

White matter loss in healthy ageing: A postmortem analysis

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Received 24 April 2007; received in revised form 23 October 2007; accepted 27 October 2007

Available online 20 February 2008

Abstract

Age-related brain changes are widely documented. Because of differences in measurement methods and case selection, the reported effects of age on regional grey and white matter brain volumes, however, are much more pronounced and widespread in neuroimaging than in postmortem studies. Consequently, the magnitude of the effect that is specific to chronological age remains unresolved. We present postmortem volume measurements for 26 cortical, subcortical and white matter regions, in 24 human brains aged 46–92 years, free of neuropathological abnormalities. Significant age-related loss was observed in anterior and posterior white matter but not in total grey matter volumes. Further analyses on five cortical subregions previously reported to exhibit large age-related loss on MRI yielded negative results. These analyses demonstrate smaller changes with age than those reported in imaging studies. Although this discrepancy between postmortem and imaging studies may partly be explained by the increase in noise of the neuroimaging data with age, our results suggest that healthy brain ageing is a process affecting predominantly white matter not grey matter.

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Keywords: Aging; Brain measurements; Grey matter; MRI; Point counting; Volumetric; White matter

1. Introduction

Morphological changes in brain structures are widely documented with ageing. Postmortem and *in vivo* magnetic resonance imaging (MRI) studies of healthy brains have reported somewhat different findings regarding the location, extent and severity of these changes with ageing. In postmortem studies of healthy human brains, more prominent age-related changes are reported in the white matter (WM) than in the grey matter (GM). Marner et al. (2003) found a 40% reduction in the total length of myelinated fibres between the age of 20 and 80 years (see also Peters, 2002). In contrast, Pakkenberg and Gundersen (1997) reported that the overall loss of neurons was less than 10% over the same age range. In addition, the total number of glial cells in the neocortex was similar in young and older adult brains (Pakkenberg et al., 2003). Also, age-related rates of change

in neocortical volumes, surface areas, WM and total brain weight are similar between men and women, despite sex differences in overall brain size (men > women; e.g., Double et al., 1996) and in the morphology of brain subregions (e.g., left planum temporale: women > men; Harasty et al., 1997; Pakkenberg and Gundersen, 1997). These changes with age occur despite a larger total neuronal count in men than in women (Pakkenberg and Gundersen, 1997).

In contrast to postmortem studies, MRI investigations have reported widespread age-related changes in GM and WM, particularly pronounced in the prefrontal region (DeCarli et al., 2005; Head et al., 2004; Rettmann et al., 2006; Sullivan and Pfefferbaum, 2003). For example, a cross-sectional study of 200 healthy adults aged 20–80 years reported 3–5% linear shrinkage per decade in the lateral and orbital prefrontal cortical regions, prefrontal WM, somatosensory cortex, and, to a lesser degree, motor cortex (Raz et al., 2004). The inferior parietal lobule and its adjacent WM, however, showed an increase in volume with age. Findings regarding the hippocampus and surrounding structures

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in the medial temporal region were not as definite. As such, Raz and colleagues reported a >6% shrinkage/decade in the hippocampal region after the age of 50 years. Other studies, however, found stable hippocampal volumes, but a decline in other medial temporal lobe volumes, with age (e.g., Sullivan et al., 2005) and a recent meta-analysis also described smaller than previously reported volumetric changes in the medial temporal region (Van Petten, 2004). This brain region is of particular interest given its contribution to memory function, memory decline being a common complaint in healthy ageing and one of the early signs of Alzheimer's disease. Longitudinal MRI studies, which address potential cohort effects, have provided mixed information: the majority report significant brain volume reductions over time (Du et al., 2006; Rettmann et al., 2006; Smith et al., 2007), but some find little or no change (Mueller et al., 1998; Pfefferbaum et al., 1998).

Some of the measurement variability between postmortem and imaging studies may be explained by differences in participant selection. Most MRI studies on ageing carefully exclude subjects with overt diseases that may have a direct or indirect impact on CNS function, but it is not possible to exclude preclinical structural changes without postmortem confirmation at the cellular level. Further, non-CNS factors (e.g., hypertension, cholesterol) are known to have an effect on neocortical and WM integrity (e.g., Pakkenberg and Gundersen, 1997; Resnick et al., 2003; Scahill et al., 2003) yet these factors are rarely considered fully. As such, the increasingly large variance in volume measurements with age reported by Scahill et al. (2003) would suggest that, at least in their study, not all older participants were free of neurodegenerative changes.

Because of these age-related pathological processes (cardiovascular or cerebrovascular disease; extra- or intracellular alterations), the independent contribution of chronological age to morphological changes in brain volumes remains to be fully explained. To date, no postmortem study has examined the effect of age on discrete brain subregions. In order to address this gap in knowledge, we conducted a comprehensive and careful postmortem volumetric analysis of 24 human brains free of neuropathological abnormalities at the cellular level from individuals aged between 46 and 92 years, who showed no psychiatric or neurological disorder prior to death. This study provides volumetric information for 26 GM and WM brain regions. It also examines the relations with chronological age in the brain regions reported to be most sensitive to the effects of age in MRI studies.

