Regional Frontal Cortical Volumes Decrease Differentially in Aging: An MRI Study to Compare Volumetric Approaches and Voxel-Based Morphometry

Danielle J. Tisserand,* Jens C. Pruessner,† Ernesto J. Sanz Arigita,‡ Martin P. J. van Boxtel,* Alan C. Evans,† Jelle Jolles,* and Harry B. M. Uylings‡§

*Brain & Behaviour Institute, Maastricht University, 6229 GP Maastricht, The Netherlands; †McConnell Brain Imaging Center, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada; ‡Netherlands Institute for Brain Research, Graduate School Neurosciences Amsterdam, KNAW, The Netherlands; and §Department of Anatomy, VU Medical Center, Amsterdam, The Netherlands

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Recent neuroimaging studies suggest that the frontal lobes are the part of the brain most profoundly affected by the aging process. The present study investigated whether subregions within the frontal cortex show different patterns of brain aging. Magnetic resonance images of 57 healthy participants between 21 and 81 years old were used to measure regional frontal gray matter volumes in three ways: a manual tracing method, a semiautomatic “Talairach boxes” volumetric method, and voxel-based morphometry. Seven regions within each hemisphere were manually traced: precentral gyrus, inferior frontal gyrus, dorsolateral frontal cortex, ventral medial region, lateral orbital region, anterior cingulate, and frontal pole. With the semiautomatic approach, four regions were measured: lateral, orbital, and medial frontal regions and frontal pole. Advancing age was strongly associated with decreases in the volume of the whole frontal cortex. Differential age effects on the volumes of frontal subregions were dependent on the method applied. According to the manual approach, age-related volume decreases were strongest in the lateral and orbital frontal gray matter. The semiautomatic and voxel-based analyses found that age effects were most prominent within the lateral frontal and cingulate regions. Overall, it was concluded that although semiautomated and voxel-based methods can provide a reasonable estimate of regional brain volume, they cannot serve as a substitute for manual volumetry.

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Key Words: MRI; aging; brain anatomy; frontal cortex; volumetry; voxel-based morphometry.

INTRODUCTION

As people age, a general reduction in brain volume can be observed. Recent magnetic resonance imaging (MRI) studies have shown that such age-related decreases in brain volume are not homogeneous across all brain regions. Atrophy seems to be especially prominent in the frontal lobes (Coffey, 1993; Cowell et al., 1994; Raz et al., 1997; Salat et al., 1999; Tisserand et al., 2001) and the regions with which they have dense reciprocal projections, such as the thalamus (Van der Werf et al., 2001) and striatum (Rubin, 1999; Raz, 2000). Whether this atrophy is due to white or gray matter loss is still an open question. For instance, in a study with participants across the adult age range, a negative association between frontal white matter volume and age was found, but it was small compared to gray matter reductions within the frontal lobes (Raz et al., 1997). In some other studies, the opposite was found; i.e., age-related changes were most obvious in white matter, but only in subjects over 70 years of age (Salat et al., 1999; Courchesne et al., 2000; Jernigan et al., 2001; Salat et al., 2001). Hence, it is possible that a decrease in white matter does not start until late in life, while the gray matter atrophy takes place gradually during the adult years.

The disproportionate tissue loss in the frontal cortex strongly supports the frontal theory of cognitive aging (Moscovitch and Winocur, 1995; West, 1996; Phillips and Della Sala, 1999). This theory suggests that changes in frontal structure and/or function are responsible for cognitive problems often seen in older people, such as attentional difficulties, forgetfulness, and lack of cognitive flexibility. Evidence for frontal lobe involvement in these functions comes from studies with patients with frontal dysfunctions and from functional imaging data (Cummings, 1993; Knight et al., 1999; Cabeza, 2000). Nevertheless, the large frontal cortex is both structurally and functionally heterogeneous and a distinction should be made between several subregions.

Only few studies have considered the effect of age on the volume of different substructures within the pre-
frontal cortex (PFC). Raz et al. (1997) distinguished between three "classical" regions: orbitofrontal, dorsolateral prefrontal, and anterior cingulate cortex (Fuster, 1980; Cummings, 1995). In participants ranging from 20 to 80 years of age, they found a strong negative association between age and volume of the dorsolateral and orbitofrontal cortex, but not between age and the anterior cingulate. In contrast, using a similar subdivision and a similar age range, we found the strongest age-associated decrease within the anterior cingulate and dorsolateral region and a smaller reduction in orbitofrontal cortex, but not between age and the anterior cingulate. In this way, the variation in brain volumes could be attributed to normal aging and not to age-extrinsic, disease-related factors (Van Boxtel et al., 1998).

