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# Effects of age on tissues and regions of the cerebrum and cerebellum

Terry L. Jernigan[a,b,f]*, Sarah L. Archibald[b], Christine Fennema-Notestine[b], Anthony C. Gamst[c], Julie C. Stout[d], Julie Bonner[e], John R. Hesselink[a,f]

[a]*Veterans Affairs San Diego Healthcare System, San Diego, CA, USA*
[b]*Brain Image Analysis Laboratory, Department of Psychiatry, University of California, San Diego, CA, USA*
[c]*Department of Neurosciences, University of California, San Diego, CA, USA*
[d]*Department of Psychology, Indiana University, Bloomington, IN, USA*
[e]*Chicago Medical School, North Chicago, IL, USA*
[f]*Department of Radiology, University of California, San Diego, CA, USA*

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**Abstract**

Normal volunteers, aged 30 to 99 years, were studied with MRI. Age was related to estimated volumes of: gray matter, white matter, and CSF of the cerebrum and cerebellum; gray matter, white matter, white matter abnormality, and CSF within each cerebral lobe; and gray matter of eight subcortical structures. The results were: 1) Age-related losses in the hippocampus were significantly accelerated relative to gray matter losses elsewhere in the brain. 2) Among the cerebral lobes, the frontal lobes were disproportionately affected by cortical volume loss and increased white matter abnormality. 3) Loss of cerebral and cerebellar white matter occurred later than, but was ultimately greater than, loss of gray matter. It is estimated that between the ages of 30 and 90 volume loss averages 14% in the cerebral cortex, 35% in the hippocampus, and 26% in the cerebral white matter. Separate analyses were conducted in which genetic risk associated with the Apolipoprotein E $\epsilon$4 allele was either overrepresented or underrepresented among elderly participants. Accelerated loss of hippocampal volume was observed with both analyses and thus does not appear to be due to the presence of at-risk subjects. MR signal alterations in the tissues of older individuals pose challenges to the validity of current methods of tissue segmentation, and should be considered in the interpretation of the results. 

*Keywords:* Aging; Human; Magnetic resonance imaging; Gray matter; White matter; White matter abnormality; Cerebrum; Cerebellum; Hippocampus; Frontal lobes; Apolipoprotein E $\epsilon$4 allele

## 1. Introduction

Early autopsy studies of neurologically normal cases revealed age-related decreases in brain weight [10,17] and brain volume [9,18]; and provided evidence that such decreases were accompanied by some degree of neuronal loss in cerebral cortex, brain stem structures, and basal ganglia [5,6,32]. Magnetic resonance imaging (MRI) of the brain was the first method with sufficient resolution to permit investigation of age-related change in individual cortical and subcortical structures in living human subjects. MRI reveals evidence of volume loss, in the form of increased cerebrospinal fluid (CSF) spaces, in many older, medically-normal individuals. We previously described age-related brain morphologic changes measured with MR morphometric techniques in subjects ranging in age from 30 to 79 years. Increasing age was associated with increasing ventricular and cortical sulcal CSF and with substantial reduction in both cortical and subcortical compartments of the gray matter. Cortical gray matter losses occurred in widespread regions, including the structures on the mesial surface of the temporal lobe. Subcortical gray matter losses were also observed, particularly in the striatum. The prevalence of signal hyperintensities within the white matter increased in the older subjects; however, we did not observe a significant decrease in the volume of the cerebral white matter [19,22].

Numerous other studies have applied MR morphometric techniques in studies of normal aging. These have confirmed volume decreases in cortical gray matter [2,31,33, 34,39], cerebral hemispheres [7,8], and basal ganglia structures [27,30,35], and increases in volumes of [15,28], or

* Corresponding author. Tel.: +1-858-622-5882; fax: +1-858-622-5890.

*E-mail address:* tjernigan@ucsd.edu (T.L. Jernigan).

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Table 1
Demographic Information: Mean (Std. Dev.)

| | *N* | Age | Education |
|---|---|---|---|
| All subjects | 78 | 62.9 (18.0) range 30–99 | 15.4 (2.7) range 10–20 |
| Men | 37 | 61.4 (18.8) range 30–89 | 16.0 (2.2) range 12–20 |
| Women | 41 | 64.2 (17.4) range 32–99 | 14.9 (3.0) range 10–20 |

Table 2
Apolipoprotein E ϵ4 allele carriers in the sample by age group

| APOE E4 Status | Age groups | | | |
|---|---|---|---|---|
| | 30–64 | 65–79 | 80–99 | Totals |
| Not genotyped | 29 (88%) | 2 (6.5%) | 4 (28.5%) | 35 (45%) |
| No E4 | 0 | 18 (58%) | 4 (28.5%) | 22 (28%) |
| Heterozygotes | 3 (9%) | 11 (35.5%) | 6 (43%) | 20 (26%) |
| Homozygotes | 1 (3%) | 0 | 0 | 1 (1%) |
| Totals | 33 (42%) | 31 (40%) | 14 (18%) | 78 (100%) |

odds of [8], signal hyperintensities in the white matter. In some studies, such as our previous study, white matter volume did not appear to change with age [2,19,33]. However, results of some recent studies suggest that significant white matter volume loss does occur, at least in some cerebral regions [15,34,36]. The present study extends our earlier work by: examining a larger age range; providing detailed volumetric characterization of multiple subcortical and limbic structures; providing lobar measures of cortical gray matter, underlying white matter, and signal abnormalities; providing measures of cerebellar gray and white matter; and applying statistical techniques for comparing the age-effects in different regions. In addition, specific problems associated with tissue segmentation in older individuals are highlighted and discussed.

## 2. Methods

### *2.1. Subjects*

Seventy-eight normal adult volunteers (41 women and 37 men, ranging in age from 30 to 99 years—mean 64, s.d. 17.4) were examined for age-associated changes in regional brain volumes. Of the seventy-eight individuals, 71 identified themselves as Caucasian, 3 as Hispanic, 3 as African-American, and 1 individual has unknown racial/ethnic identity. All subjects were screened for any current and significant medical, psychiatric, intellectual, or neurological disorders. Middle-aged and elderly subjects underwent physical examination, had normal cognitive function, and were living independently. Common medical conditions of the elderly, such as hypertension and cardiac conditions, were not exclusions if the subjects were stable and their conditions were considered to be well controlled with medications. Subjects were excluded if screening resulted in any history consistent with stroke, or if they scored above 4 on a modified Hachinski Ischemia Scale [16]. The subject characteristics are summarized in Table 1.

Among the elderly subjects, 43 were followed within the University of California, San Diego, Alzheimer's Disease Research Center (ADRC) and were genotyped for the presence of the Apolipoprotein E ϵ4 allele: 21 were ϵ4+ (20 heterozygotes and 1 homozygote for the Apo E ϵ4 allele) and 22 were ϵ4−. Thus genetic risk for Alzheimer's Disease (AD) was more prevalent in the elderly subjects within this subset of our elderly subjects than in the general population. The genotype information available for the full sample is summarized in more detail in Table 2. The study of genetic risk factors, per se, is a high priority within the ADRC and the center attempts to maintain a normal control population in which adequate numbers of at-risk individuals are available for such studies. We selected a sample of 21 of the ϵ4+ subjects from the ADRC so that we could compare directly the brain morphology of ϵ4+ with matched ϵ4− volunteers from the ADRC. This resulted in overrepresentation of genetic risk within our full sample relative to the general population. It should be noted that non-ADRC participants of the present study (representing 45% of the sample) were screened using similar criteria to those applied in the ADRC, but no genotyping was performed for this group, and therefore there is presumably no overrepresentation of genetic risk in these subjects relative to rates in the general population.