2. Methods

2.1. Participants

The brains of 24 individuals (13 men, 11 women) were collected from Royal Prince Alfred Hospital and from the New South Wales Institute of Forensic Medicine in Sydney with consent from relatives. The Human Ethics Committees of the Central Sydney Area Health Services, the University of Sydney, and the University of New South Wales approved this study, which also complies with the statement on human experimentation issued by the National Health and Medical Research Council of Australia.

Table 1
Demographic characteristics of study participants

Case	Sex	PM delay (h)	Age (yrs)	Brain weight (g)	Brain volume (ml)	Cause of death
1	M	23	46	1666	1390	Acute myocardial ischaemia
2	F	6	50	1302	1115	Myocardial infarction
3	M	25	54	1350	1197	Blood loss
4	M	17	55	1441	1209	Complications during cardiac surgery
5	F	19	57	1469	1281	Respiratory failure
6	M	26	58	1649	1513	Coronary artery occlusion
7	M	10	61	1334	1109	Sepsis
8	F	16	64	1289	1140	Perforated bowel
9	F	4	69	1339	1197	Dissecting abdominal aortic aneurysm
10	F	8	70	1183	1033	Respiratory failure
11	M	27	71	1433	1165	Post-operation complications
12	M	8	72	1441	1251	Respiratory failure
13	M	44	74	1306	1179	Cardiac/respiratory arrest
14	M	17	74	1240	992	Acute myocardial infarction
15	F	29	75	1064	887	Metastatic carcinoma
16	F	26	77	1199	956	Heart failure
17	M	35	79	1540	1348	Myocardial ischaemia
18	M	45	82	1362	1054	Metastatic malignant melanoma
19	F	12	84	1290	1083	Pulmonary oedema
20	M	21	85	1481	1229	Respiratory failure
21	M	12	86	1407	1194	Pneumonia
22	F	19	88	1159	964	Cardiac/respiratory arrest
23	F	24	91	1250	1103	Ruptured abdominal aortic aneurysm
24	F	41	92	1130	930	Perforated peptic ulcer

Note. PM delay: postmortem delay. Hippocampal neuronal counts for cases 1, 7, 11, 12, 14 and 19 have been published previously (Harding et al., 1998).

Stringent clinical inclusion criteria were used and case selection was based on an absence of significant neurological or cardiovascular disease, as well as other illnesses or injuries involving the central nervous system before death. Medical status was based on the examination of medical files and the standard questionnaires to relatives and treating general practitioners at the time of death. Further standardised longitudinal data were available on some of the older participants, who had been enrolled prospectively in our brain donor programme. Postmortem macroscopic and microscopic investigations were conducted to confirm the absence of neuropathological changes due to premorbid processes or changes secondary to the cause of death. Presence of vascular risk factors or cause of death was found not to be associated with abnormal neuronal density or with any neuropathological abnormalities.

The participants' demographic characteristics, cause of death and postmortem delay interval are shown in [Table 1](#).

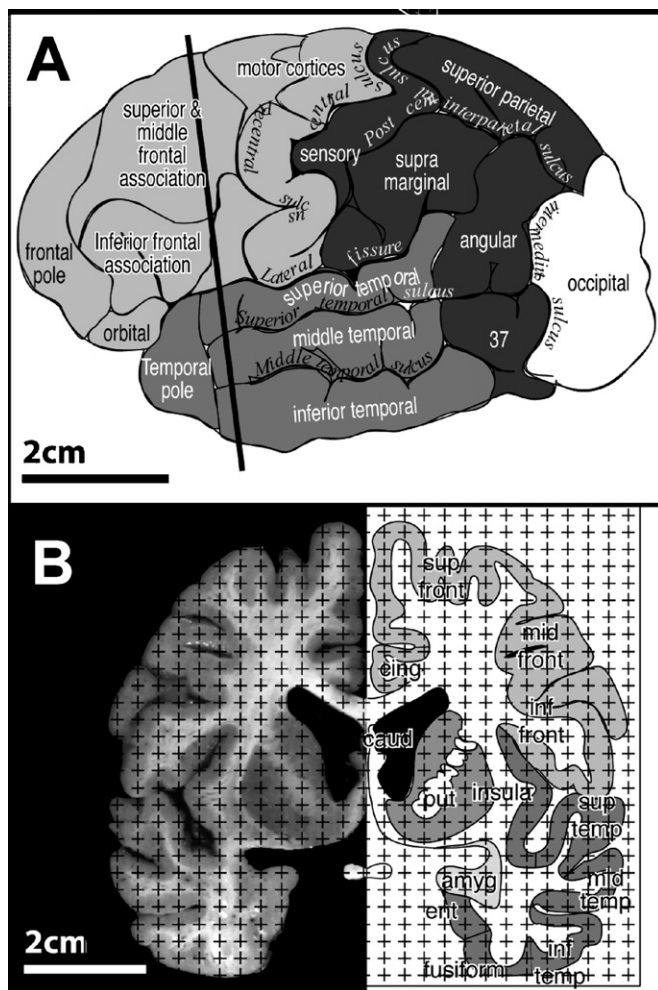


Fig. 1. (A) Cartoon of the brain surface showing cortical subregions painted with coloured dyes prior to sectioning. Black bar indicates the location of the coronal brain section shown in B. (B) Coronal brain slice overlaid with the 3848-point grid (left) and the corresponding brain cortical and subcortical subregions (right).