MATERIALS AND METHODS

Subjects

The study sample comprised 57 healthy and cognitively normal persons, between 21 and 81 years of age (mean ± SD = 55.7 ± 16.2 years). The group consisted of 34 men (mean age ± SD = 54.0 ± 16.2 years) and 23 women (mean age ± SD = 58.1 ± 16.4 years). All participants were rigorously screened for presence of health-related problems with a health questionnaire and a medical interview (Van Boxtel et al., 1998). Individuals were excluded if there was a history of hypertension, cerebrovascular or chronic neurological disease, systemic disorders, or major psychiatric illnesses (Tisserand et al., 2000). Cognitive status of subjects older than 35 years (n = 38) was assessed with the Mini-Mental State Examination (Folstein et al., 1975), and a cutoff score of >24 was used for inclusion (mean ± SD: 28.5 ± 1.8). Written informed consent was obtained from all participants. The research protocol was approved by the Medical Ethical Committee of the Academic Hospital Maastricht.

MRI Acquisition and Analysis

MRI scans were acquired with a 1.5 T Gyroscan ACS-II MRI scanner (Philips, Best, The Netherlands). T1-weighted images were obtained in the coronal plane (perpendicular to the anterior commissure–posterior commissure [AC–PC] line). A three-dimensional (3D) gradient fast field echo sequence was applied with TR = 23 ms, TE = 7 ms, and a flip angle of 30°. Slice thickness was 1.5 mm with no interslice gap. The image matrix was 256 × 256 and the field of view was 230 mm.

The same preprocessing steps were applied for all three methods. This procedure consisted of three steps. First, the image files were corrected for MR signal nonuniformities due to magnetic field inhomogeneities in the scanner (Sled et al., 1998). Second, the original images were linearly transformed into stereotaxic space (Talairach and Tournoux, 1988) using an automatic registration program developed at the McConnell Brain Imaging Center of the Montreal Neurological Institute (Collins et al., 1994). This transformation results in an alignment along the AC–PC axis and accounts for individual differences in global brain size and shape. After this resampling the MRI volume con-
sisted of 181 axial slices, and the voxel size was $1 \times 1 \times 1$ mm. Finally, images were classified into different maps of gray matter, white matter, and cerebrospinal fluid (Evans et al., 1996; Collins and Evans, 1999). This procedure included the removal of all extracranial tissue and the cerebellum and has been validated before (Collins et al., 1994). The gray matter map was used to determine regional cortical volumes in both the manual and the semiautomatic approaches and to determine gray matter density in the voxel-based analysis.

Manual Volumetric Tracing Method

The manual tracing analysis was performed with the software package DISPLAY developed at the Montreal Neurological Institute (e.g., Pruessner et al., 2000, 2001). This program allows simultaneous viewing in coronal, sagittal, and horizontal sections and a 3D surface rendering. Editing of the images can be performed on the triplanar (2D) sections but also on the rendered surface (Figs. 1 and 2). This is important because tracing sulci, in particular those on the lateral side of the brain, is rather complicated using only 2D sections. Seven frontal regions were outlined within each hemisphere and were all measured by the same rater (D.T.). The total time needed to measure the volumes of the frontal subregions of one individual was approximately 10 h. This parcellation method slightly differs from the ones described before (Rademacher et al., 1992; Crespo-Facorro et al., 1999). Based upon knowledge about the connectivity and cytoarchitecture within the frontal lobes (Groenewegen and Uylings, 2000; Uylings et al., 2000a) and guided by several anatomical brain atlases (Nieuwenhuys et al., 1981; Ono et al., 1990; Duvernoy, 1991), the following rules were applied for delineation of the regions of interest.

