Neuroanatomical Correlates of Memory Deficits in Tuberous Sclerosis Complex

Tuberous sclerosis complex (TSC) is a multisystem syndrome classically associated with the occurrence of focal brain dysplasias. We used structural magnetic resonance imaging to test for neuroanatomical abnormalities in TSC (tubers, white matter lesions, and subependymal nodules) and to explore the relationships between these lesions and computational morphometric abnormalities of gray and white matter distribution. We tested memory function in TSC and investigated the relationship between memory function and both morphometric variation and lesion load. Patients demonstrated deficits bilaterally in volume of subcortical gray matter regions including thalamus, basal ganglia, insula, and cerebellum, as well as white matter deficits bilaterally in intrahemispheric tracts. Morphometric deficits could not be explained as local effects of lesions. Patients demonstrated deficits in executive working memory and recall memory, sparing recognition. Structure-function mapping showed long-term and working memory function was positively correlated with gray matter density (in thalamus, caudate nucleus, and frontal cortex) but not with lesion load. The neuroanatomical endophenotype of TSC is more extensive than previously recognized and comprises abnormalities in the distribution of gray and white matter in addition to classical lesions. Normal intelligence quotient patients with TSC show a profile of long-term and working memory impairment that is related to gray matter deficits in thalamus and basal ganglia components of fronto-striatal circuits.

Keywords: cognition, memory, morphometry, MRI, neurogenetics, neuroimaging, tuberous sclerosis complex

Introduction

Tuberous sclerosis complex (TSC) is a multisystem genetic disorder with an autosomal dominant pattern of inheritance and an incidence of approximately 1:6000 births (Osborne and others 1991). Two causative genes have been identified: TSC1, located on chromosome 9q34 (van Slagenhorst and others 1997), and TSC2, located on chromosome 16p13.3 (Nellist and others 1993). The disorder has a highly variable clinical phenotype, with a wide range of physical and neuropsychiatric disorders including epilepsy, mental retardation, autism spectrum disorders, and hyperkinetic disorder (Prather and de Vries 2004; de Vries and others 2005). The most severely affected cases can be profoundly handicapped, whereas some individuals are largely unscathed by the disease (Hunt and Dennis 1987). The neuropathology of TSC has classically been described in terms of cortical tubers or hamartomata, white matter abnormalities, and subependymal nodules (SENs) (Scheithauer and Reagan 1999). SENs are located immediately beneath the ependymal epithelium lining the ventricles and may undergo neoplastic transformation to form subependymal giant cell astrocytomas (Scheithauer and Reagan 1999). Cortical tubers are clusters of developmentally abnormal cells that have radially migrated to cortex from the periventricular zone; the track of migration is indicated by the persistence of heterotopic dysplastic neurons in adult white matter (Gomez 1988; Braffman and others 1992; Scheithauer and Reagan 1999).

Most prior neuroimaging studies of TSC have focused on the number and distribution of tubers and nodules; for example, seeking to correlate indices of neuroradiological "lesion load" with epilepsy, mental retardation, autism, or other psychopathological outcomes (Jambaque and others 1991; Shepherd and others 1995; Bolton and Griffiths 1997; Goodman and others 1997; Sert and others 1999; Jambaque and others 2000; Weber and others 2000; Bolton and others 2002; O’Callaghan and others 2004). One such finding is an inverse correlation between neuroradiological "lesion load" (number of tubers or nodules) and global cognitive function (intelligence quotient [IQ]), although findings have been inconsistent; see Ridler and others (2004) for review. Tubers are located predominantly in neocortical regions, especially frontal and parietal cortex, as well as anterior cingulate cortex. Nodules are embedded in ventricle walls and extend into subcortical nuclei, most frequently into the caudate nucleus, putamen, and thalamus (Ridler and others 2004). However, we have previously used computational morphometry to demonstrate structural abnormalities of gray and white matter over and above the classical lesions in patients with TSC (Ridler and others 2001). Specifically, we demonstrated bilaterally symmetric gray matter deficits in thalamus, basal ganglia, and medial temporal lobe, as well as extensive, bilaterally symmetric deficits in white matter constituting major intrahemispheric tracts. An issue arising from this prior study, which is directly addressed by the current work, is the possibility that gray and white matter morphometric abnormalities might simply be attributable to the classical lesions of the disorder.