As described in a separate report [12], comparisons of the two matched subgroups of elderly ADRC subjects, with and without genetic risk, suggested that they were remarkably similar on most measures examined in the present study. The ϵ4+ subjects did appear to have proportionately less subcortical gray matter than did the ϵ4− subjects. This difference was due primarily to lower estimated volumes of the lenticular nucleus in the ϵ4+ subjects relative to the ϵ4− subjects. Subsequent investigation suggested that this apparent difference in lenticular nucleus volume may be illusory. It is likely that the presence of signal elevation surrounding the vessels that course through and are adjacent to the putamen and pallidum leads to inflation of lenticular nucleus volume estimates in some individuals. Such inflation of lenticular volumes appeared to occur more frequently in the ϵ4− individuals. Because the elevated signal appears to surround vessels, blood pressure and history of clinically diagnosed hypertension were investigated as a possible source of this group difference. Forty percent of the ϵ4+ group ($N = 8$) and only 15% of the ϵ4− group ($N = 3$) had a positive history of hypertension ($t = 1.8$; $p = .08$), although the groups did not differ on diastolic or systolic blood pressure readings taken near the time of the scan (ϵ4− 137.7 (15.1)/75.3 (10.1); ϵ4+ 133.3 (11.5)/78.3 (6.8)). Hypertension, then, did not appear to be the primary cause of greater abnormal signals in the ϵ4− group. There-

fore, the conclusion was that there was no evidence for advanced neurodegeneration in our sample of $\epsilon 4+$ subjects, although subtle differences in brain structure may exist between these subgroups of the normal population. It should be emphasized that all of the genotyped older subjects examined in the present study were fully assessed and were cognitively normal at the time of imaging ($\epsilon 4-$: Dementia Rating Scale = 141.2 (2.8), Mini-Mental State Examination = 29.7 (0.6); $\epsilon 4+$: Dementia Rating Scale = 140.4 (2.5), Mini-Mental State Examination = 29.4 (0.8)).

### 2.2. Imaging protocol

Three whole-brain image series were collected for each subject. The first was a gradient-echo (SPGR) T1-weighted series with TR = 24 ms, TE = 5 ms, NEX = 2, flip angle = 45 degrees, field of view of 24 cm, and contiguous 1.2 mm sections. The second and third series were fast spin-echo (FSE) acquisitions yielding two separate image sets: TR = 3000 ms, TE = 17 ms, ET = 4 and TR = 3800 ms, TE = 102 ms, ET = 8. For all series, the field of view was 24 cm. Section thickness for the FSE series was 4 mm, no gaps (interleaved).

### 2.3. Image analysis

The image-analytic approach is similar to that used in our previous anatomical studies [19,20,22], but represents a significant elaboration of these methods as described previously [21]. Trained anatomists who were blind to subject diagnosis, age, gender or any other identifying information subjected each image dataset to the following image analysis procedures: 1) interactive isolation of intracranial regions from surrounding extracranial tissue, 2) three-dimensional digital filtering of the matrix of pixel values representing brain voxels to reduce inhomogeneity artifact, 3) reslicing of the volume to a standard orientation, 4) tissue segmentation using semi-automated algorithms, and 5) neuroanatomical region-of-interest analysis. These procedures were performed on the lower resolution FSE image volumes; however, the spatially registered SPGR volumes were visualized throughout the analyses to guide the operators. Each procedure is described briefly below.

Brain is first isolated from extracranial areas in the images, i.e., from surrounding tissue that is in some instances contiguous with brain tissue and similar in signal value. This process results in a new volume within which the positions of brain voxels are coded, i.e., a mask. This "stripping" method was applied to 6 pairs of image volumes (i.e., to 12 separate volumes) and the within-pair discrepancies were examined. Each pair represented 2 FSE volumes obtained on different occasions in the same individual. Discrepancies in brain volume were small, ranging from .03% to 1.25% with a mean of .54%.

Filtering is applied to reduce nonbiological signal drift across the field of view, which is presumably due to field inhomogeneity and susceptibility effects. A three-dimensional, high-pass filter is applied, with two iterations, separately to the "stripped" proton density (PD) weighted and T2-weighted FSE image volumes (i.e., the filter is applied to the "brain" voxels only). First, a roughly cubic near-neighbor averaging filter is applied to produce a smoothed dataset; then the original volume is divided by the smoothed dataset on a voxel-by-voxel basis; and finally each voxel value is multiplied by the mean voxel value of the original dataset. The dimensions of the cubic smoothing filter were chosen by subjective evaluation of the results obtained with a series of filter sizes and were set at approximately 30 mm. That is, the set of voxels averaged to create each voxel value in the smoothed dataset spans 33 voxels in the x and y directions, and 7 voxels in the z direction (i.e., it measures 31 mm $\times$ 31 mm $\times$ 28 mm). This method is a 3D elaboration of the 2D filtering method used in our previous anatomical studies.

After filtering, the image datasets are resectioned in a standard coronal plane defined relative to the decussations of the anterior and posterior commissures and the structural midline. Registration of the T1-weighted and spin-echo data sets is accomplished so that registered sections from all three data sets are available to the operators when attempting to resolve anatomical boundaries.

The tissue classification procedure is an interactive, supervised process. Operators manually designate the positions of three sets of tissue samples, one for each of the target tissues (gray, white, and CSF), on the resectioned images. The goals are to obtain samples in standard anatomical locations, within regions of homogeneous tissue; and to avoid artifacts and tissue abnormalities (such as ischemic damage). Samples are selected in locations that appear to be homogeneous and free of signal abnormalities both in the section sampled and in the adjacent sections. In most cases, the operators select samples in 6 gray matter locations (bilaterally in the caudate nucleus, putamen, and the pulvinar of the thalamus); in 4 white matter locations (bilaterally in the suprasylvian white matter at the level of the pulvinar, and in similar locations at the level of the caudate/putamen); and in 4 locations within CSF-filled structures (2 samples are taken within the frontal horns, and 2 more posterior samples are taken at approximately the level of the trigones of the cerebral ventricles). The sample voxel values are then analyzed using simple regression techniques to separate first all brain parenchymal voxels from CSF voxels, and then gray matter voxels from white matter voxels. The regression coefficients obtained in these simple analyses are then applied to classify each voxel within the volume as most similar to CSF, gray matter, or white matter. Two anatomists, working independently, applied the tissue classification method to 11 brain MRI volumes. Interoperator reliability estimates were .92 for white matter volume, .95 for gray matter volume, and .99 for CSF volume. Furthermore, scan-rescan reliability was estimated by applying the segmentation procedures independently and