Precentral gyrus (Brodmann area 4 and ventral part of area 6). The posterior border, i.e., the bottom of the central sulcus, was traced on the surface-rendered image. This sulcus is generally continuous, starting at the medial surface and ending near the Sylvian fissure (Ono et al., 1990). The anterior border, the bottom of the precentral sulcus, was traced in a similar fashion. Frequently, this sulcus is interrupted (Ono et al., 1990). The shortest possible line was drawn to connect the various segments. In case of two parallel sulci, the most posterior sulcus was traced, to ensure that the precentral region consisted of only one gyrus. The ventral border was the Sylvian fissure.

Anterior cingulate (Brodmann areas 24 and 32). The anterior cingulate cortex was traced using the sagittal and coronal views. The dorsal border consisted of the bottom of the paracingulate sulcus. When this sulcus was interrupted (which is frequently the case, e.g., Paus et al. (1996)), the shortest possible line between sulci was drawn. In no case was the cingulate sulcus taken as the dorsal border. The posterior border was located three slices posterior to the slice where the anterior commissure is most clearly visible (Fig. 1, plane A). The anterior border was the first deep sulcus measured from the genu of the corpus callosum, usually the cingulate sulcus. This sulcus was also taken as the ventral border. The ventral-posterior border consisted of the most caudal slice on which the inner curvature of the corpus callosum was visible (Fig. 1, plane C). This border was chosen to ensure that area 25 was not included in this region.

Frontal pole (Brodmann area 10). Because it is difficult to apply anatomical criteria near the anterior tip of the frontal cortex, a pragmatic cutoff point was used. This region was therefore defined as all gray matter anterior to the line $y = 44$ (Fig. 1, plane D), which encompassed approximately 25 coronal slices (2.5 cm). Such a cutoff line could be used because the images were spatially normalized and therefore the position of this line was approximately similar in each subject. Only in the case of the anterior cingulate was this rule not applied; i.e., that region was measured completely, even if it included tissue rostral of the line $y = 44$.

Orbitofrontal cortex (Brodmann areas 11, 12, and 47). This can be subdivided into a ventral medial and a lateral orbital part (Elliott et al., 2000; Öngür & Price, 2000). The ventral medial part consists of Brodmann areas 11 and 12. All three planes were used to trace the borders of this region. Based upon our cytoarchitectonic experience (E.S.A. and H.U.), the posterior border was defined as the inner curvature of the corpus callosum (Fig. 1, plane C), and the anterior border consisted of the frontal pole region. The lateral border was the crown of the olfactory sulcus, which is the first orbital sulcus when moving laterally from the midline. The complete gyrus was traced on the five most ventral axial slices; dorsal to this point, only the gray matter medially to the white matter band of this gyrus was included. Dorsally, this region was limited by the anterior cingulate region. The lateral orbital region consists of Brodmann area 47. The posterior border consisted of the posterior tip of the corpus callosum (Fig. 1, plane B). On coronal sections, this is seen as a bright spot just below the lateral ventricles. The anterior border was formed by the frontal pole region. The medial border was the one with the ventral medial region. The lateral border was the circular sulcus of the insula on the more posterior slices and the anterior horizontal ramus of the Sylvian fissure on the more anterior slices. The dorsal border was the AC-PC line.

Inferior frontal gyrus (Brodmann areas 44 and 45). The ventral border consisted of the anterior horizontal ramus of the Sylvian fissure. The dorsal border was the inferior frontal sulcus. In most cases, these sulci were clearly visible and they were traced on the surface...
The posterior border was the precen- 
tral sulcus, and the anterior border was the frontal 
pole region.

Dorsolateral frontal (Brodmann areas 8, 9, and 46). 
This region had as lateral ventral border the inferior 
frontal sulcus and as medial border the paracingulate
sulcus. The posterior border was defined by the precentral sulcus and the anterior border by the frontal pole region. The dorsolateral region was traced on the surface rendered image.

Reproducibility of measurements. To determine the test-retest reliability of the measurements, all frontal volumes of five randomly selected brains were measured twice by the same rater. Intraclass correlation coefficients (ICCs) were determined using a one-way analysis of variance model (Bartko and Carpenter, 1976). ICCs take into account both within-subject and between-subject variance and are therefore a very sensitive method to assess the reproducibility of measurements. As can be concluded from Table 1, the reliability of the manual volumetric approach was high: all ICCs were >0.88, except for the right precentral gyrus (r = 0.76).