Mean age (women: 74.3 ± 13.8 years; men: 69 ± 12.9 years) and postmortem delay (women: 18.6 ± 11.1 h; men: 23.9 ± 11.8 h) were not significantly different between sexes. As expected, however, the average absolute total brain volume was larger in men (1216 ± 139 ml) than in women (1063 ± 121 ml), $t(22) = 2.857$, $p = 0.009$. There was no effect of postmortem delay on any regional volume measurements.

2.2. Brain preparation and region determination

Each brain was weighed at autopsy and the volume determined by fluid displacement. Following fixation for 14 days in 15% neutral buffered formalin, the weight and volume was remeasured to determine formalin-induced shrinkage. This shrinkage was minimal ($0.8 \pm 1\%$ from fresh brain volume) and in keeping with previous reports using this protocol (Halliday et al., 2003). The cerebellum and brainstem were separated from the cerebrum by sectioning through the cerebral peduncles. After determining the weight and volume of the cerebrum, the length of each hemisphere was measured before being embedded in 3% agarose, sectioned in 3 mm coronal slices, photographed and printed (magnification: $1\times$).

The entire cortical volume was measured following the method published previously (Halliday et al., 2003; Kril et al., 1997). Briefly, cortical gyri were painted with coloured dyes prior to sectioning to aid in the identification of regions in the coronal plane. Twenty-three neocortical regions were identified as described by Halliday et al. (2003) and measured (Fig. 1A). In addition, volumes of WM (anterior, posterior) and total basal ganglia structures (caudate, putamen, nucleus accumbens, internal globus pallidus, external globus pallidus) were also calculated. Detailed description of the regions of interest is presented by Halliday et al. (2003).

2.3. Volume determination

The volume of each cortical and subcortical subregion was determined by a point counting procedure. All regions in each brain slice photograph were identified. The slice photograph was randomly overlaid with a grid of 3848 points (area: $286 \text{ mm} \times 196 \text{ mm}$), and the number of points falling on each region identified, and the total number of points within each brain slice counted (Fig. 1B). This measurement method is reliable and highly reproducible with an average coefficient of error for each regional volume measure of 0.032 ± 0.008 (range: 0.021–0.048) and intra-class correlation coefficients >0.95 for each regional volume (Halliday et al., 2003).

The volume of each region was calculated by multiplying the sum of the points falling on a given structure by the volume represented by each point (volume/point = total number of points counted/cerebrum volume; average of 0.05 ml). This method is routinely used in our laboratory to measure regional volumes postmortem (Double et al., 1996; Halliday et al., 2003; Harasty et al., 1997) and approximates current point counting procedures used in MRI studies of brain volumes.

2.4. Statistical analyses

Effects of sex and laterality were investigated using repeated-measures analyses of covariance (ANCOVA). Age was introduced as a covariate to control for a possible cohort effect. The effect of chronological age on brain volume changes was investigated using regression analyses.

3. Results

3.1. Sex and hemispheric differences on absolute brain subregion volumes

A mixed-design, repeated-measures ANCOVA on the absolute subregional volume measurements, with sex as a between-subject factor, brain hemisphere as within-subject factor and age as covariate, revealed a significant main effect of sex, $F(1,22) = 7.979$, $p = .010$, $\eta^2 = .28$, and subregional volumes, $F(25,500) = 54.435$, $p < .001$, $\eta^2 = .72$, but not of laterality, $F(1,22) < 1$. A significant two-way interaction between subregional volumes and sex was also present, $F(25,500) = 5.880$, $p = .005$, $\eta^2 = .22$. Importantly, these analyses showed that none of the interactions with laterality reached statistical significance. Consequently, for each brain subregion, left and right volume measurements were summed into one global measure prior to examining age and sex effects further.

3.2. Regression analyses on absolute white and grey matter volumes

Mean total WM and GM volumes were 470.3 ± 92.6 ml (female: 417.6 ± 85.0 ml; male: 514.8 ± 75.6 ml) and 616.3 ± 62.4 ml (female: 587.5 ± 39.8 ml; male: 640.8 ± 68.8 ml), respectively. The contribution of age and sex on total WM and GM volumes was established using linear regression analyses. Both age ($b = -3.47$; $p = .004$) and sex ($b = 78.9$; $p = .010$; where female = 1 and male = 2) contributed to the total WM volume regression model [$F_{\text{obt}}(2,21) = 11.64$, $p < .001$], explaining 53% of the score variance. In contrast, only sex was a significant predictor of total GM volume [$b = 53.33$; $F_{\text{obt}}(1,22) = 5.13$, $p = .034$], explaining 19% of the score variance. Age did not contribute to this regression model. These analyses showed that the effect of age was limited to WM volume. Not surprisingly, given the known larger brain size in men than in women, sex contribution to score variance was present on both measures.