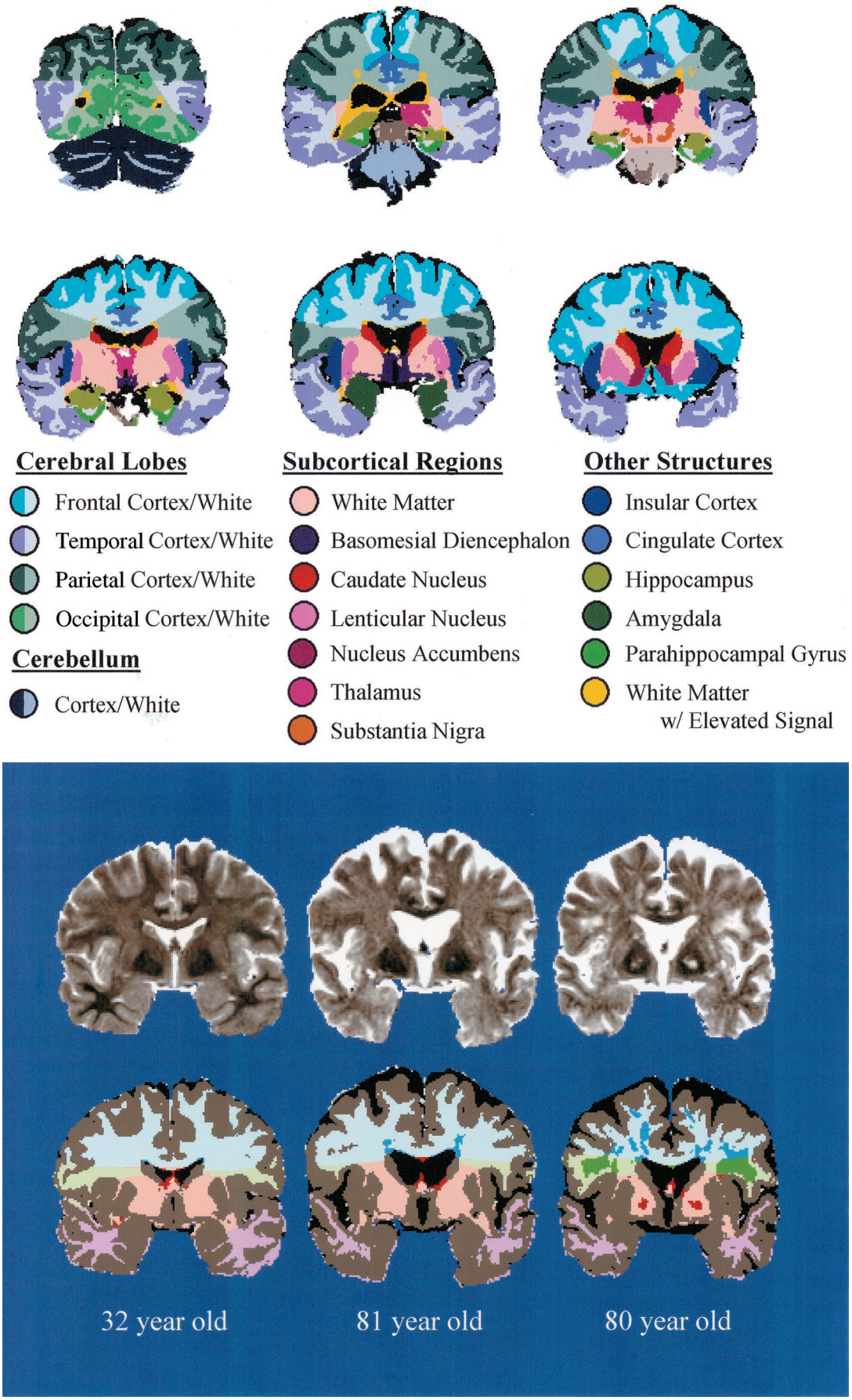
Cerebral Lobes
Frontal Cortex/White
Temporal Cortex/White
Parietal Cortex/White
Occipital Cortex/White
Cerebellum
Cortex/White
Subcortical Regions
White Matter
Basomesial Diencephalon
Caudate Nucleus
Lenticular Nucleus
Nucleus Accumbens
Thalamus
Substantia Nigra
Other Structures
Insular Cortex
Cingulate Cortex
Hippocampus
Amygdala
Parahippocampal Gyrus
White Matter
w/ Elevated Signal
32 year old
81 year old
80 year old

Fig. 1. Example of fully processed images showing structural boundaries. All white matter voxels with elevated signal are shown in yellow; however, the locations of such voxels within different lobes are also coded as shown in Figure 2.

blindly to 11 pairs of image volumes from individuals imaged twice. Scan-rescan reliability estimates for the white matter, gray matter, and CSF volumes were .94, .94, .99, respectively.

Anatomists circumscribe regions on the tissue-segmented images. Standardized rules are applied for delineating a set of subcortical structures and cortical regions. Subcortical structures include the cerebral ventricles, the caudate nucleus, the nucleus accumbens, the lenticular nucleus, the thalamus, the substantia nigra, and a region referred to as basomesial diencephalon (which includes septal nuclei, mamillary bodies and other hypothalamic structures, the bed nucleus of the stria terminalis, and the diagonal band of Broca). Cortical regions include the temporal lobe, frontal lobe, parietal lobe, occipital lobe, cingulate cortex, and insular cortex. Separate measures are obtained of three mesial temporal lobe structures: the hippocampus, the amygdala and adjacent entorhinal and perirhinal cortex (amygdala +), and the parahippocampal gyrus. The four major cortical lobes are drawn to include cortical gray matter, underlying white matter, and CSF. Volumes of each tissue are estimated separately within each lobe, and white matter and CSF volumes are also measured in a deep subcortical zone not within any of the cortical lobes. Gray matter and adjacent CSF of the cingulate cortex and insular cortex are defined separately. Representative fully-processed images from a normal brain, illustrating the regional boundaries of the measured brain structures, are shown in Fig. 1. Region of interest analysis of 10 tissue-segmented brain datasets was performed independently by two anatomists. Interoperator reliability for estimated volumes of the 15 primary gray matter structures ranged from .85 to .99, with reliability for most measures exceeding .95.

As described above, cerebral white matter was subdivided into the four major cerebral lobes and a deep zone surrounding the basal ganglia and diencephalon. Within these regions, some voxels have signal values that exceed those of the voxels selected for the samples of normal white matter, i.e., they fall in the distribution of signal values obtained from gray matter voxels. These voxels are classified with our tissue segmentation methods as "gray matter", but are isolated from true gray matter voxels in the anatomical analysis described above. Such voxels (in white matter regions) with elevated signal values are coded as abnormal white matter and summed separately for each white matter region. Fig. 2 illustrates the boundaries of the white matter regions and the appearance of such voxels within these regions in one younger subject and two selected older subjects from the present study.

It should be noted that all voxels with signal values equal to or exceeding those of normal gray matter are classified as "gray matter" with the present methods. When such voxels are located within the normal boundaries of gray matter structures they are included in those structures, even if they appear to be hyperintense on one or more of the registered MR images. This may in some instances lead to inflation of volume estimates of the affected gray matter structures. This problem will be discussed further below. To determine whether voxels with elevated signal are within white matter or gray matter structures, anatomists examine both flanking sections as well as the section in question. The goal is to determine whether the voxels could represent a gray matter structure (such as a cortical gyrus or sulcus) which is not readily identifiable in the present section but is partially volumed within that section (i.e., present in the same location on a flanking section). Only if such voxels are present in locations that could not plausibly be gray matter structures (i.e., they are clearly within white matter regions) are the voxels then coded as white matter abnormality. It should be noted, however, that such voxels include those associated with diffuse white matter pallor and enlarged peri-vascular spaces as well as frank white matter hyperintensities.

### 2.4. Statistical methods

The relationship between age and the following tissue volumes was examined: cortical gray matter volumes of the major lobes, the insula, the cingulate, and the parahippocampal gyrus; gray matter volume of the cerebellum; volumes of the gray matter of the hippocampus, amygdala+, thalamus, basomesial diencephalon, caudate nucleus, lenticular nucleus, nucleus accumbens, and substantia nigra; white matter volumes (including white matter with elevated MR signal values) of the four major lobes, of the cerebellum, and of the deep subcortical zone; the volumes of white matter tissue having elevated MR signal values in the major lobes and the deep subcortical zone; and the volumes of cortical sulcal CSF, ventricular CSF, and cerebellar CSF. Initial examination of plots of tissue volumes against participants' ages suggested that changes in some tissue compartments were nonlinear, but apparently monotone. Given this, linear regression was deemed of questionable validity for assessing the age effects. Instead, kernel smoothing was

Fig. 2. Example of T2 weighted images (top row) and associated segmented images (bottom row) for three different subjects: from left to right, a 32 year old, an 81 year old, and an 80 year old. The boundaries of the white matter regions are color-coded and voxels with elevated signal are shown in a darker shade. Parietal lobe = green; frontal lobe = blue; temporal lobe = purple; deep subcortical region = red.

used to produce a nonparametric estimate of the monotone regression function of volume on age [25], and the strength of association was measured using the Spearman correlation coefficient. The latter was chosen in part because it is relatively robust to the effects of leverage and outliers, and in part because it is sensitive to any monotone association between the regression variables (here: volume or relative volume and age). The volumes were proportionalized to the total volume of the cranial vault (supratentorial vault for cerebral measures and infratentorial vault for cerebellar measures) to control for individual differences in head size. This was deemed appropriate given that there was no expected, nor was there evidence for, any association between head size and age in this adult sample.