The cognitive manifestations of this complex and highly variable neuroanatomical phenotype are not yet clearly defined. There are now a few studies seeking to investigate the neuropsychological profile of individuals with TSC; see Prather and de Vries (2004) for a recent review. These authors suggested that attentional-executive deficits are consistent findings in the disorder and proposed that such functional abnormalities were due to aberrations in fronto-striatal circuits (Alexander and others 1986). There have, however, been no systematic investigations of memory functions in TSC nor any attempt to examine the neuroanatomical substrates of specific cognitive deficits.

The present study was designed with three main aims in mind. First, to confirm and replicate the finding of morphometric abnormalities in the brain and to test that these were not simply attributable to tubers, white matter lesions, or SENs;
second, to investigate the profile of memory function in normally intelligent adults with and without TSC; third, to explore correlations between memory performance and both brain morphometry and lesion load in search of the putative neuroanatomical substrate of memory deficits in TSC.

Materials and Methods

Subjects
Twenty-five adults with TSC (17 males, 8 females) and 25 healthy comparison subjects (16 males, 9 females) were studied. The groups were well matched for age, sex, handedness, and IQ; see Table 1 and Supplementary Data Table 1 for details. All participants had normal intelligence as assessed by both the National Adult Reading Test (Nelson and Willison 1991) and their age-adjusted scores on the Wechsler Abbreviated Scale of Intelligence (Wechsler 1999). All participants with TSC satisfied standard operationalized diagnostic criteria (Roach and others 1998). No subject suffered from other neurological or psychiatric disorder (except depression, which was present in 2 patients). Patients were interviewed using a structured questionnaire to ascertain the presence or absence of epilepsy and the characteristics of their epilepsy, that is, age at onset, duration and type of seizures, and current medication details (see Table 1 and for more details see Supplementary Data Table 1). At the time of the magnetic resonance imaging (MR), antiepileptic treatment included monotherapy or polytherapy with carbamazepine (patients \( N = 7 \)), lamotrigine (\( N = 4 \)), phenytoin (\( N = 3 \)), sodium valproate (\( N = 2 \)), levetiracetam (\( N = 1 \)), olanzapine (\( N = 1 \)), oxcarbazepine (\( N = 1 \)), phenobarbital (\( N = 1 \)), prednisolone (\( N = 1 \)), and vigabatrin (\( N = 1 \)). Note that 9 of the patients had never suffered from seizures. All healthy volunteers satisfied the following inclusion criteria: no personal or family history of neurological or psychiatric disorder, no history of prolonged loss of consciousness, no contraindication to MRI. The study was approved by the Local Research Ethics Committee (Addenbrooke’s NHS Trust, UK), and all participants gave informed written consent.

Structural MRI data acquisition
We used a GE Signa system (General Electric, Milwaukee, WI) operating at 1.5 T at the Magnetic Resonance Imaging and Spectroscopy Unit, Addenbrooke’s Hospital, Cambridge, UK. A preliminary localizing scan in the sagittal plane was used to identify anterior and posterior commissures and to prescribe acquisition of a dual-echo, fast spin-echo (FSE) data set in a near-axial plane parallel to the intercommissural line. Whole-brain coverage was provided by 40 contiguous, interleaved, 4-mm-thick proton density-weighted and \( T_2 \)-weighted images acquired with the following parameters: repetition time (TR) = 5625 ms; echo times (TEs) = 20 ms and 102 ms with an 8-echo train length; matrix size = 256 x 256; and field of view = 22 cm, giving an in-plane resolution of 0.859 mm. The total acquisition time was 12 min and 10 s. \( T_2 \)-weighted fluid-attenuated inversion recovery (FLAIR) data were also acquired in the same (near-axial) orientation as the dual-echo FSE data with TR = 10 002 ms, TE = 112.5 ms with 2 excitations, inversion time = 2250 ms; matrix size = 256 x 256, slice thickness = 4 mm. The total acquisition time was 14 min and 40 s.