Because of the sex difference in body and brain size and the associated large score variance, these analyses ascertained only large effects and were not able to identify small, region-specific changes related to age. For this reason, we transformed each absolute subregion volume measurement into a percentage of the female (or male) mean total brain volume (i.e., transformed volume = [absolute volume/female (or male) mean total brain volume] \times 100), in order to min-

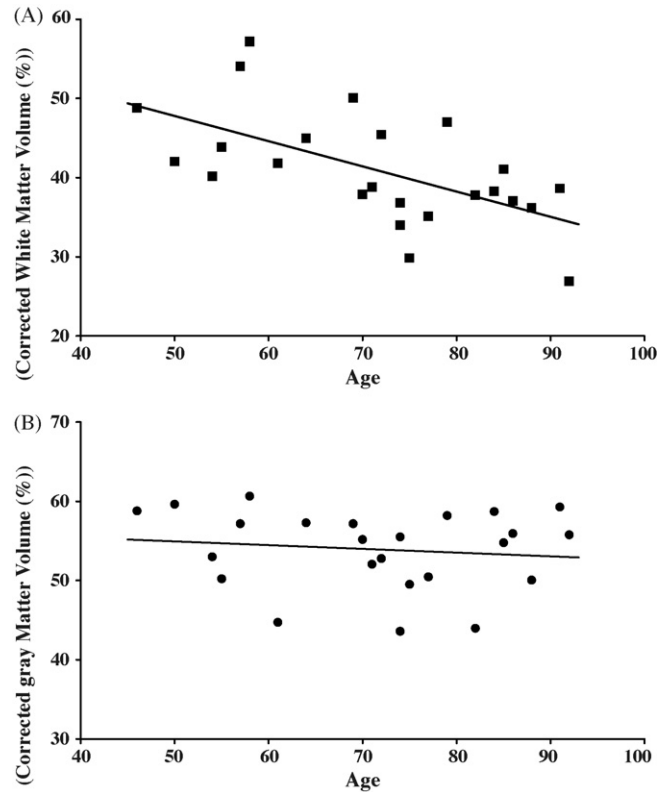


Fig. 2. Linear regression analyses showing the relations between (A) total corrected white matter volume and age [$Y = (63.72 - 0.318 \times \text{age})$; $R^2 = .360$; $p = .002$], and (B) total corrected grey matter volume and age [$Y = (57.33 - 0.048 \times \text{age})$; $R^2 = .016$; $p = .553$].

imise measurement variance and to eliminate the variance in score associated with sex.

3.3. Regression analyses on transformed total white and grey matter volumes

Repeated regression analyses on transformed total white and grey matter volumes confirmed the removal of score variance related to sex: only age contributed to the total WM volume regression model [$b = -0.318$; $F_{\text{obt}}(1,22) = 12.38$, $p = .002$], explaining 36% of the score variance. Neither age nor sex was a significant predictor of total GM volume (see Fig. 2). These analyses also confirmed that the age effect was specific to WM volumes and did not affect GM.

3.4. Regression analyses on transformed subregion volumes

Descriptive characteristics of the transformed subregion volumes are presented in Table 2. To identify more specifically the contribution of age to WM and GM, we investigated 5 cortical subregions that have been reported to exhibit age-related changes most consistently in previous MRI studies (e.g., Raz, 2005). These subregions were: hippocampus, dorsolateral prefrontal, orbitofrontal, entorhinal, and supra-

Table 2
Descriptive statistics (mean \pm S.E.) and correlations with age for total white matter, total grey matter and subregion volumes

	Mean \pm S.E.	<i>r</i>	<i>p</i>
WM total	40.98 \pm 1.45	−0.600	0.002
Anterior WM	27.59 \pm 0.95	−0.532	0.007
Posterior WM	13.36 \pm 0.59	−0.616	0.001
GM total	53.93 \pm 1.02	−0.127	0.553
Limbic regions			
Amygdala	0.30 \pm 0.01	−0.186	0.384
Hippocampus	0.83 \pm 0.03	−0.205	0.336
Entorhinal	0.35 \pm 0.03	−0.205	0.338
Posterior parahippocampal	0.25 \pm 0.01	−0.093	0.665
Anterior cingulate	1.07 \pm 0.03	0.113	0.600
Posterior cingulate	0.84 \pm 0.03	−0.355	0.088
Frontal cortex			
Frontal pole	7.32 \pm 0.21	−0.088	0.683
Orbitofrontal	1.83 \pm 0.09	0.038	0.860
Superior/middle frontal	2.48 \pm 0.10	0.096	0.662
Inferior frontal	1.02 \pm 0.07	0.028	0.898
Motor cortices	3.47 \pm 0.10	−0.186	0.383
Temporal cortex			
Temporal pole	1.96 \pm 0.08	−0.291	0.168
Superior temporal	1.98 \pm 0.06	−0.117	0.586
Middle temporal	1.52 \pm 0.05	0.044	0.839
Inferior temporal	1.18 \pm 0.05	0.075	0.727
Fusiform	0.61 \pm 0.03	−0.230	0.280
Parietal cortex			
Area 37	1.91 \pm 0.10	0.012	0.955
Angular	2.49 \pm 0.08	0.171	0.426
Supramarginal	2.33 \pm 0.12	−0.365	0.080
Superior parietal	3.27 \pm 0.13	0.156	0.467
Postcentral	2.06 \pm 0.07	−0.282	0.182
Other regions			
Insula	0.75 \pm 0.03	−0.330	0.116
Basal ganglia	4.01 \pm 0.12	−0.302	0.151
Occipital	15.2 \pm 0.38	−0.009	0.967

Note. Significant correlations are indicated in bold. GM = grey matter; WM = white matter.

marginal cortices. Given the contribution of age to WM shown above, we examined anterior and posterior WM volumes separately, to determine whether an anterior–posterior gradient was present as previously reported on some MRI studies (Head et al., 2004; Sullivan and Pfefferbaum, 2003). We restricted regression analyses to these regions, in order to limit the risk of Type I error.