Additional analyses were performed to compare the rates of change with age in volume between various regions and tissue types. For these analyses, one takes the ratio of the two volumes for each subject and computes the Spearman correlation between this ratio and age. A large Spearman correlation coefficient indicates that one of the volumes changes more rapidly than the other over some portion of the age range and no less rapidly over the other portions. It is worth noting that these volume ratios (and hence the results of the correlation analysis) are independent of whether or not one uses the proportionalized or non-proportionalized measures (because, within a single subject, the proportionalization constant is the same for both volumes)—the choice of which volume is numerator and which denominator also has no effect (because we are using a rank-based correlation measure). The analysis also has the advantage that the validity of the results is not influenced by inhomogeneities of variance either between the two measures compared or across the age-range.

## 3. Results

Estimates of the strength of (monotone) association between age and the gray matter volumes are given in Table 3. There is strong evidence for age-related decline in the volume of the cerebral cortex, and this appears to be particularly pronounced in frontal cortex. Indeed the secondary analyses comparing rates of decline in cortical regions suggest that the decline in frontal cortex volume is significantly faster than is the decline in parietal lobe or insular cortex (rho's: frontal vs. parietal, .33, $p < .05$; frontal vs. insular, .38, $p < .05$). There is also definite evidence for loss of cerebellar gray matter volume.

The results suggest moderate age-related loss of total subcortical gray matter in this sample; however, age effects on individual structures show wide variability. Modest age-related volume loss is observed in the caudate nucleus and nucleus accumbens; however no significant age-related change was detectable in amygdala, diencephalic structures, lenticular nucleus, or substantia nigra. In striking contrast to this is the rapid age-related loss measured in the hippocampus. Indeed comparisons of rate of decline in the hippocampus with that in other structures suggests that it declines significantly more rapidly in volume than any cortical volume, even the frontal cortex volume (rho for hippocampal volume vs. frontal cortex volume: .50, $p < .001$).

Table 3
Correlations of proportionalized gray matter volumes with age (Spearman's rho)

| Gray matter reductions | | | |
|---|---|---|---|
| Cerebral cortex | −.53# | Subcortical structures | −.40# |
| Frontal cortex | −.56# | Hippocampus | −.65# |
| Parietal cortex | −.21 | Amygdala | −.08 |
| Occipital cortex | −.33* | Thalamus | −.13 |
| Cingulate cortex | −.33* | Basomesial diencephalon | −.21 |
| Temporal neocortex | −.43# | Caudate nucleus | −.35* |
| Parahippocampal | −.38* | Lenticular nucleus | .00 |
| Insular cortex | −.20 | Nucleus accumbens | −.33* |
| | | Substantia nigra | .13 |
| Cerebellar Gray | −.56# | | |

* $p < .05$.
# $p < .001$.

Significantly accelerated age-related loss in the hippocampus relative to that in other gray matter regions raised further concern about the over-representation of $\epsilon 4+$ subjects within the elderly participants in the present study. It was feared that the striking hippocampal changes could somehow be related to the high proportion of at-risk subjects in the sample, in spite of the lack of $\epsilon 4$-related differences in the hippocampal volumes in the matched-group comparisons. Therefore the associations of age with the hippocampal volume and the cerebral cortex volume were re-computed, removing all $\epsilon 4+$ subjects from the analysis. Note that this results in a sample in which genetic risk is *underrepresented* among the elderly subjects relative to unselected community samples. The strength of the monotone association between age and both volumes remained very high (rhos = −.67 for both measures, $p < .001$). Most importantly, the comparison of rate of decline of the two volumes again strongly suggests accelerated decline in the hippocampus relative to that in the cerebral cortex (rho for hippocampal volume vs. frontal cortex volume: .55, $p < .001$).

The age-related changes in the white matter measures are summarized in Table 4. Volume decreases in white matter in both cerebrum and cerebellum were highly significant, as were increases in the volume of cerebral white matter with elevated MR signal values.

Comparisons of the rate of decline in gray and white matter suggest that for both the cerebrum and the cerebellum, the rate of decline is significantly more rapid in white matter than in gray matter (rho's: cerebrum, .35, $p < .05$; cerebellum, .27, $p < .05$).

Comparisons of rate of decline of white matter volume in different cerebral regions suggests that the white matter of all four major lobes declines more rapidly than does the

Table 4
Correlations of proportionalized white matter measures with age (Spearman's rho)

| White matter changes | | |
|---|---|---|
| | Proportional volume of white matter | Proportional volume of white matter signal elevation |
| Cerebral white matter | −.63# | .76# |
| Frontal lobe | −.61# | .79# |
| Parietal lobe | −.63# | .67# |
| Occipital lobe | −.57# | .57# |
| Temporal lobe | −.48# | .69# |
| Deep subcortical | −.35* | .53# |
| Cerebellar white matter | −.49# | — |

* $p < .05$.
# $p < .001$.

white matter of the deep subcortical region (rho's: temporal lobe, .32, $p < .05$; frontal lobe, .48, $p < .001$; parietal lobe, .39, $p < .05$; occipital lobe, .49, $p < .001$). Increase in white matter hyperintensity is also more rapid in the white matter of the cerebral lobes than in the deep subcortical region (rho's: temporal lobe, .45, $p < .001$; frontal lobe, .67, $p < .001$; parietal lobe, .54, $p < .001$; occipital lobe, .44, $p < .001$). In addition, the increase in frontal lobe hyperintensity is more rapid than that in temporal and parietal lobes (rho's: frontal vs. temporal lobe, .50, $p < .001$; frontal vs. parietal lobe, .34, $p < .05$) but not than in the occipital lobe.

Finally, the increases in CSF compartments are presented in Table 5. All three CSF compartments show rapid increases with age. There was no evidence for a significant difference in the rate of change in sulcal and ventricular volumes.

## 4. Discussion

Although, ideally, models of the effects of advancing age on the brain would be based on observed changes over time in individual subjects, in practice, longitudinal methods are attended by a number of significant logistical complications. The cross-sectional data presented here describe differences between subjects of different ages, not age changes *per se*. We are therefore attempting to extrapolate age-changes from age-differences. The validity of such functions based on age-differences depends upon the absence of any unintended differences between age cohorts that may have structural or functional consequences. For example, if differences in body size (and hence brain size) exist between age cohorts then a difference in brain size between younger and older subjects could be interpreted as shrinkage when in fact none occurs. Modeling of age-changes from age-differences, therefore, should always be done cautiously. These concerns notwithstanding, these results describe, in greater detail than was previously available, gross structural age-differences in gray and white matter tissue compartments of both the infratentorial and supratentorial cranial vaults. Apparent age-related volume loss in gray and white tissue compartments was observed, as were marked increases in CSF and in the volume of white matter exhibiting signal elevation on MR. Linear estimates of regional volume changes are presented in Table 6. Furthermore, these results suggest significant variability in the rate of change across different tissue compartments, and across different regions within each compartment.