Semiautomatic “Talairach Boxes” Method

For this approach, the BrainImage software (Subramaniam et al., 1997), developed at the National Institute of Health, was used in combination with custom software developed at the Maastricht Brain and Behaviour Institute. The Talairach grid system divides the brain into boxes, based upon the location of the AC and PC and the outer boundaries of the brain (Talairach and Tournoux, 1988). The only nonautomatic

<table>
<thead>
<tr>
<th></th>
<th>Left</th>
<th>Right</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior cingulate</td>
<td>0.88</td>
<td>0.98</td>
</tr>
<tr>
<td>Dorsolateral frontal</td>
<td>0.97</td>
<td>0.96</td>
</tr>
<tr>
<td>Inferior frontal</td>
<td>0.98</td>
<td>0.94</td>
</tr>
<tr>
<td>Ventral medial</td>
<td>0.99</td>
<td>0.93</td>
</tr>
<tr>
<td>Lateral orbital</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Frontal pole</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Precentral gyrus</td>
<td>0.89</td>
<td>0.76</td>
</tr>
<tr>
<td>Total frontal</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>
step in this procedure consists of marking the two commissures. Subsequently, a grid consisting of 1056 boxes (11 anterior–posterior × 8 left–right × 12 dorsal–ventral) is automatically placed over the brain (Fig. 3). Because the image files were spatially transformed prior to this analysis, the volume of each box was constant across all subjects (i.e., approximately 3.2 cm³). Using these boxes, specific regions of interest can be defined. It was previously shown that volumes of regions defined with the aid of the grid have a high correspondence to manually traced volumes of the same regions (Andreasen et al., 1994). Furthermore, a great number of functional neuroimaging studies have referred to the Talairach coordinate system to localize and compare brain regions between individuals.

In the present study, “Talairach boxes” representing the frontal lobes were subdivided into four regions based upon anatomical knowledge and with the use of the Talairach atlas (Talairach and Tournoux, 1988): lateral prefrontal region, anterior cingulate region, orbital prefrontal region, and frontal pole region.

This subdivision is a slightly adapted version of the one described in Tisserand et al. (2001), to ensure the best possible comparison between the manual and the semiautomatic methods. The complete frontal region was defined as the region starting at the frontal tip and ending posteriorly at a plane orthogonal to the AC–PC line, at one third of the distance between the AC and the PC (see Fig. 3 for an example). The definition of the subregions was based upon the landmarks as defined under Manual Volumetric Tracing Method. The frontal pole consisted of the most rostral quarter of the region between the frontal tip and the AC. The anterior cingulate region included the boxes encompassing the cingulate and paracingulate sulci. The lateral PFC region included all boxes containing the inferior frontal and dorsolateral region as described under Manual Volumetric Tracing Method. The orbitofrontal region included all ventral medial and orbital boxes. When a box included two different regions, it was assigned to the region which contributed most of the tissue in the box. A random image file was used to assign the boxes to the various regions, based upon the anatomical characteristics of this image. Subsequently, five image files were inspected to test whether this subdivision fitted the individual brains best or whether boxes needed to be shifted from one region to the neighboring one. Then, after agreement had been reached, the gray matter maps of all 57 brains were analyzed using this standard protocol. The approximate total time needed to measure the volumes of frontal subregions of one individual was 15 min. All ICCs of the semiautomatically determined frontal volumes were 1.0 (data not shown).

In Table 2, a comparison of the frontal regions as defined by the manual and semiautomatic methods is presented.

### Voxel-Based Morphometry

The gray matter images, resulting from the normalization and classification as described before, were smoothed using a Gaussian kernel of 10 mm full-width at half-maximum. These gray matter density maps were used to localize age-related volume losses. Because this approach is fully automated, test–retest reliability is perfect. VBM analyses were performed with software developed at the Montreal Neurological Institute (e.g., Paus et al., 1999; Pruessner et al., 2001; Watkins et al., 2001).

### Statistical Analysis

Pearson product-moment correlations between the semiautomatically and the manually traced regions were calculated to compare these volumetric methods. Furthermore, the effect of age on the frontal subregions, measured with both volumetric methods, was examined by using linear regression analysis. Because the brain images were spatially normalized before measurements were started, no further correction for individual variation in head size was required. To investigate whether the age-related atrophy was disproportional within the frontal lobe, total brain volume and age were entered successively into a regression model with the regional frontal gray matter volumes as dependent variables (Tisserand et al., 2000).

