanner technical features, it may be hypothesized that different acquisition parameters were responsible for different tissue contrast, thus affecting SPM tissue segmentation. For a subgroup of 90 subjects (Brescia n=37, Milan n=18, Verona n=35), TIV was computed with another type of software, SIENAX (an adaptation of SIENA for cross-sectional measurement) (21). SIENAX relies less than SPM on tissue contrast features and more on cranial size and shape. We compared TIV calculated with SIENAX and SPM separately for the three scanners, and found that the two TIV measures differed only marginally (Brescia 1367±126 *vs* 1374±134 cc, difference of 0.51%; Milan 1395±93 *vs* 1399±109, difference of 0.29%; Verona 1354±109 *vs* 1372±115, difference of 1.33%). As the maximum difference of SPM-based TIV among groups was as large as 6.44% (1336±131 in Brescia and 1422±133 in Milan), this suggests that scanner features explain only a minor proportion of the TIV differences.

However, in order to correct for the potential different contrast of the three scanners among GM, WM, and CSF, linear regression models were built in which GM, WM, and CSF volumes were the dependent and scanners were the independent variables. The PG scanner was chosen as the reference and the SV and SI scanners were treated as dummy variables, with age, gender and TIV as covariates. A regression coefficient for the SV and SI scanners significantly different from zero would indicate that the scanner underestimates (in the case of negative coefficients) or overestimates (positive coefficients) the tissue of interest, compared with the SI scanner. In order to equalize volume estimates from the images of the three scanners, the amount of the over- or underestimate (corresponding to the parameter estimate) was added or subtracted to the observed GM, WM and CSF values. The equalized volumes were then

Table 1 - *Characteristics of 71 and 158 right-handed healthy men and women.*

	Women			**Men**		
	≤60 n=95	**>60 n=63**	**All n=158**	**≤60 n=38**	**>60 n=33**	**All n=71**
Education, years	10 (4)	7 (4)	9 (4)	12 (5)	10 (5)	11 (5)
MMSE	29 (2)	27 (2)	28 (2)	29 (1)	28 (2)	29 (2)
TIV, cc	1290 (100)	1322 (107)	1303 (104)	1469 (126)	1509 (144)	1488 (135)

Values denote mean (standard deviation); MMSE: mini mental state examination; TIV: total intracranial volume.

Table 2 - *Fractional brain volumes in 71 and 158 right-handed healthy men and women.*

	Women			**Men**			***p***		
	≤60 n=95	**>60 n=63**	**all n=158**	**≤60 n=38**	**>60 n=33**	**all n=71**	**gender**	**age**	**gender × age**
Gray matter	578 (24)	530 (32)	559 (36)	569 (27)	518 (30)	545 (38)	0.011	<0.001	ns
	-48			*-51*					
right	288 (12)	265 (16)	279 (18)	284 (14)	260 (15)	273 (19)	0.035	<0.001	ns
	-23			*-24*					
left	290 (12)	265 (16)	280 (18)	285 (14)	259 (15)	272 (19)	0.005	<0.001	ns
	-25			*-26*					
White matter	272 (16)	265 (15)	269 (16)	270 (15)	254 (20)	262 (19)	0.007	<0.001	0.053
	-7			*-16*					
right	135 (8)	132 (8)	134 (8)	135 (8)	125 (11)	130 (10)	0.010	<0.001	0.015
	-3			*-10*					
left	137 (8)	133 (8)	135 (8)	135 (8)	128 (10)	132 (9)	0.007	<0.001	0.168
	-4			*-7*					

Values denote mean (standard deviation) of volumes equalized by scanner and normalized by total intracranial volume (see methods). *p*: significance on ANOVA; ns: interaction factor was not significant and significance of the effects was computed in a model not including interaction term. Figures in italics: difference between younger and older groups (age effect).

normalized on TIV (fractional volumes). All analyses were carried out on fractional volumes.

The effect of age, gender, and laterality on the variables of interest was studied by analysis of variance (ANOVA) models, in which age and gender were between-subjects and laterality within-subjects factors. Full factorial models were first built and the highest-order interaction tested for significance. When this did not prove significant, a model without the interaction was built. The final model was that in which the lowest-order interaction was significant. Pearson's correlation coefficient (and 95% confidence interval, CI) were computed between age and brain volumes and Fisher's *z* scores were used to compare correlations. The relationship between age and WM volumes was also tested with a quadratic regression function.