Table 5
Correlations of proportionalized CSF measures with age (Spearman's rho)

| Increases in cerebrospinal fluid | |
|---|---|
| Cortical sulcal CSF | .83# |
| Cerebral ventricular CSF | .74# |
| Cerebellar CSF | .75# |

# $p < .001$.

### 4.1. Regional variability in gray matter loss

The estimated volumes of cerebral cortex are plotted against age in Fig. 3. In this figure, the best fitting monotone smooth is drawn through the points. It should be noted that the values obtained for the volume of the cerebral cortex in this sample closely resemble those obtained by Hubbard and Anderson [18] when they measured the volumes of the entire cerebral cortex in the brains of 19 individuals, ranging in age from 23 to 95, examined at autopsy. They expressed their values, which ranged from .45 to .39, as proportions of the volume of the total cranial vault. The average comparable value of our cerebral cortex volume in 30 year olds (expressed as a proportion of total cranial vault) is .45 and the average for 90 year olds is .40. Note that the proportional values provided in the figure are expressed relative to volume of the supratentorial cranial vault, not total cranial vault (including the infratentorial vault) as was used by Hubbard and Anderson.

Comparison of linear estimates of the volume of the cortex at age 30 and age 90 suggests loss of approximately 14% over this age range (Table 6). The most rapid loss of gray matter volume was observed in the hippocampus, shown plotted against age in Fig. 4. Linear estimates of the volume of this structure at ages 30 and 90 suggest that 35.5% of the structure's volume is lost (Table 6). Statistically, the rate of loss in this structure exceeds that of the cortex. Comparison of the plots reveals that this is due to particularly steep decline in the oldest subjects in the sample.

The present findings represent the first systematic comparison of rate of volume loss in hippocampus and neocortex. The finding of accelerated loss in hippocampus is somewhat surprising, given the inconsistency of previous results regarding the degree of age-related loss present in this

Table 6
Linear estimates of regional volume changes from age 30 to 90. STCN = Supratentorial cranial vault. Note that the % change of STCN values for the measures in rows marked by an asterisk in the table sum to zero; i.e., the estimated losses in gray and white matter are equal in amount to the estimated increases in cerebrospinal fluid

| Region | Proportion of STCN | | % Change of STCN | % Change of 30 yr old volume |
|---|---|---|---|---|
| | $X$ = 30 yrs | $X$ = 90 yrs | | |
| *All gray matter | .569225 | .491105 | −7.8 | −13.7 |
| Cortical gray | .537652 | .461572 | −7.6 | −14.2 |
| Hippocampal gray | .009292 | .005992 | −0.3 | −35.5 |
| Subcortical gray | .031485 | .029565 | −0.2 | −6.1 |
| *White matter | .387492 | .284952 | −10.2 | −26.5 |
| Elevated white | .004852 | .029872 | +2.5 | +516 |
| *Cortical fluid | .035821 | .179461 | +14.4 | +400 |
| *Ventricular fluid | .007126 | .042886 | +3.6 | +500 |

structure. Sullivan et al. [39] reported no age-related loss in hippocampus, while highly significant losses were reported by Murphy et al. [31]; and clinical ratings of peri-hippocampal fluid increases also suggested age-related hippocampal atrophy [13]. Although the sample sizes were similar for these studies, the age-range examined was extended in the latter two studies, as it was in the present study, relative to the former. Thus, hippocampal loss in the relatively younger sample of Sullivan et al. [39], may have been more modest. This is consistent with the apparent acceleration of hippocampal loss in the oldest subjects of the present study.

The increased prevalence of Apo E $\epsilon$4+ subjects among the elderly subjects of this sample raises concerns that the rate of hippocampal loss observed in the present study may

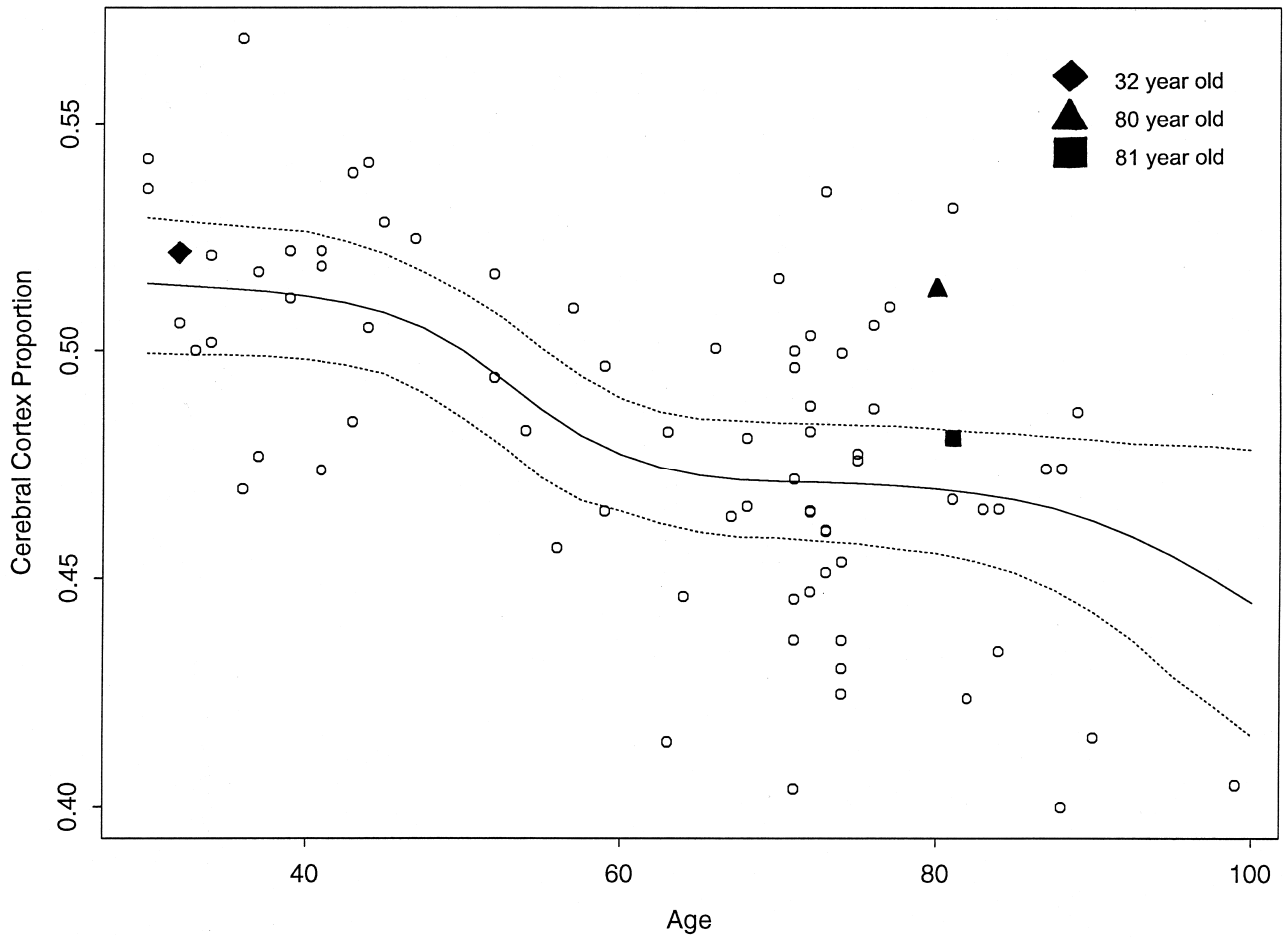


Fig. 3. Proportionalized estimated volumes of cerebral cortex by age with monotone smooth (—) and variability bands (····). Values for the 3 subjects shown in Figure 2 are coded with distinctive symbols.