To localize age-related decreases in gray matter density with the VBM approach, a linear regression model was applied to the normalized and smoothed gray matter maps of all subjects (Wright et al., 1995; Ashburner and Friston, 2000). The statistical significance of the relation between age and signal intensity was assessed for each voxel, after removal of the effect of sex. This method, based upon Gaussian random field theory, corrects for multiple comparisons in a given search volume, in this case the gray matter maps of all subjects (Friston et al., 1996; Worsley et al., 1996).

### Table 2

<table>
<thead>
<tr>
<th>Talairach boxes</th>
<th>Manual tracing</th>
<th>Approximate Brodmann area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral frontal</td>
<td>Inferior frontal gyrus</td>
<td>44, 45</td>
</tr>
<tr>
<td></td>
<td>Dorsolateral frontal</td>
<td>8, 9, 46</td>
</tr>
<tr>
<td>Anterior cingulate</td>
<td>Anterior cingulate</td>
<td>24, 32</td>
</tr>
<tr>
<td>Orbital frontal</td>
<td>Ventral medial</td>
<td>11, 12</td>
</tr>
<tr>
<td></td>
<td>Lateral orbital</td>
<td>47</td>
</tr>
<tr>
<td>Frontal pole</td>
<td>Frontal pole</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Precentral gyrus</td>
<td>4, 6</td>
</tr>
</tbody>
</table>

Note. The clustering of the manually traced frontal cortical areas as presented in this table was performed to allow for a comparison between the two volumetric methods (cf. Table 5).
**RESULTS**

Frontal Cortical Volumes—A Methodological Comparison

To enable a comparison between the two volumetric methods, several of the manually traced frontal areas were merged to match the four semiautomatically measured regions ("Talairach boxes") as described in Table 2. Left and right regional volumes were combined. Pearson product-moment correlations between the volumes as measured with each of the two methods were calculated. A high correspondence was found between the manual and the semiautomatic method for the frontal cortex as a whole \((r = 0.98; \text{Table 3})\). Equally, the correlations between both methods within the four large subregions were adequate, ranging from 0.85 (orbital frontal) to 0.93 (lateral frontal). As can be seen in Fig. 4, the semiautomatically measured volumes of all regions were smaller (frontal pole) or larger (other regions) than the manually traced volumes. The fact that the correlations nevertheless were high suggests that the volumes differed in a systematic fashion.

Effects of Age on the Frontal Cortical Volumes

The age effects will first be described for the manual tracing method, followed by the results of the semiautomatic ("Talairach boxes") approach. Age accounted for a considerable part of the variation in all of the manually determined frontal cortical volumes (Table 4), ranging from 18% (frontal pole) to 44% (inferior frontal). To investigate whether the frontal volume decreases were disproportional, i.e., to adjust for the fact that age-related volume losses also occur in other parts of the brain, analyses were repeated, controlling for the total brain volume. It was found that, after correction, older age was significantly associated \((P < 0.01)\) with smaller dorsolateral and inferior frontal, ventral medial, and lateral orbital frontal volumes, but not with smaller volumes of the anterior cingulate, frontal pole, and precentral gyrus. Although there were significant differences between males and females in regional frontal volumes (Table 3), no sex \times age interactions were found.

To compare the two volumetric methods, analyses as described for the manually traced frontal volumes were repeated with the semiautomatic method, using the four frontal regions specified in Table 2 (Fig. 4, Table 5). As with the manual tracing method, age was strongly associated with lateral frontal volumes. Differences between the two volumetric methods are clearly noticeable, however. In contrast to the manual tracing approach, the semiautomatic method showed a lack of disproportional age effects on the volume of the orbital frontal cortex, whereas a significant disproportional age effect became apparent on the volume of the anterior cingulate cortex.

Effects of Age on Gray Matter Assessed with Voxel-Based Morphometry

The association between age and regional gray matter volume was also investigated with a voxel-based linear regression analysis, resulting in a 3D t-statistic map. In this paper, we will focus on the results for the frontal lobes. Significant age-related decreases in gray matter density \((t < -5.0, P < 0.01, \text{corrected})\) were found throughout the frontal cortex. The highest associations with age \((t < -8.0; \text{Table 6})\) were found within the anterior cingulate, the lateral orbitofrontal area predominantly in the right hemisphere, and within the region of the inferior frontal cortex bilaterally, extending into the insula. The largest clusters were located in the cingulate and lateral frontal regions.