Age was a significant predictor to both anterior [$b = -0.185$; $F_{\text{obt}}(1,22) = 8.69$, $p = .007$; $R^2 = .28$] and posterior [$b = -0.133$; $F_{\text{obt}}(1,22) = 13.46$, $p = .001$; $R^2 = .38$] WM volumes. The regression models indicated a loss of 27% of anterior WM and 37% of posterior WM between the age of 46 and 92 years. The difference in R^2 (i.e., difference in explained variance) between the two WM volumes was not statistically significant, indicating that the effect of age on anterior and posterior WM subregions was similar. In contrast, age did not contribute significantly to any of the regression models for the GM subregions: dorsolateral prefrontal ($b = -0.003$; $p = \text{ns}$), orbitofrontal ($b = -0.208$; $p = \text{ns}$), hip-

pocampus ($b = -0.002$; $p = \text{ns}$), entorhinal ($b = -0.002$; $p = \text{ns}$) and supramarginal ($b = -0.016$; $p = \text{ns}$).

In the light of this lack of contribution of chronological age to specific GM volumes, we conducted exploratory analyses to determine whether any of the remaining subregion brain volumes would show an association with age. None of the Pearson correlation coefficients between age and GM subregions was significant, even before correcting for multiple measurements (Table 2).

4. Discussion

This postmortem study reports the comprehensive volumetric measurements of distinct cortical regions, WM and basal ganglia volumes, using point-counting methods in 24 healthy adults. Our investigations demonstrated that the impact of chronological age on healthy brain structures appears limited to WM volumes, with no anterior–posterior gradient, and no evidence of change in GM volumes.

To our knowledge, no postmortem study has examined the effect of age on such a large number of well-defined brain regions in a sample of subjects spanning five decades. These subjects were very healthy: they were free of neurological and neurodegenerative diseases prior to death; and macro- and microscopic postmortem investigations confirmed the absence of neuropathological changes in these brains. Unlike other postmortem studies, we reported analyses on transformed (proportional) volumes. These analyses allowed the examination of ageing effects on brain volumes independently from possible sex and cohort differences.

4.1. Effects of age on white matter integrity

Changes in the brain WM are increasingly common with ageing, even in the absence of a neuropathological process. These microscopic lesions are due to cellular (myelin pallor, gliosis) and vascular abnormalities (increased perivascular space, reduced perfusion) and may be more predominant in the frontal regions. In this study, a significant association was present between age and WM volumes. The regression analyses indicated that the estimated age-related loss of WM was present in anterior and posterior WM volumes. The estimated age-related loss of anterior WM was 27% compared to a 37% loss in the posterior WM between the age of 46 and 92 years. Although the age-related loss of brain tissue appeared more severe in the posterior WM, the difference in WM loss between the two WM compartments was not significant. The age-related loss in WM volume is somewhat more pronounced than that reported by Marner et al. (2003). These authors found a 23% loss of WM volume between 20 and 80 years in their postmortem study. The continuing myelinating process of nerve fibres that has been reported to take place until the fifth decade of life (Bartzokis et al., 2003) probably accounts for this difference. Marner et al. (2003) also found the absolute WM volume to be 13% bigger in men compared

to women; density of myelinated fibres was, however, similar across sexes. In this study, the uncorrected total WM volume was 23% larger in male brains than in female brains.

In addition to volume measurements, markers of age-related WM changes in *in vivo* MRI studies have included calculation of WM lesion burden and change in WM properties on diffusion tensor imaging. Lesions in the WM present as hyperintense signal on MRI T2 weighted images (de Leeuw et al., 2001; Piguet et al., 2003). Typically, these lesions are reported separately for the periventricular region and for the deep WM. Both types of lesions increase in prevalence with age but only lesions in the deep WM are thought to indicate an abnormal pathological process, most likely of vascular origin. Reduction in fractional anisotropy on diffusion tensor imaging is also reported with ageing (Head et al., 2004; Sullivan and Pfefferbaum, 2003). This change reflects a decrease in WM fibre organisation and fibre integrity. Evidence from neuroimaging studies differs from that arising from postmortem investigations in an important way: MRI studies tend to favour a frontal gradient of WM change with chronological age. This frontal ageing predilection is reported for volume measurements (greater shrinkage in frontal than posterior WM) (Raz and Rodrigue, 2006), for the number of hyperintense lesions in the deep WM (de Leeuw et al., 2001), as well as for WM structural integrity as measured by diffusion tensor imaging with significantly lower anisotropy measures in older than in young adults but only in the anterior WM (Pfefferbaum et al., 2005); although see Madden et al. (2004).

In a longitudinal study over a 4-year period, Resnick et al. (2003) also reported a predominantly frontal decline in WM volume in a sample of 92 individuals aged 59–85 years at enrolment. This effect, however, disappeared when the analyses were limited to 24 very healthy individuals who remained free of medical condition and showed no cognitive impairment. This suggests that the higher sensitivity of the frontal region to changes in WM integrity may well reflect a pathological process rather than age-associated decline (Artero et al., 2004). Alternatively, discrepancy between *in vivo* and postmortem studies may also result from differences in boundary definition between anterior and posterior WM compartments.