RESULTS

The age of the 71 men in this study was 58.9±11.6 and of the 158 women 57.2±10.3 ($F_{1,227}=14.5$, $p=0.246$ on ANOVA). Table 1 shows that older age and female gender were associated with lower educational level ($F_{1,227}=11.7$, $p=0.001$ and $F_{1,227}=15.8$, $p<0.0005$). As expected, men had greater TIV than women ($F_{1,227}=135.2$, $p<0.0005$; difference between means= 179 cc, 95% CI 145 to 213). Younger subjects had higher MMSE scores than older persons ($F_{1,221}=18.3$, $p<0.0005$; difference= 1.1, 95% CI 0.6 to 1.6) and men had higher scores than women, although they failed to reach the critical statistical threshold ($F_{1,221}=5.1$, $p=0.134$; difference= 0.4, 95% CI –0.1 to 0.9).

Gray matter

A strong age effect was detected on fractional GM in both genders and in the right and left hemispheres (Table 2, Fig. 1a), Pearson's correlation coefficients ranging between –0.73 and –0.80 (Table 3). A gender effect (Table 2) was detected in both hemispheres, men having less fractional GM than women (difference= 5.8, 95% CI 0.6 to 11.1 in the right and 7.4, 95% CI 2.2 to 12.6 in the left hemisphere). This can also be appreciated in Figure 1a, in which the regression lines of GM volumes of women are above those of men at all ages. Gender × age interactions were not significant (Table 2).

Figure 2a shows that men have slightly, but not significantly, more GM in the right ($p=0.261$ on paired *t*-test) and women in the left hemisphere ($p=0.010$ on paired *t*-test). Figure 2b shows that pairwise differences between left and right GM were negative (greater GM in right hemisphere) in men and positive (greater GM in left hemisphere) in women (–0.56±4.2 *vs* 0.99±4.8), the difference being significant (Fig. 2b). It should be emphasized that the magnitude of the asymmetry is very low, the left-right difference in men being –0.21% and 0.35% in women.

White matter

The effect of age on fractional WM was different in men and women in both hemispheres (Table 2, Fig. 1b), gender × age interaction terms being significant on ANOVA (Table 2), indicating that the association of WM with age was stronger in men. This result is confirmed by the observation that Pearson's correlation coefficients were significantly higher in men (r=–0.51)