**DISCUSSION**

Comparison of the Manual and Semiautomatic Volumetric Methods

In this study two volumetric approaches to measure frontal cortical subregions were compared. Guided by
knowledge about the cytoarchitecture and connectivity within the frontal lobes (Groenewegen and Uylings, 2000; Uylings et al., 2000a), seven frontal cortical regions were manually outlined in each hemisphere: precentral gyrus, inferior frontal gyrus, dorsolateral prefrontal, anterior cingulate, ventral medial region, orbital region, and frontal pole. This method proved to be highly reliable and reproducible in measuring the volume of these frontal subregions. However, this approach was very labor intensive (approximately 10 h per brain). This makes such an approach rather unattractive for the analysis of datasets involving large numbers of subjects. For that purpose, a second, semi-automatic method (Subramaniam et al., 1997), based upon the Talairach coordinate system (Talairach and Tournoux, 1988), was used to determine frontal cortical volumes. Four regions were distinguished using this approach: lateral prefrontal, orbital frontal, anterior cingulate, and frontal pole. Application of this method took less than 15 min per brain, and the reproducibility was perfect. Correlations between the total and the regional frontal cortical volumes as measured with both volumetric methods were high.

The definition and number of functionally distinct macroscopic regions within the brain remain, to a certain extent, uncertain. Therefore, a somewhat crude
subdivision may be just as accurate as a more detailed and anatomically correct parcellation in determining a relation with factors such as age. To test this, both volumetric methods were used to examine age-related changes in regions of the frontal lobe gray matter volume.

With the manual method, age was found to explain almost half (42%) of the individual variation in gray matter volume of the frontal lobes as a whole. This age effect was significantly disproportional relative to volume changes within the whole brain. This is in accordance with studies showing that particularly the frontal lobes are affected by increasing age (Coffey, 1993; Cowell et al., 1994; Raz et al., 1997; Salat et al., 1999; Tisserand et al., 2001). Furthermore, the present study is one of the first to show on a detailed scale that there are regional differences in the amount of age-related frontal atrophy. For example, the least volume losses were found in the precentral and frontal polar regions. The fact that volume decrease was only moderate in the precentral area is not surprising, given the evidence that primary sensory and motor areas are less vulnerable to age influences than association areas (Kemper, 1994). The finding that volume losses were also limited in the region of the frontal pole has not been reported before. Remarkably, several older studies have found the largest decreases in neuronal densities in exactly these two frontal regions (Haug, 1985; Kemper, 1994). However, the correctness of these older

<table>
<thead>
<tr>
<th>TABLE 4</th>
<th>Explained Variance ($R^2$) of Age on Frontal Cortical Volumes (Manual Tracing Method), Uncorrected and Corrected for Global Decreases in Brain Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, uncorrected for brain volume</td>
<td>Age, corrected for brain volume</td>
</tr>
<tr>
<td>Anterior cingulate</td>
<td>0.30*</td>
</tr>
<tr>
<td>Dorsolateral frontal</td>
<td>0.39*</td>
</tr>
<tr>
<td>Inferior frontal</td>
<td>0.44*</td>
</tr>
<tr>
<td>Ventral medial</td>
<td>0.40*</td>
</tr>
<tr>
<td>Lateral orbital</td>
<td>0.41*</td>
</tr>
<tr>
<td>Frontal pole</td>
<td>0.18*</td>
</tr>
<tr>
<td>Precentral gyrus</td>
<td>0.20*</td>
</tr>
</tbody>
</table>

* $P < 0.01.$
cell counting methods has been criticized in recent years: age-related neuronal loss appears to be only mild (Uylings et al., 2000b). Cortical atrophy is now thought to be the result of cell shrinkage rather than a decrease in neuronal number (Kemper, 1994; Uylings et al., 2000b).