4.2. Effects of age on grey matter subregions

Contrasting with the analyses performed on WM measurements, we found no effect of age on either the global GM measure or the discrete GM volumes reported to be prone to age-related changes. The greater sensitivity of frontal, compared to non-frontal, cortical regions to ageing processes reported in the majority of neuroimaging studies (e.g., DeCarli et al., 2005; Rettmann et al., 2006) was absent in this sample of very healthy individuals. These findings are consistent with the limited loss of neocortical neurons (~10%) reported between the age of 20 and 90 years (Pakkenberg and Gundersen, 1997). These results also confirm previous

work from our group that reported an age-related reduction in brain volume due to changes in WM, with GM remaining essentially unaffected by age (Double et al., 1996).

Similar to previous postmortem studies (e.g., Pakkenberg and Gundersen, 1997), we found no age effects on basal ganglia volumes. These results support the view that, when present, changes in these subcortical structures are pathognomonic of such neurodegenerative disorders as Parkinson's disease or Huntington's disease. Variable age-related changes in basal ganglia are reported in neuroimaging studies. Generally, these changes, if significant, are of a smaller magnitude than those seen in the neocortex (e.g., Walhovd et al., 2005; see also Raz and Rodrigue, 2006). The discrepancy between postmortem and *in vivo* findings likely arises from the difficulty in obtaining accurate measurements of small brain regions with automated parcellation methods and the greater risk of partial voluming due to voxel size and decreased signal-to-noise observed in MRI studies with ageing.

4.3. Can the differences between postmortem and MRI results be explained?

The reported discrepancy between postmortem and neuroimaging findings would suggest that the two bodies of results are incompatible. Several plausible explanations may resolve these differences. Although most MRI volumetric studies report GM changes with a frontal predominance, at least two longitudinal studies have demonstrated that these changes may be reduced, or even eliminated, when analyses are limited to very healthy subjects (Mueller et al., 1998; Resnick et al., 2003). These two studies, which included participants with a similar age range to this sample, suggested that the age-related changes reported in cross-sectional studies may have resulted from the inclusion of individuals with preclinical dementia and/or pathology. When such individuals were excluded, little brain loss was observed over time, even in individuals over the age of 85 years. This finding underlies the inherent difficulty that exists in investigating healthy ageing in an organ which may harbour neuropathological changes, such as seen in Alzheimer's disease, up to 30 years prior to the clinical manifestation of the disease (Braak and Braak, 1991). In this study, subjects were considered only if they had shown no sign of neurological or psychiatric disorder prior to death. In addition, the selected brains were included in the sample only after careful examination to ensure that no neuropathological changes were identified under the microscope.

Properties of MRI scanning also contribute to the differences reported between postmortem and MRI studies. MRI measurement accuracy is limited by image resolution (i.e., voxel size). Because a voxel contains a single tissue value only, voxels located on the edge of a brain structure (e.g., WM/GM boundary) contain signal from different tissue types, which will not be reflected in the voxel signal value. Such voxels may or may not be included in the measurement, resulting in partial voluming artefact. Thus, the

extent of partial voluming of voxels depends on voxel dimensions: the smaller the voxel size, the higher the resolution (and less partial voluming), resulting in increased accuracy of measurements. The risk of partial voluming is particularly pronounced for small brain structures (e.g., subcortical nuclei, hippocampus). Partial voluming was shown to contribute significantly to differences between postmortem and MRI cortical and subcortical volume measurements in pig and human brains (Garcia-Finana et al., 2003; Jelsing et al., 2005).

Even in healthy individuals, age-related cellular changes are known to affect the magnetic properties of brain tissues, which give rise to decreased signal-to-noise ratio and reduced GM/WM contrast on MRI. This reduced contrast between GM and WM tissues affects MRI volumetric measurements and may account in part for the differences between postmortem and *in vivo* brain volume measurements. The possible inclusion of *in vivo* cases with preclinical neurodegenerative cellular changes may also exaggerate the age contribution previously reported.

4.4. Strengths and limitations of the study

The major strength of this study lies in the quality of the sample. All selected subjects were carefully screened: they were free of any neuropathological changes at the time of death and all had been free of any significant neurological disorders during life. The application of stringent inclusion and exclusion criteria has allowed us to investigate the effects of age on brain structures in their own rights, that is independently from co-occurring effects of age-associated brain disorders. The size of this highly selected, and therefore, small sample may be perceived as a possible limitation to our results. It is also worth emphasising, however, that postmortem studies remain extremely labour intensive: they require hours of tissue preparation prior to any measurements taking place and, whilst very accurate, the point counting technique remains a slow and laborious process, particularly when measuring a number of discrete regions, as was the case in this study. These limitations certainly prevent the inclusion of large groups of subjects and, compared to most current MRI studies, which are limited only by hardware and software performance, the sample size may indeed appear small. Despite these limitations, the sample size and number of regions measured in this study compare very favourably, however, to other recent postmortem studies (Pakkenberg and Gundersen, 1997; Rabinowicz et al., 2002).

Previous MRI studies have reported significant age-related changes in the cerebellum (Paul et al., 2007; Raz et al., 2005). In this study, the cerebellum was not included in the analyses as it was not processed identically to the cerebrum with respect to slice thickness and orientation, preventing direct comparisons of cerebral and cerebellar findings. The presence of age-related cerebellar WM and GM changes in this sample can, therefore, not be ascertained.