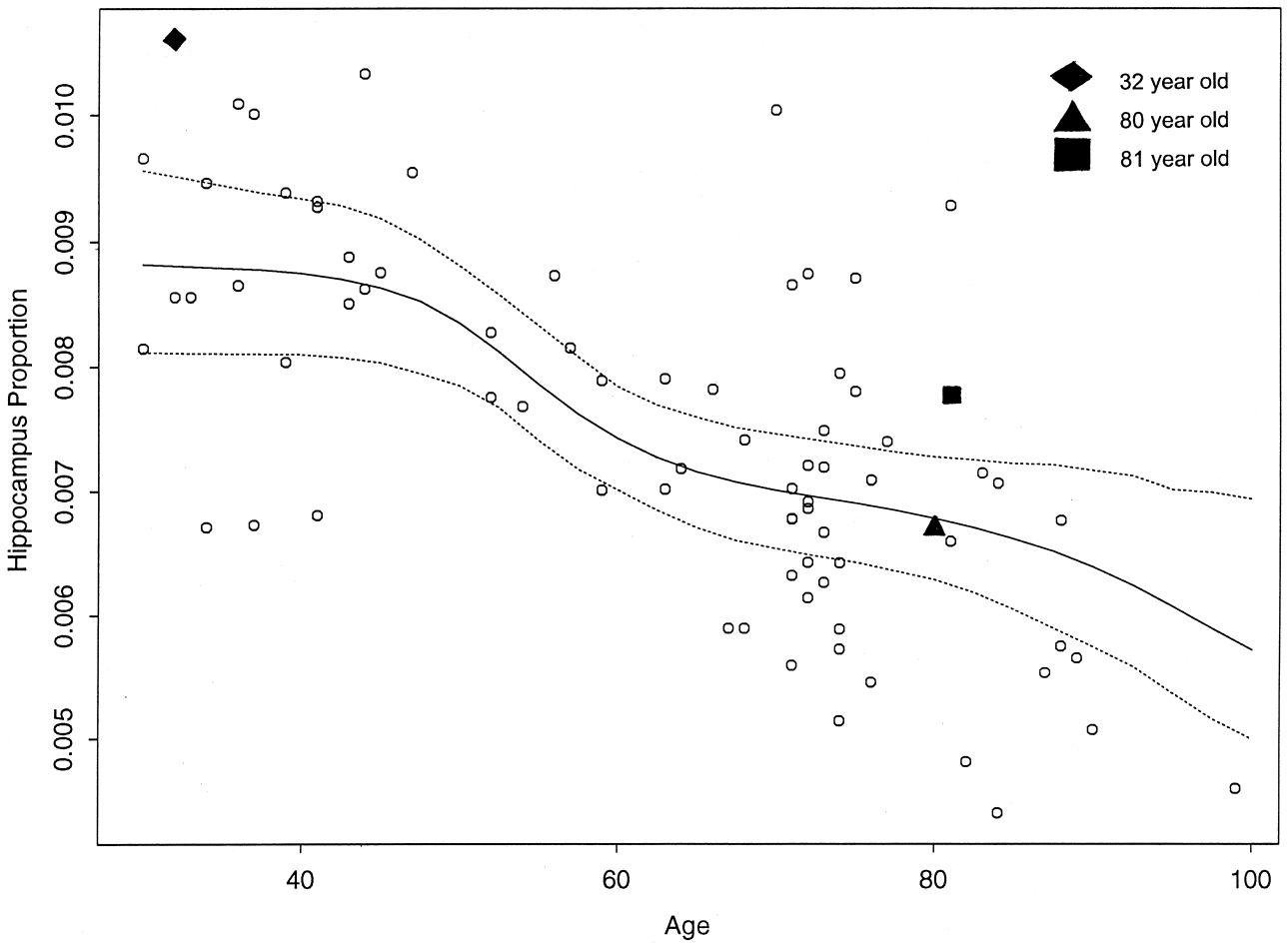


Fig. 4. Proportionalized estimated volumes of hippocampal gray matter by age with monotone smooth (—) and variability bands (····). Values for the 3 subjects shown in Figure 2 are coded with distinctive symbols.

exceed that in the general population. However, in separate analyses [12] there was no evidence for hippocampal volume loss (or loss in any cortical volume) in the $\epsilon 4+$ subjects relative to matched $\epsilon 4-$ controls; in fact, the mean volumes were strikingly similar. Furthermore, the age-differences on the hippocampal and cerebral cortex volumes were examined and compared in a separate analysis in which all subjects known to be $\epsilon 4+$ were excluded. Since the great majority of the elderly subjects in the present sample were genotyped, this resulted in a sample in which $\epsilon 4$ genetic risk was almost certainly underrepresented, specifically among the elderly subjects. This analysis revealed very similar and equally striking effects on the hippocampal and cerebral cortex volumes, and suggested that the acceleration of hippocampal volume loss over cerebral cortex volume loss is not diminished in populations free of increased genetic risk. Thus it is unlikely that age-functions observed for the gray matter measures in the present study were significantly influenced by Apo E $\epsilon 4$ status.

### *4.2. Prominence of white matter loss*

In several previous studies, age-related changes in white matter volume failed to reach significance [2,19,33]. However the present findings are consistent with those of recent studies in which age-related white matter volume loss was observed [15,34,36]. Possible reasons for the inconsistency of these findings include differences in age-range examined, accuracy of the segmentation methods used, or methods for handling white matter signal elevation (see discussion below).

The age-related decline in cerebral white matter volume observed in the present study is shown graphically in Fig. 5. The shape of the function describing cerebral white matter loss differs from that for cerebral gray matter. Cerebral white matter volume appears to remain relatively stable until age 70 after which the decline is rapid. Comparison of linear estimates of cerebral white matter volume at ages 30 and 90 suggests loss of approximately 26.5% (Table 6). This loss is greater than that observed in cerebral gray matter. Thus, in spite of a later onset of significant cerebral white matter volume loss, this loss ultimately exceeds that of cerebral gray matter in clinically normal older subjects past the age of 70. Some earlier studies included very few individuals past the age of 75, and, given the function shown in Fig. 5, it is not surprising that such studies show only very modest white matter volume loss.