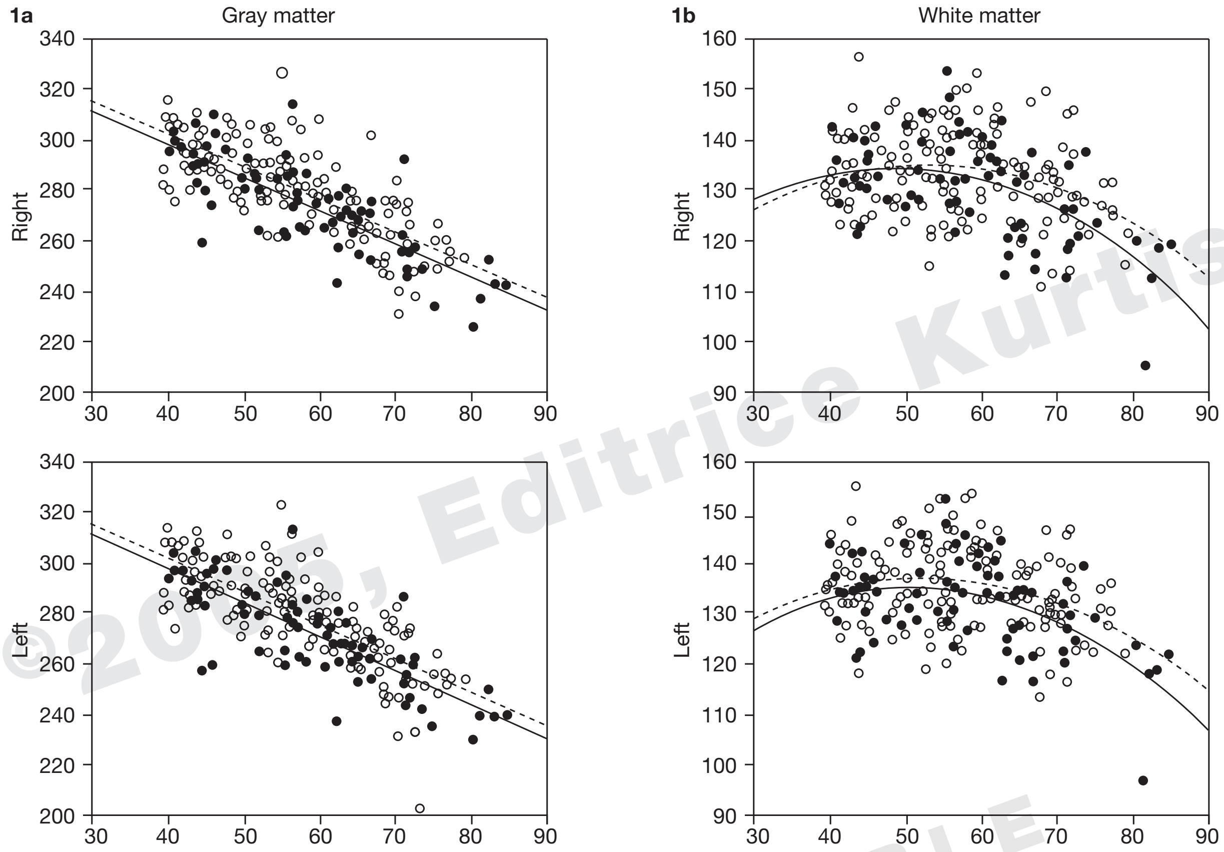

Fig. 1 - *Age effect on fractional brain volumes in 71 healthy men (filled dots) and 158 women (open dots). Lines: linear (1a) and quadratic (1b) regression functions of brain volumes on age in men (solid line) and women (dashed line).*

than in women (r=–0.19) (Table 3). It should be noted that the amount of WM at younger ages is not significantly different in men and women (272±16 *vs* 270±15) and that differences develop only with advancing age (265±15 *vs* 254±20).

The association between age and WM was best-fitted with a quadratic function. As can be seen in Figure 1b, WM volumes slightly increased until approximately 50 years of age and decreased thereafter. This pattern of change was found for both right and left hemispheres and for males (right hemisphere: regression coefficient –0.019, 95% CI –0.032 to –0.005, *p*=0.007; left: –0.019, 95% CI –0.032 to –0.007, *p*=0.003) and females (right: –0.017, 95% CI –0.029 to –0.005, *p*=0.005; left:

Table 3 - *Pearson's correlation coefficients of fractional brain volumes with age in 71 and 158 right-handed healthy men and women.*

	Women		**Men**		***p***
	r	**(95% CI)**	**r**	**(95% CI)**	
Gray matter	–0.74	–0.79 to –0.66	–0.80	–0.87 to –0.70	0.308
right	–0.73	–0.81 to –0.65	–0.79	–0.86 to –0.69	0.327
left	–0.75	–0.81 to –0.67	–0.79	–0.86 to –0.69	0.498
White matter	–0.19	–0.34 to –0.04	–0.48	–0.64 to –0.28	0.023
right	–0.18	–0.33 to –0.03	–0.51	–0.66 to –0.32	0.009
left	–0.19	–0.34 to –0.04	–0.43	–0.60 to –0.22	0.066

CI: confidence interval; *p*: significance on Fisher's *z* score.

–0.016, 95% CI –0.028 to –0.005, *p*=0.006) although WM loss was faster in men.

Figure 3a shows that the greater effect of age on WM in men is different in right and left hemispheres. Figure 3b shows that in both men and women pairwise differences between left and right WM are positive (greater WM in left hemisphere). An interaction between age and gender was also present, in that in women pairwise differences were similar in young and old subjects (left-right difference 1.79, 95% CI 1.26 to 2.32, and 1.61, 95% CI 0.59 to 2.62), whereas in men they were smaller in young and larger in older subjects (0.38, 95% CI –0.43 to 1.18 and 2.85, 95% CI 2.02 to 3.67). Again, the magnitude of the asymmetry is very low, left-right differences in young and older men being 0.28 and 2.22% and 1.30 and 1.21% in young and older women. The greater WM loss in older men in the right hemisphere was tested with an ANOVA model in which side (right *vs* left fractional WM volumes) was a within-subjects factor. As Figure 3b shows, the interaction of side with age and gender was significant (F=9.22; *p*=0.003).