Structural MRI Data: Segmentation, Normalization and Parcellation
MRI data were segmented and probabilistic maps of gray matter, white matter, and cerebrospinal fluid (CSF) were created for each subject. Tissue classification maps were coregistered with a customized template image in standard stereotactic space (Talairach and Tournoux 1988), using a 12-parameter affine transformation. Both segmentation and normalization were implemented using BAMM software (http://www.bic.mni.mcgill.ca/software; Brummer and others 1997; Suckling, Brummer, and others 1999; Suckling, Sigmundsson, and others 1999).

To facilitate later regional parcellation of the gray matter maps, each of these was also coregistered with the Montreal Neurological Institute (MNI) single-subject high-resolution \( T_2 \)-weighted image (MNI; http://www.bic.mni.mcgill.ca) using an identical procedure. The automated anatomical labeling (AAL) regionally parcellated template image (Schmahmann and others 1999; Tzourio-Mazoyer and others 2002), also in the space of the MNI \( T_1 \) image, was then used to estimate regional mean gray matter densities in each of 116 regions, which were aggregated to give 10 lobar and major subcortical structure measures in each hemisphere for each subject.

MRI Data: Statistical Analysis
Group differences in gray matter, white matter, and CSF volume were explored separately by fitting a linear regression model at each intracerebral voxel. Loci of significant between-group differences were identified by a nonparametric permutation test of suprathreshold voxel clusters, as described in detail elsewhere (Bullmore and others 1999; Suckling and Bullmore 2004). Thresholds for statistical significance were set such that the clusterwise probability of type I error \( P < 0.005 \) (for gray and white matter) and the expected number of false-positive tests was less than one per map.

MRI Data: Neuroradiological Assessment
To explore the relationship between neuroradiological lesion load, cognitive function, and morphometric abnormalities, we identified tubers and nodules by expert neuroradiological examination of the FLAIR and FSE data. Lesion locations were manually marked on the imaging data in native space, and maps of lesion location were then coregistered in standard space by the same procedures used to coregister the gray matter maps; see Ridler and others (2004) for detail. This procedure allowed us to estimate lesion load both in terms of whole-brain number of tubers and also in terms of lesion density (percent region occupancy) in each of the 10 lobar and major subcortical regions of interest defined by appropriately aggregating sublobar regional volumes defined by the MNI parcellated template image (Schmahmann and others 1999; Tzourio-Mazoyer and others 2002). The regional distribution of lesions in this group of patients and the relationships between the classical lesions are presented in detail elsewhere (Ridler and others 2004).

Neuropsychological tests
All subjects were examined using a battery of neuropsychological tests (for test details see Table 2). Group means and standard deviations (SDs) were estimated for all outcome measures of each test, and between-group differences were assessed by \( t \) tests. In order to provide comprehensive yet parsimonious measurements of cognitive functioning, the dimensionality of the neuropsychological data was reduced by summarizing the neuropsychological battery into cognitive domains; see Table 2. We computed a summary score of performance in each domain for each subject by standardizing all individual test scores

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Adults with TSC</th>
<th>Healthy volunteers</th>
<th>Between-group differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size (( n ))</td>
<td>25</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Gender, male/female</td>
<td>17.8</td>
<td>16.9</td>
<td></td>
</tr>
<tr>
<td>Handedness, right/left</td>
<td>19.6</td>
<td>20.5</td>
<td></td>
</tr>
<tr>
<td>Average age (SD)</td>
<td>39.3 (10.7)</td>
<td>34.3 (11.0)</td>
<td>( r = -1.620 ) (df = 48), ( P = 0.111 )</td>
</tr>
<tr>
<td>Average WASI IQ (SD)</td>
<td>116.9 (12.5)</td>
<td>116.7 (10.8)</td>
<td>( r = -0.049 ) (df = 48), ( P = 0.962 )</td>
</tr>
<tr>
<td>Average NART IQ (SD)</td>
<td>114.1 (9.3)</td>
<td>115.4 (5.1)</td>
<td>( r = 0.698 ) (df = 48), ( P = 0.548 )</td>
</tr>
</tbody>
</table>

Note: WASI IQ, Wechsler Abbreviated Scale of Intelligence (Wechsler 1999); IQ, NART IQ, National Adult Reading Test (Nelson and Willison 1991) IQ; Seizure free, patients who had not suffered from a seizure in the year preceding testing; Seizures currently uncontrolled, all patients who had suffered from a seizure in the last 12 months despite antiepileptic medication.