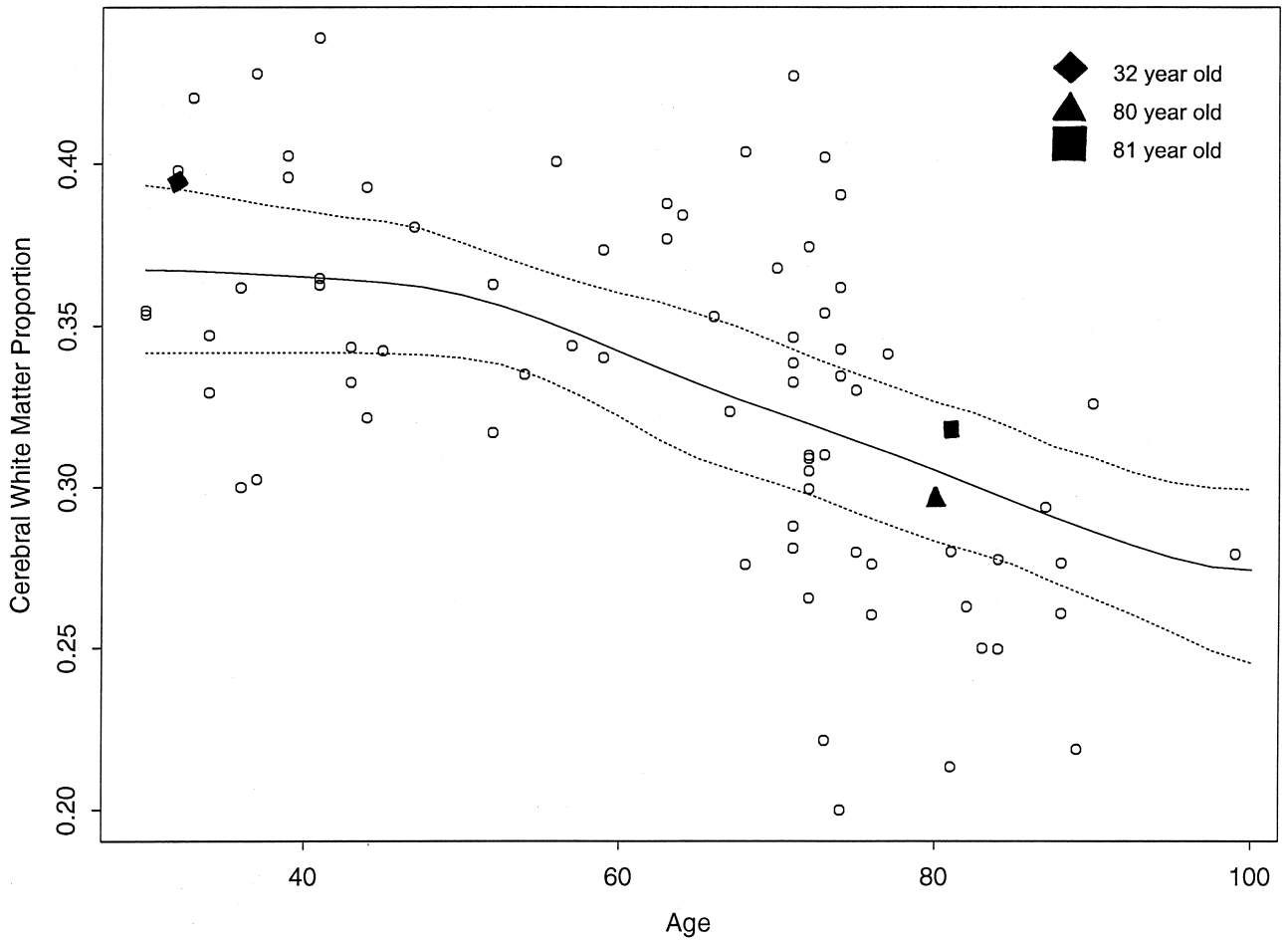


Fig. 5. Proportionalized estimated volumes of cerebral white matter by age with monotone smooth (—) and variability bands (----). Values for the 3 subjects shown in Figure 2 are coded with distinctive symbols.

It should be noted that the same pattern observed within the cerebrum, of more rapid decline of white matter volume relative to gray matter volume, was also observed within the cerebellum.

### *4.3. Prevalence of signal hyperintensities within white matter regions*

As in previous studies, there was clear evidence for age-related increase in the amount of cerebral white matter exhibiting signal elevation on MRI. Fig. 6 shows estimated volumes of "altered" white matter plotted against age for the present sample. Examination of the regional variability in age-related signal alteration revealed that the white matter of all lobes showed more age-related change than did white matter of the deep subcortical region. Furthermore, the increase in these abnormalities within the frontal lobe was more rapid than in the temporal or parietal lobes. These changes have been linked to vascular risk factors [24,26,38]. Attempts to link them to functional impairment in the normal elderly have been mixed. Some authors have reported no apparent association between degree of white matter abnormality and performance measures [1,4,11]. However others have linked the changes to neuropsychological impairment, particularly in attention and speed of information processing [3,24,40]. White matter abnormalities in normal elderly were also associated with performance decrements in the results of a recent meta-analysis of previous aging studies [14]. Thus they may no longer be considered to be entirely benign. Some individuals examined in the present study had mild hypertension or a history of hypertension; however, no relationship between blood pressure level or history of hypertension and the presence of white matter hyperintensity was observed.

### *4.4. Age-related change in subcortical structures*

The present study examined for the first time the relative age-related losses in three separate divisions of the basal ganglia: the caudate nucleus, the more ventrally lying nucleus accumbens, and the lenticular nucleus. The results suggest that the caudate and nucleus accumbens decline in volume across the age range and the rate of decline may be more rapid than in the lenticular nucleus, although the

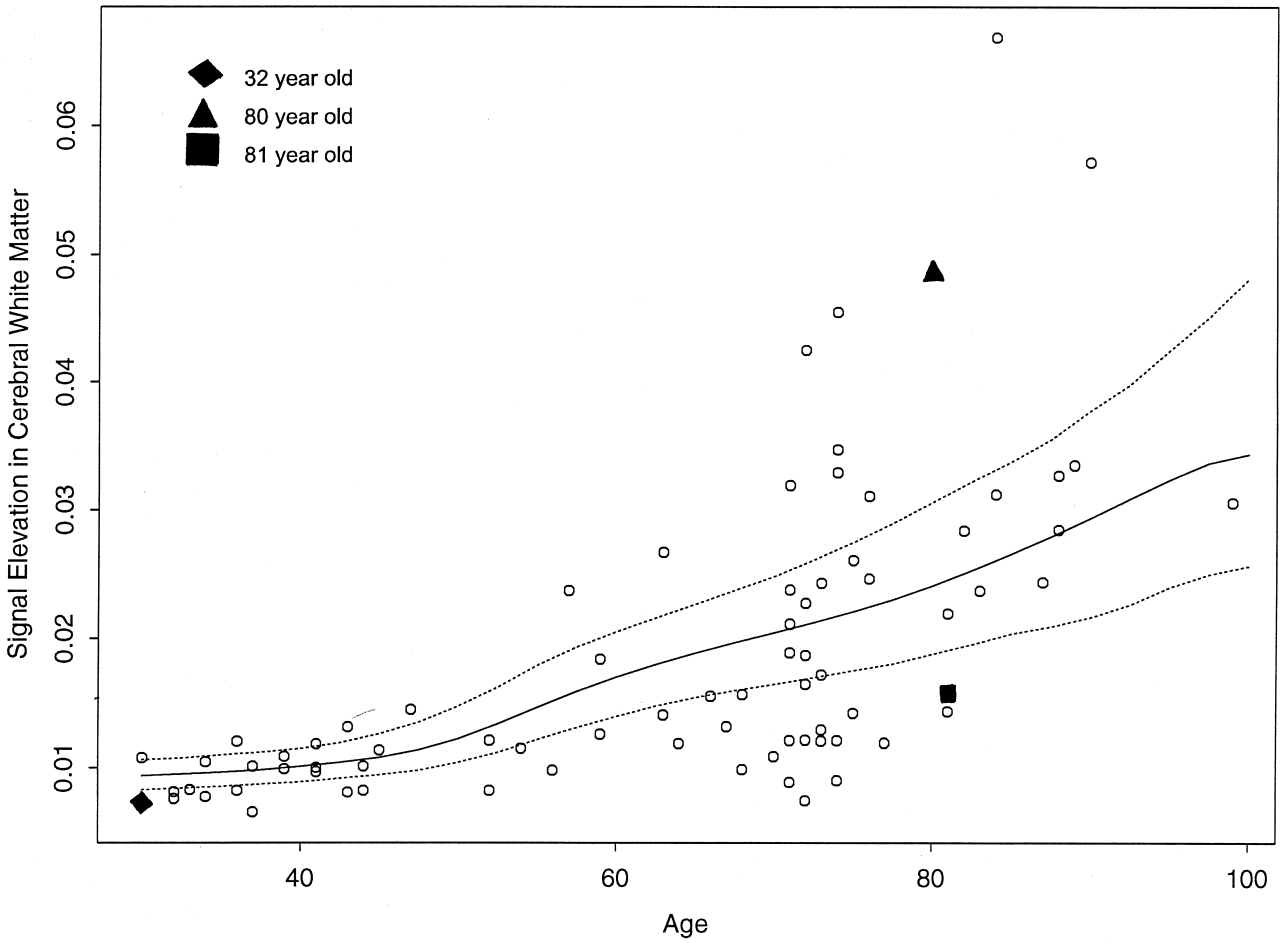


Fig. 6. Proportionalized estimated volumes of white matter signal elevation by age with monotone smooth (—) and variability bands (----). Values for the 3 subjects shown in Figure 2 are coded with distinctive symbols.

results are equivocal in this regard. The present study provided little evidence for any age-related volume loss in the latter structure. This is surprising, since age-related volume reductions in lenticular nucleus [30,31] or putamen [27] have been reported previously; and age-related increase in globus pallidus hypointensity [29] might have been expected to reduce the apparent size of the lenticular nucleus in older subjects. One distinction between these studies and the present study is that the former included subjects below the age of 30. Prominent age-related (probably maturational) reductions in lenticular volume occur in young adults [23,37] and these could be expected to amplify any age-effects observed in samples including young adults. Indeed, in our previous morphometric study of aging [19], in which the youngest subject was 30 years of age, the modest volume reductions in the lenticular nucleus just failed to reach significance.