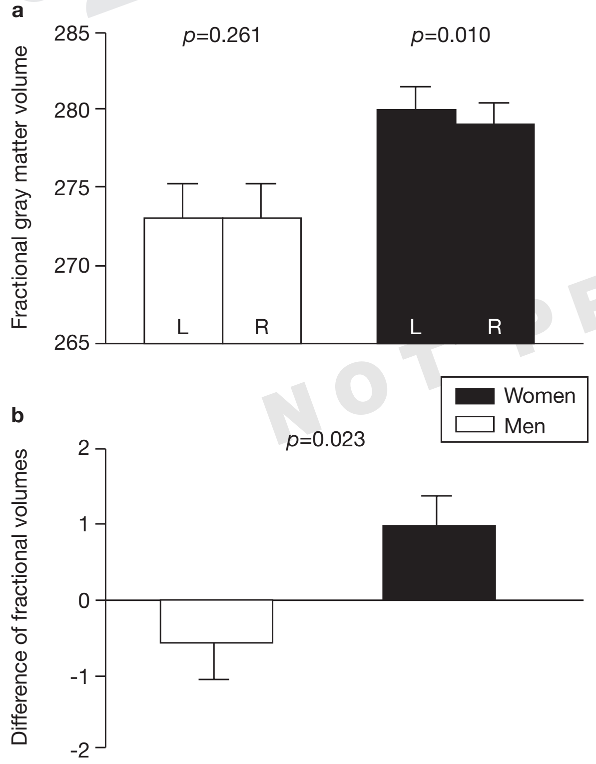

Fig. 2 - *Fractional left (L) and right (R) GM volumes (a) and left minus right difference (b) as a function of gender. Error bars: standard error;* p*: significance (a) on t-test for paired samples and (b) of gender main effect on ANOVA, with left minus right fractional volume as dependent variable and gender and age as factors. Age × gender interaction and age main effect were not significant.*

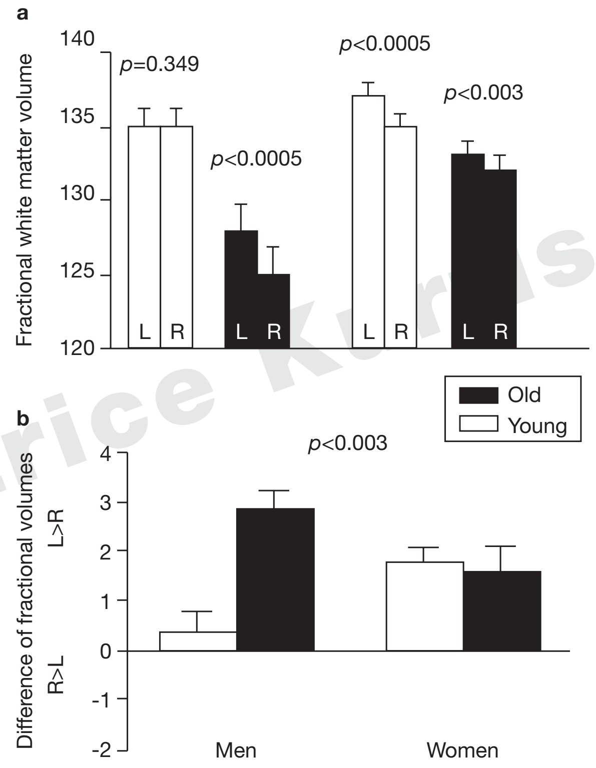

Fig. 3 - *Fractional left (L) and right (R) WM volumes (a) and left minus right difference (b) as a function of age and gender. Young and old are ≤60 and >60 years. Error bars: standard errors;* p*: significance (a) on t-test for paired samples and (b) of gender × age interaction on ANOVA (right vs left WM was inserted as a within-subject factor), indicating that differences between columns in men is greater than in women.*

DISCUSSION

We found that women have leftward asymmetry, and that GM volumes are negatively correlated with age in both genders. WM is more strongly associated with age in men than in women, and the age-related WM loss in men is particularly marked in the right hemisphere. These findings are important in that they show where aging affects brain structure and allow us to formulate hypotheses on pathogenetic mechanisms.

Our finding of greater fractional GM in women confirms the results of Gur et al., who used a different method of volume extraction (11), and of Luders et al. (12), who used one similar to ours. This fact was interpreted as compensation for the smaller intracranial space in women, so that the higher percentage of GM makes more tissue available for computation of information. Although the mean age of the subjects in the above studies was more than 30 years younger than in ours, it is noteworthy that we found this effect also in our much older population. However, it should be noted that literature data are discordant, in that some studies found no difference between genders (6, 7) whereas others found greater GM in men at all ages (22). Differences in subject selection strategies and brain analysis tools may account for these discrepancies.