Average age (SD) 39.3 (10.7) 34.3 (11.0)
Average WASI IQ (SD) 116.9 (12.5) 116.7 (10.8)
Average NART IQ (SD) 114.1 (9.3) 115.4 (5.1)
Seizure free, medicated 4
Seizure free, unmedicated 7
Intractable spasms 6

Age of onset 8.67 (9.5)
Seizures currently uncontrolled 7
Seizure free, medicated 5
Seizure free, unmedicated 4
Intractable spasms 6

Ridler and others 2004) for detail. This procedure allowed us to estimate lesion load both in terms of whole-brain number of tubers and also in terms of lesion density (percent region occupancy) in each of the 10 lobar and major subcortical regions of interest defined by appropriately aggregating sublobar regional volumes defined by the MNI parcellated template image (Schmahmann and others 1999; Tzourio-Mazoyer and others 2002). The regional distribution of lesions in this group of patients and the relationships between the classical lesions are presented in detail elsewhere (Ridler and others 2004).

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(subtracting healthy group mean and dividing by healthy group SD) and averaging them within domain for each subject. This procedure reduced the total number of psychological variables per subject from 37 raw scores to 6 domain-specific Z-scores.

Structure-function Analysis of Neuropsychological and MRI Data

Associations between cognitive domain scores of interest and gray matter volume were tested by computational morphometry separately for each domain, and corroborated at a regional level of analysis, using a simple linear regression model with cognitive domain score as the independent variable. Voxel-level statistic maps, representing local strength of association between each cognitive domain score and gray matter structure (across all subjects), were tested for statistical significance using a nonparametric permutation test of the mass or sum of suprathreshold voxel clusters (Bullmore and others 1999; Suckling and Bullmore 2004). For whole-brain maps, the size of each clusterwise test was set such that the expected number of false-positive tests in each map was less than 1: clusterwise \( P < 0.005 \).

Cortical and subcortical areas of significant association identified in this way by whole-brain mapping were corroborated by fitting the same regression model to gray matter volumes in corresponding regions defined by the AAL template image and testing the strength of regional association by a \( t \)-test on the standardized regression coefficients (allowing a direct comparison with analysis performed on identically parcellated radiological measures of classical lesions). We also fitted an analysis of covariance (ANCOVA) model to estimate the main effect of parcellated radiological measures of classical lesions (allowing a direct comparison with analysis performed on identically parcellated radiological measures of classical lesions). We also fitted an ANCOVA model to estimate the main effect of diagnostic group (healthy volunteers vs. people with TSC), the effect of cognitive domain scores, and the group \( \times \) cognitive performance interaction on gray matter volume in the regions of association identified in the morphometric analysis of the entire sample. The same process was completed independently for each cognitive domain score.

Results

Structural MRI Data: Case-Control Differences

Whole-Brain and Total Tissue Class Volumes

There were no significant differences in whole-brain volume (2-sample \( t \)-test, \( t = -0.236, df = 48, P < 0.814 \)) or total gray matter (\( t = 0.529, df = 48, P < 0.599 \)), white matter (\( t = -0.687, df = 48, P < 0.495 \)), or CSF (\( t = -0.553, df = 48, P < 0.583 \)) volumes when patients with TSC were compared with healthy volunteers. Note that these whole-brain measures were estimated prior to spatial normalization of the images in each subject’s native space; the subsequent morphometric results, estimated after spatial normalization to a standard template image, will have effectively controlled for intersubject differences in global brain size.

Computational Morphometry of Gray Matter

The patients with TSC demonstrated extensive and bilaterally symmetric deficits of gray matter in the cerebellum, midbrain, thalamus, basal ganglia (caudate, putamen, and globus pallidus), and insula. There were also deficits in anterior cingulate cortex and right medial temporal lobe; see panel D of Figure 1. Over all these regions of deficit, gray matter volume was reduced by approximately 19% in patients with TSC (2-sample \( t \)-test, \( t = 7.132, df = 48, P < 0.001 \)).