It should also be noted, however, that anatomists circumscribing the lenticular nucleus in the present study often observed what appeared to be areas of signal elevation, particularly near lenticulo-striate vessels, within the lenticular nucleus. This phenomenon is illustrated graphically in Fig. 2. The sections on the left are the T2-weighted MR image and the corresponding processed image from a young subject, and the middle and right sections are similar sections from two elderly subjects. As can be seen, while there is evidence for brain volume loss in both older subjects (in the form of increased CSF spaces) the two differ markedly in the degree of signal alteration in the cerebral white matter. Such signal elevation can be observed within and surrounding the putamen and globus pallidus of the older subjects. These areas of increased signal are more common in older individuals, and the presence of this signal elevation can result in a subtle increase of the apparent size of this structure. This can occur because white matter voxels within or just adjacent to the lenticular nucleus may acquire signal values within the gray matter range, and are therefore indistinguishable from normal gray matter voxels. Furthermore, even hyperintense voxels are classified as gray matter with our methods, and if they are located within the normal boundaries of gray matter structures they are included as part of these structures. For this reason, it is possible that an underlying volume reduction in putamen or globus pallidus was masked in the present study.

There was also no evidence for volume loss in amygdala or substantia nigra in the present study. This conflicts with

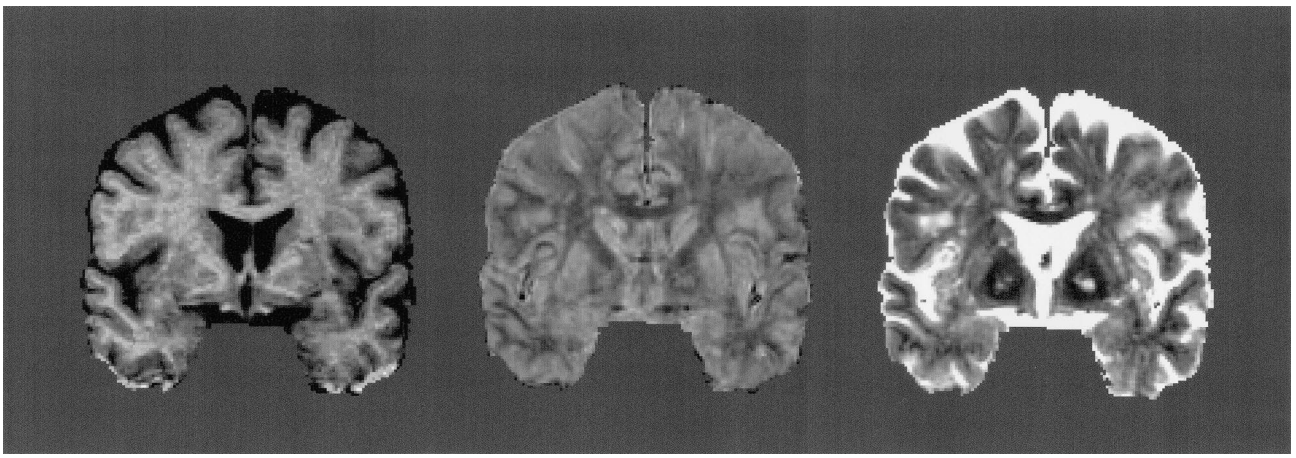

Fig. 7. Additional MR images of the section from the healthy elderly 80 year old subject presented on the right in Figure 2: T1-weighted (SPGR), proton density weighted (fast spin echo), and T2-weighted (fast spin echo).

previous findings of age-related loss in these structures. The reason for this discrepancy is unclear, but again, the presence of signal elevation in these regions may have resulted in poor sensitivity of our methods for estimating gray matter volumes in some of the older individuals.

### *4.5. Challenges to tissue segmentation in older individuals: conflating gray and white matter volume changes with white matter signal changes*

As has been noted by previous investigators [15], some otherwise well-validated segmentation methods, particularly substantially automated methods, result in the classification of some voxels within white matter as gray matter. This is not, strictly speaking, a segmentation error, as some lightly myelinated areas of the white matter have MR signal characteristics identical to gray matter. Clearly, the number of such voxels increases substantially in many older individuals, such as the older subject shown in Fig. 2. The full set of MR images, including the T1-weighted SPGR image, the PD image, and the T2-weighted image of the normal subject shown on the right in Fig. 2 are shown in Fig. 7, to help illustrate the challenge that such white matter changes pose when tissue segmentation is applied in such individuals. All tissue segmentation schemes that classify voxels as gray, white, or CSF will misclassify such voxels, since these changes represent the shift of white matter signal values toward, into, and ultimately beyond, the range of signal values characteristic of gray matter. Methods that attempt to classify hyperintense voxels separately have the potential to reduce the errors, however, they will not succeed entirely, because many areas of white matter signal elevation can not be distinguished from gray matter on signal characteristics alone. Inspection of the different MR images of Fig. 7 reveals that the areas of affected white matter have signal values characteristic of other tissues in all sections, i.e., segmentation of T1-weighted volumes is also complicated by these changes.

Without further anatomically-based coding of such voxels, as was applied in the present study, the age-related increase in their number leads to a systematic underestimation of cerebral gray matter loss, and a systematic overestimation of the loss of cerebral white matter. Indeed, our results suggest that failure to recode white matter voxels with elevated signal as "white matter" would have led to a reduction in the estimate of proportional loss of gray matter with age from 14% to 9%, while the present estimate of proportional (cerebral) white matter volume loss, 26.5%, would have been inflated to 33.3%. While these errors are unlikely, on their own, to result in a failure to detect significant changes in the estimated volumes, they do represent significant distortions within parametric models of age-related morphological change. Since automated segmentation methods are particularly common in the image-analysis paths employed for functional imaging, these results could have unintended, and unappreciated, effects on functional imaging results in older subjects.

For these reasons, results of volumetric studies, and indeed, of functional imaging studies employing tissue segmentation in the image analysis path, should be interpreted cautiously when elderly individuals are included. Further development of tissue segmentation methods appropriate for use in older individuals is needed. It is possible that tissue segmentation based on signal values could be combined with methods employing spatial "probability" maps that code the likelihood that a voxel is within white matter. Such methods could help to distinguish affected white matter voxels from otherwise similar voxels in gray matter structures.

## 5. Summary and conclusions

The major novel findings of the present study are: that age-related losses in the hippocampus are significantly accelerated relative to gray matter losses elsewhere in the

brain; that there are disproportionate effects on the frontal lobes in terms of cortical volume loss and increases in white matter signal hyperintensity; and that the later occurring loss of cerebral and cerebellar white matter is ultimately greater than loss of gray matter in very elderly normal individuals. Differing sample composition and methodological problems may underlie some apparent discrepancies between the results of different studies of age-related change in brain morphology. Persisting methodological problems, particularly relating to age-associated changes that raise the signal of white matter into the gray matter range, continue to complicate the interpretation of tissue segmentation results in older individuals.

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