When comparing the two approaches with respect to age effects, the results of the semiautomatic “Talairach boxes” method were highly similar to those of the manual tracing approach for the frontal cortex as a whole. However, for the frontal subregions the results were largely dependent on the method applied. That is, despite equal preprocessing steps, and despite the fact that the data from both methods were highly correlated, there were clear differences in age effects on frontal cortical subregions. These differences were the most striking for the anterior cingulate and orbitofrontal regions. With the manual tracing method the age effects on the volume of the anterior cingulate were relatively small, whereas with the semiautomatic method they were relatively large. Conversely, the orbital region was the most severely affected region according to the manual tracing method and the least affected according to the semiautomatic approach. A possible explanation for this discrepancy is that part of the orbital frontal cortex was included in the anterior cingulate Talairach boxes. All boxes are complementary; hence when a frontal subregion is excluded from one Talairach region, it automatically belongs to the neighboring region. The volume of the anterior cingulate region was indeed larger according to the semiautomatic approach than when calculated with the manual method. Furthermore, visual inspection of the images (by overlaying the Talairach boxes on the manually outlined regions) demonstrated that the anterior cingulate boxes included tissue from the ventral medial cortex and, to a lesser extent, the medial wall of the superior frontal gyrus, which is part of the dorsolateral region. Due to the size of the boxes it was impossible to be more precise in determining the borders between regions. Another explanation for the discrepancy between the methods with respect to age effects could be that more variance was present in the data of one of the two approaches. Indeed, the standard error tended to be larger for the semiautomatically measured frontal volumes than for the manually traced volumes.

The results of the semiautomatic “Talairach boxes” method are consistent with our previous findings (Tisserand et al., 2001), i.e., age-related volume decreases are strongest in the medial frontal cortex. This is evident, because in that study the same subjects were examined with the same method (with some small adjustments). It also shows that our current attempt to

### TABLE 5

<table>
<thead>
<tr>
<th>Cortical Region</th>
<th>Method</th>
<th>Age, uncorrected for brain volume</th>
<th>Age, corrected for brain volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior cingulate</td>
<td>Talairach</td>
<td>0.49*</td>
<td>0.11*</td>
</tr>
<tr>
<td></td>
<td>Manual</td>
<td>0.30*</td>
<td>0.03</td>
</tr>
<tr>
<td>Lateral frontal</td>
<td>Talairach</td>
<td>0.40*</td>
<td>0.09*</td>
</tr>
<tr>
<td></td>
<td>Manual</td>
<td>0.45*</td>
<td>0.12*</td>
</tr>
<tr>
<td>Orbital frontal</td>
<td>Talairach</td>
<td>0.24*</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Manual</td>
<td>0.47*</td>
<td>0.10*</td>
</tr>
<tr>
<td>Frontal pole</td>
<td>Talairach</td>
<td>0.22*</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Manual</td>
<td>0.18*</td>
<td>0.00</td>
</tr>
<tr>
<td>Total frontal</td>
<td>Talairach</td>
<td>0.42*</td>
<td>0.07*</td>
</tr>
<tr>
<td></td>
<td>Manual</td>
<td>0.42*</td>
<td>0.07*</td>
</tr>
</tbody>
</table>

* P < 0.01.

### TABLE 6

Results of the Voxel-Based Morphometry Analysis: Negative Associations (t < -6; P < 0.0001) between Frontal Gray Matter Density and Age

<table>
<thead>
<tr>
<th>Cortical Region</th>
<th>Brodmann area</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>t value</th>
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<tbody>
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<td>Frontal pole left</td>
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<td>10</td>
<td>21</td>
<td>55</td>
<td>-9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>30</td>
<td>55</td>
<td>15</td>
</tr>
<tr>
<td>Lateral orbital left</td>
<td></td>
<td>47</td>
<td>31</td>
<td>34</td>
<td>-12</td>
</tr>
<tr>
<td>Lateral orbital right</td>
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<td>47</td>
<td>-28</td>
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<td>-14</td>
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<tr>
<td>Anterior cingulate</td>
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<td>35</td>
<td>19</td>
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</tr>
<tr>
<td></td>
<td>24/32</td>
<td>-6</td>
<td>31</td>
<td>40</td>
<td>-8.30</td>
</tr>
<tr>
<td></td>
<td>24/32</td>
<td>1</td>
<td>17</td>
<td>-11</td>
<td>-8.08</td>
</tr>
<tr>
<td>Dorsolateral frontal left</td>
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<td>42</td>
<td>-1</td>
<td>-6.37</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>19</td>
<td>29</td>
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<td>-6.81</td>
</tr>
<tr>
<td></td>
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<td>18</td>
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<tr>
<td>Inferior frontal left</td>
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<td>52</td>
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<td>1</td>
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</tr>
<tr>
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<td>-15</td>
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<td>Inferior frontal right</td>
<td>44/45</td>
<td>-44</td>
<td>21</td>
<td>-2</td>
<td>-8.01</td>
</tr>
</tbody>
</table>

Note. X, Y, Z are the coordinates in Talairach space; t value is the peak value.
match the “Talairach regions” as closely as possible to the manually defined anatomical regions did not change the findings with respect to age.