Regional Volumes of Gray Matter

The patients with TSC demonstrated significant gray matter deficits in basal ganglia, thalamus, and cerebellum regions defined by a parcellated anatomical template image; see Table 3. It is important to note that these regional deficits remained significant even after gray matter volumes in the patient group were combined with tuber and nodule volumes in the corresponding regions, implying that the gray matter deficits were not simply attributable to classification of subcortical tubers or nodules as white matter or CSF; see Figure 2.

Computational Morphometry of White Matter

The patients with TSC demonstrated extensive and bilaterally symmetric deficits of white matter in the territory of major intrahemispheric tracts including the superior and inferior longitudinal fasciculi, subgyral white matter in the inferior parietal and medial temporal lobes, the tapetum, and the optic radiation. Over all these regions of deficit, white matter volume was reduced by approximately 15% in patients with TSC (\( t = 11.151, df = 48, P < 0.001 \)). There were also areas of white matter excess (\( t = -6.041, df = 48, P < 0.001 \)), which complemented and were located adjacent to the regions of gray matter deficit already described in cerebellum, thalamus, basal ganglia, and insula.

Computational Morphometry of CSF

The patients with TSC demonstrated significantly increased CSF volume in the lateral ventricles bilaterally and in the third ventricle. The relative increase of CSF in these regions was 28% (\( t = -4.5, df = 48, P < 0.001 \)). There was also an area of relative decrease of extracerebral CSF by 24% (\( t = 4.9, df = 48, P < 0.001 \)). These changes were in the absence of obstructive hydrocephalus.

Relationships between Morphometric Abnormalities and Radiological Lesions

The frequency and distribution of lesions in this sample of patients with TSC is presented in detail elsewhere (Ridler and others 2004). A summary of the lesion load profile is presented

Table 2

Neuropsychological tests summarized under six cognitive domains

<table>
<thead>
<tr>
<th>Domain</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Long-term memory</td>
<td>Rey-Osterrieth Complex Figure—delayed recall (Rey 1941; Osterrieth 1944)</td>
</tr>
<tr>
<td></td>
<td>CANTAB nonverbal paired associate learning (level 6)—total errors (Sahakian and others 1988; Fowler and others 1993)</td>
</tr>
<tr>
<td></td>
<td>WMS-III Logical Memory—inmediate and delayed recall (Wescaler 1997)</td>
</tr>
<tr>
<td></td>
<td>WMS-III Verbal Paired Associates—inmediate and delayed recall (Wescaler 1997)</td>
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<tr>
<td></td>
<td>Camden faces—total number correct (Warrington 1996)</td>
</tr>
<tr>
<td></td>
<td>Long-term memory encoding and recognition Task—number correct deep and shallow encoding levels (Craik and Lockhart 1972; Fletcher and others 2003)</td>
</tr>
<tr>
<td>2. Verbal working memory</td>
<td>WMS-III Digit Span—forward and backward (Wescaler 1997)</td>
</tr>
<tr>
<td></td>
<td>Verbal Sternberg (loads 1, 3, and 5)—total number correct (Stenberg 1969a, 1969b, 1975)</td>
</tr>
<tr>
<td>3. Spatial working memory</td>
<td>CANTAB spatial span—span (Owen and others 1990)</td>
</tr>
<tr>
<td></td>
<td>CANTAB spatial working memory—strategy score, both between- and within-search errors (Owen and others 1996; Joyce and Robbins 1991)</td>
</tr>
<tr>
<td></td>
<td>Spatial Sternberg task (loads 1, 3, and 5)—total number correct (Stenberg 1969a, 1969b, 1975)</td>
</tr>
<tr>
<td>4. Inhibition</td>
<td>MARS go/no-go Task—percent inhibition, omission, and prepotent errors (Rubia and others 2001)</td>
</tr>
<tr>
<td></td>
<td>MARS stop task—omission and prepotent errors (Rubia and others 2001)</td>
</tr>
<tr>
<td></td>
<td>MARS switch task—number correct for switch and repeat trials, omission, and prepotent errors (Rubia and others 2001)</td>
</tr>
<tr>
<td>5. Processing speed</td>
<td>Spatial Sternberg task—learning slope and 0-intercept (Stenberg 1969a, 1969b, 1975)</td>
</tr>
<tr>
<td></td>
<td>Verbal Sternberg task—learning slope and 0-intercept (Stenberg 1969a, 1969b, 1975)</td>
</tr>
<tr>
<td></td>
<td>MARS II go/no-go—mean reaction time (Rubia and others 2001)</td>
</tr>
<tr>
<td></td>
<td>MARS II stop—mean reaction time and stop signal delay (Rubia and others 2001)</td>
</tr>
<tr>
<td></td>
<td>MARS II switch—mean reaction time (Rubia and others 2001)</td>
</tr>
<tr>
<td></td>
<td>Long-term memory encoding and recognition task—mean reaction times for correct and incorrect responses (Craik and Lockhart 1972; Fletcher and others 2003)</td>
</tr>
<tr>
<td>6. Visuospatial skills</td>
<td>Rey-Osterrieth Complex Figure Test—copy (Rey 1941; Osterrieth 1944)</td>
</tr>
</tbody>
</table>