In summary, this study reports age- and sex-related changes in brains spanning five decades and free from neuropathological changes. Our analyses of carefully defined 23 cortical regions, basal ganglia and WM volumes demonstrate much smaller changes with ageing than reported in imaging studies. The changes are found in the anterior and posterior WM to a similar degree with little evidence for substantive GM involvement. Although the discrepancy between these postmortem data and imaging results may in part be explained by the increase in noise of the neuroimaging data with ageing, our results suggest that healthy brain ageing is a process affecting more predominantly the white matter than the grey matter.

Conflicts of interest

All authors declare that they have no actual or potential conflicts of interest.

Acknowledgements

This research was supported by the National Health and Medical Research Council (NHMRC) of Australia, the Medical Foundation of the University of Sydney, the Australian Brain Foundation and Sandoz Foundation for Gerontological Research. O. Piguet is supported by an NHMRC Neil Hamilton Fairley Postdoctoral Fellowship (222909). G. Halliday and K.L. Double are Fellows of the NHMRC of Australia.

References

- Artero, S., Tiemeier, H., Prins, N.D., Sabatier, R., Breteler, M.M., Ritchie, K., 2004. Neuroanatomical localisation and clinical correlates of white matter lesions in the elderly. *J. Neurol. Neurosurg. Psychiatry* 75, 1304–1308.
- Bartzokis, G., Cummings, J.L., Sultzer, D., Henderson, V.W., Nuechterlein, K.H., Mintz, J., 2003. White matter structural integrity in healthy aging adults and patients with Alzheimer disease: a magnetic resonance imaging study. *Arch. Neurol.* 60, 393–398.
- Braak, H., Braak, E., 1991. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol.* 82, 239–259.
- de Leeuw, F.E., de Groot, J.C., Achten, E., Oudkerk, M., Ramos, L.M., Heijboer, R., Hofman, A., Jolles, J., van Gijn, J., Breteler, M.M., 2001. Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam scan study. *J. Neurol. Neurosurg. Psychiatry* 70, 9–14.
- DeCarli, C., Massaro, J., Harvey, D., Hald, J., Tullberg, M., Au, R., Beiser, A., D'Agostino, R., Wolf, P.A., 2005. Measures of brain morphology and infarction in the framingham heart study: establishing what is normal. *Neurobiol. Aging* 26, 491–510.
- Double, K.L., Halliday, G.M., Kril, J.J., Harasty, J.A., Cullen, K., Brooks, W.S., Creasey, H., Broe, G.A., 1996. Topography of brain atrophy during normal aging and Alzheimer's disease. *Neurobiol. Aging* 17, 513–521.
- Du, A.T., Schuff, N., Chao, L.L., Kornak, J., Jagust, W.J., Kramer, J.H., Reed, B.R., Miller, B.L., Norman, D., Chui, H.C., Weiner, M.W., 2006. Age effects on atrophy rates of entorhinal cortex and hippocampus. *Neurobiol. Aging* 27, 733–740.