Our manual measurements are in agreement with results in the study by Raz et al. (1997), pointing to a disproportional volume decrease in the orbitofrontal cortex. By contrast, a recent study (Salat et al., 2001) using a manual volumetric approach found a relative preservation of the orbitofrontal cortex as compared to three other frontal regions. However, the subjects that they studied were older than those in the present study and those of Raz et al. (1997). Furthermore, the anterior cingulate region could not be reliably measured in that study and was therefore excluded from the analysis. The results from these studies can therefore not easily be compared.

To conclude, when manual measures are taken as anatomical reference, the semiautomatic “Talairach boxes” approach does not seem to be accurate enough to quantify brain volumes, unless the regions are as large as complete lobes (as in Andreasen et al., 1994; Goldszal et al., 1998; Resnick et al., 2000).

Comparison of Volumetric Methods and Voxel-Based Morphometry

To consider manual delineation of cerebral subregions the “gold standard” might be debatable. Despite the high ICCs in the present study, manual demarcation of regions often is at the expense of reproducibility (e.g., Salat et al., 2001), particularly in regions where anatomical borders are ill defined, such as the highly convoluted frontal cortex. Furthermore, manually outlining cortical regions is a time-consuming operation. Alternatively, the effect of age on gray matter volume can be investigated with voxel-based morphometry. The advantage of such an approach is that it is completely automated, and it allows determination of changes within highly local regions of the brain. However, VBM can provide only a qualitative, not a quantitative, measure of brain volume. In the present study, the strongest associations between frontal gray matter density and age were found within the anterior cingulate and inferior frontal cortex. These findings coincide with the results of two other studies that used VBM to study age effects (Good et al., 2001; Goto et al., 2001). However, there is clearly a discrepancy between the findings of our VBM and volumetric analyses. One explanation for this discrepancy could be that the manually determined frontal cortical regions were still too large to adequately capture focal age-related decreases in local volumes. For instance, the VBM analysis revealed that within the anterior cingulate region the effect of age was larger for the dorsal part than for the ventral part. Another explanation for the differences between the methods is a lower sensitivity of VBM to the detection of changes in brain areas with large anatomical variability. It has been shown that even major sulci vary considerably in terms of continuity and branching (Ono et al., 1990; Thompson et al., 1996, 2001). This variability (even after nonlinear warping and smoothing; e.g., Uylings et al., 2000a; Bookstein, 2001; Davatzikos et al., 2001) leads to false-negative results (i.e., a failure to detect true effects) and may have influenced our VBM results. For instance, consistent with other voxel-based studies (Good et al., 2001; Goto et al., 2001) the greatest age-related reductions in gray matter density were found near the inferior frontal and cingulate sulci, which have a relatively fixed position in the brain (Ono et al., 1990; Paus et al., 1996; Thompson et al., 2001). On the other hand, in the dorsolateral prefrontal cortex, where the folding pattern is highly variable across individuals (Ono et al., 1990; Thompson et al., 2001), virtually no age-related decrease in gray matter density was found, similar to the results obtained by Good et al. (2001) and Goto et al. (2001). These results differ from those of volumetric studies, which have consistently found marked reductions in gray matter volume of the dorsolateral prefrontal cortex in aging (present study; Raz et al., 1997; Tisserand et al., 2001).

To conclude, in this study it was shown that age-related decreases in regional frontal cortical volumes are differential, but the age effects are dependent (at least partially) on the method applied. Over the past few years, a lot of effort has been put into the development of more automatic methods to measure specific brain regions (Collins et al., 1995; Goldszal et al., 1998; Kabani et al., 2001). However, despite the clear advantages of automatic and voxel-based approaches (quick, perfectly reproducible, applicable to large datasets), the current findings suggest that, at present, the most accurate method is still an anatomically based manual tracing one.

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REFERENCES