Gender differences of brain lateralization have been extensively investigated as the biological substrate of the well-documented differences in cognitive performance, women performing better on language tasks and men on spatial ones. In agreement with current knowledge, in the present study we found that women had more GM in the left hemisphere independently of age, whereas men were more symmetric. However, data in the literature are conflicting, as other authors have found the opposite (11).

The quadratic association between WM and age fits data indicating that WM increases until middle age and declines thereafter (7, 23). However, Bartzokis et al. (24) and Ge et al. (7) found the peak of the curve at 38 and 40 years of age respectively, whereas in our group of subjects WM volume loss started later, around 50. These discrepancies may be due to differences in group composition and brain regions studied. First, Bartzokis et al. analyzed the relationship between age and WM limited to the frontal lobe, which is known to be more susceptible to the aging process. Second, their study investigated specifically relaxation time, which is a measure sensitive to brain myelination breakdown, the decrease in which predates the age at which WM volume begins to decrease. In fact, when the same authors investigated the relationship between WM volumes and age in frontal and temporal lobes, they found that the peak for the quadratic function was 47 years of age for temporal and 44 for frontal WM volume (25). It must also be noted that the above study was on a group of 70 healthy men, whereas ours also included female subjects, who are known to be less susceptible to age-associated diseases of WM such as cerebrovascular disease.

Studies which failed to find age-related WM loss included few subjects above the age of 40 (9, 11). To the best of our knowledge, the differential age-related loss that we found in men and women is a novel finding. This observation, together with other literature data, allow us to hypothesize that men have equal or more WM than women in younger ages but, over time, men lose more WM than women, so that in old age women have more WM than men. Indeed, in 40 men and 40 women with a mean age of 26 years, Gur et al. (11) found that men had greater WM, while Ge et al. (7) found no differences in 22 men and 32 women with a mean age of around 50. It has also recently been shown that the *corpus callosum* ages differently in men and women, the former showing greater shrinkage with advancing age (26). We speculate that the greater WM loss in men is related to vascular diseases and vascular risk factors, which are known to be more prevalent in men. This hypothesis is supported by observations indicating that hypertension is associated with smaller brain volumes (27, 28).

A further novel finding of our study is that the greater age-related WM loss in men is more marked in the right than in the left hemisphere. It may be hypothesized that the right hemisphere is more susceptible to age-associated cerebrovascular disease affecting primarily white matter and causing tissue loss. However, it has recently been demonstrated that lacunar infarcts are symmetrically distributed, whereas non-lacunar strokes are more frequent to the left in a population-based study of 1843 subjects (29). Similarly, the thickness and cross-sectional area of the vessel wall (intima and media) of the common carotid artery have been found greater to the left in untreated hypertensive patients and healthy subjects (29, 30), suggesting that WM loss in the right hemisphere is not related to vascular but to neuronal or glial factors. Different concentrations of androgen receptors in the right and left hemispheres may account for a putative glial effect on asymmetrical brain aging. It has been demonstrated that androgen receptors are present in glial cells of the white matter in primates (31), and are asymmetrically distributed in the temporal and frontal lobes (32), and those regions where androgen receptor density is higher have greater age-related brain volume loss (33). Although this scenario is far from clear, androgen receptor concentration may account for the higher susceptibility of the right hemisphere to age-related WM atrophy in men.

The slower global decline of brain volumes in women should also be discussed. Lower brain volume loss with age in women may be related to the neuro-protective effect of estrogens (34). Some studies have found a beneficial effect of hormone replacement therapy on verbal memory in surgically menopausal women (35). This effect may be due to protection against neurotoxins which boost free radical production (36) or to an increase in synaptic and dendritic spine density in some grey structures such as the hippocampus (37).

In conclusion, our results indicate greater GM and WM volumes in women, an age-related decline in GM and WM volumes in both sexes, and a greater WM loss in men. Further studies are needed in order to understand which factors, genetic or environmental, modulate the rate of GM and WM decline in men and women and in different brain regions. These data from normal elderly subjects provide a useful reference for better understanding of changes in regional brain structures associated with pathological aging.

ACKNOWLEDGEMENTS

Dr. Roberta Rossi and Dr. Lorena Bresciani assisted in data processing. The data of this study were collected thanks to an *ad hoc* grant of the Italian Ministry of Health, Ricerca Finalizzata "Archivio normativo italiano di morfometria cerebrale con risonanza magnetica (età 40+)." (RF 00.343).

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