- Garcia-Finana, M., Cruz-Orive, L.M., Mackay, C.E., Pakkenberg, B., Roberts, N., 2003. Comparison of MR imaging against physical sectioning to estimate the volume of human cerebral compartments. *Neuroimage* 18, 505–516.
- Halliday, G.M., Double, K.L., Macdonald, V., Kril, J.J., 2003. Identifying severely atrophic cortical subregions in Alzheimer's disease. *Neurobiol. Aging* 24, 797–806.
- Harasty, J., Double, K.L., Halliday, G.M., Kril, J.J., McRitchie, D.A., 1997. Language-associated cortical regions are proportionally larger in the female brain. *Arch. Neurol.* 54, 171–176.
- Harding, A.J., Halliday, G.M., Kril, J.J., 1998. Variation in hippocampal neuron number with age and brain volume. *Cereb. Cortex* 8, 710–718.
- Head, D., Buckner, R.L., Shimony, J.S., Williams, L.E., Akbudak, E., Conturo, T.E., McAvoy, M., Morris, J.C., Snyder, A.Z., 2004. Differential vulnerability of anterior white matter in nondemented aging with minimal acceleration in dementia of the Alzheimer type: evidence from diffusion tensor imaging. *Cereb. Cortex* 14, 410–423.
- Jelsing, J., Rostrup, E., Markenroth, K., Paulson, O.B., Gundersen, H.J., Hemmingsen, R., Pakkenberg, B., 2005. Assessment of in vivo MR imaging compared to physical sections in vitro—a quantitative study of brain volumes using stereology. *Neuroimage* 26, 57–65.
- Kril, J.J., Halliday, G.M., Svoboda, M.D., Cartwright, H., 1997. The cerebral cortex is damaged in chronic alcoholics. *Neuroscience* 79, 983–998.
- Madden, D.J., Whiting, W.L., Huettel, S.A., White, L.E., MacFall, J.R., Provenzale, J.M., 2004. Diffusion tensor imaging of adult age differences in cerebral white matter: relation to response time. *Neuroimage* 21, 1174–1181.
- Marner, L., Nyengaard, J.R., Tang, Y., Pakkenberg, B., 2003. Marked loss of myelinated nerve fibers in the human brain with age. *J. Comp. Neurol.* 462, 144–152.
- Mueller, E.A., Moore, M.M., Kerr, D.C.R., Sexton, G., Camicioli, R.M., Howieson, D.B., Quinn, J.F., Kaye, J.A., 1998. Brain volume preserved in healthy elderly through the eleventh decade. *Neurology* 51, 1555–1562.
- Pakkenberg, B., Gundersen, H.J., 1997. Neocortical neuron number in humans: effect of sex and age. *J. Comp. Neurol.* 384, 312–320.
- Pakkenberg, B., Pelvig, D., Marner, L., Bundgaard, M.J., Gundersen, H.J., Nyengaard, J.R., Regeur, L., 2003. Aging and the human neocortex. *Exp. Gerontol.* 38, 95–99.
- Paul, R., Grieve, S.M., Chaudary, B., Gordon, N., Lawrence, J., Cooper, N., Clark, C.R., Kukla, M., Mulligan, R., Gordon, E., 2009. Relative contributions of the cerebellar vermis and prefrontal lobe volumes on cognitive function across the adult lifespan. *Neurobiol. Aging* 30, 457–465.
- Peters, A., 2002. The effects of normal aging on myelin and nerve fibers: a review. *J. Neurocytol.* 31, 581–593.
- Pfefferbaum, A., Sullivan, E.V., Rosenbloom, M.J., Mathalon, D.H., Lim, K.O., 1998. A controlled study of cortical gray matter and ventricular changes in alcoholic men over a 5-year interval. *Arch. Gen. Psychiatry* 55, 905–912.
- Pfefferbaum, A., Adalsteinsson, E., Sullivan, E.V., 2005. Frontal circuitry degradation marks healthy adult aging: evidence from diffusion tensor imaging. *Neuroimage* 26, 891–899.
- Piguet, O., Ridley, L., Grayson, D.A., Bennett, H.P., Creasey, H., Lye, T.C., Broe, G.A., 2003. Are MRI white matter lesions clinically significant in the 'old-old'? Evidence from the Sydney older persons study. *Dement. Geriatr. Cogn. Disord.* 15, 143–150.
- Rabinowicz, T., Petetot, J.M., Gartside, P.S., Sheyn, D., Sheyn, T., de, C.M., 2002. Structure of the cerebral cortex in men and women. *J. Neuropathol. Exp. Neurol.* 61, 46–57.
- Raz, N., Gunning-Dixon, F., Head, D., Rodrigue, K.M., Williamson, A., Acker, J.D., 2004. Aging, sexual dimorphism, and hemispheric asymmetry of the cerebral cortex: replicability of regional differences in volume. *Neurobiol. Aging* 25, 377–396.
- Raz, N., 2005. The aging brain observed in vivo: differential changes and their modifiers. In: Cabeza, R., Nyberg, L., Park, D.C. (Eds.), *Cognitive Neuroscience of Aging: Linking Cognitive and Cerebral Aging*. Oxford University Press, New York.
- Raz, N., Lindenberger, U., Rodrigue, K.M., Kennedy, K.M., Head, D., Williamson, A., Dahle, C., Gerstorf, D., Acker, J.D., 2005. Regional brain changes in aging healthy adults: general trends, individual differences and modifiers. *Cereb. Cortex* 15, 1676–1689.
- Raz, N., Rodrigue, K.M., 2006. Differential aging of the brain: patterns, cognitive correlates and modifiers. *Neurosci. Biobehav. Rev.* 30, 730–748.
- Resnick, S.M., Pham, D.L., Kraut, M.A., Zonderman, A.B., Davatzikos, C., 2003. Longitudinal magnetic resonance imaging studies of older adults: a shrinking brain. *J. Neurosci.* 23, 3295–3301.
- Rettmann, M.E., Kraut, M.A., Prince, J.L., Resnick, S.M., 2006. Cross-sectional and longitudinal analyses of anatomical sulcal changes associated with aging. *Cereb. Cortex* 16, 1584–1594.
- Scahill, R.I., Frost, C., Jenkins, R., Whitwell, J.L., Rossor, M.N., Fox, N.C., 2003. A longitudinal study of brain volume changes in normal aging using serial registered magnetic resonance imaging. *Arch. Neurol.* 60, 989–994.
- Smith, C.D., Chebrolu, H., Wekstein, D.R., Schmitt, F.A., Markesbery, W.R., 2007. Age and gender effects on human brain anatomy: a voxel-based morphometric study in healthy elderly. *Neurobiol. Aging* 28, 1075–1087.
- Sullivan, E.V., Pfefferbaum, A., 2003. Diffusion tensor imaging in normal aging and neuropsychiatric disorders. *Eur. J. Radiol.* 45, 244–255.
- Sullivan, E.V., Marsh, L., Pfefferbaum, A., 2005. Preservation of hippocampal volume throughout adulthood in healthy men and women. *Neurobiol. Aging* 26, 1093–1098.
- Van Petten, C., 2004. Relationship between hippocampal volume and memory ability in healthy individuals across the lifespan: review and meta-analysis. *Neuropsychologia* 42, 1394–1413.
- Walhovd, K.B., Fjell, A.M., Reinvang, I., Lundervold, A., Dale, A.M., Eilertsen, D.E., Quinn, B.T., Salat, D., Makris, N., Fischl, B., 2005. Effects of age on volumes of cortex, white matter and subcortical structures. *Neurobiol. Aging* 26, 1261–1270, discussion 1275–1268.