Note. WMS-III is the Wechsler Memory Scale 3rd edition, CANTAB is the Cambridge Neuropsychological Test Automated Battery, and MARS II is the Maudsley Attention and Response Suppression task battery II.
in Table 3. By inspection of Figure 1, the distribution of nodules, tubers, and white matter abnormalities (panel E) is directly comparable with the distribution of morphometric deficits in gray and white matter (panel D). It is evident that there was little exact anatomical coincidence between these two measures of neuroanatomical abnormality. More quantitatively, less than 11% of the gray or white matter deficits identified using morphometric analysis coincided anatomically with the locations of radiological lesions in one or more subjects.

There was, however, some evidence for correlation between morphometric and radiological abnormalities. Total SEN volume ($r = -0.41, P = 0.043$) and total tuber count ($r = -0.53, P = 0.006$) were both negatively correlated with white matter volume in the right frontal region of relative deficit in the TSC group. Total tuber volume was also negatively correlated with gray matter volume in bilateral thalamic and basal ganglia regions of relative deficit in the TSC group ($r = -0.383, P = 0.058$), although this association was not quite significant.

At a regional level of analysis, volumes of tubers and nodules in cortical and subcortical regions, respectively, were negatively correlated with gray matter volumes both locally and remotely. For example, tuber volume in the occipital lobe was negatively correlated with local (occipital) gray matter volume ($r = -0.491, P < 0.013$); tuber volume in frontal lobe was negatively correlated with gray matter volumes of remote regions (lateral temporal lobe [$r = -0.422, P < 0.035$] and insula [$r = -0.406, P < 0.035$]); and tuber volume in lateral temporal lobe was negatively correlated with gray matter volumes of medial temporal lobe ($r = -0.480, P < 0.015$) and thalamus ($r = -0.523, P < 0.007$). There were also negative correlations between SEN volume in basal ganglia and gray matter volumes of both basal ganglia ($r = -0.393, P < 0.052$) and thalamus ($r = -0.389, P < 0.055$), but these were not quite significant.

**Neuropsychological Tests: Case-Control Differences**

There was an uneven profile of cognitive impairment in the TSC group (see Table 4). Patients were significantly impaired on
tests of long-term memory, especially immediate recall rather than recognition, and on tests of working memory, especially the backward digit span and the between-search errors score of the Cambridge Neuropsychological Test Automated Battery (Ridler and others 2004). % of subjects, percent of subjects with lesion affecting that structure; mean % occupancy, the mean percent of lesion occupancy (lesion volume in a region/total volume of the region). Percent lobe occupancy was calculated for each subject and was less than 0.05 given in bold; ** indicates a significant difference between groups on measures of inhibition, processing speed, or visuospatial function (Table 4).

This pattern of results in the raw scores was confirmed by the analysis of between-group differences on the summary scores for each domain (Table 5). Patients with TSC were significantly ($P < 0.01$) impaired on average overall tests of long-term memory, verbal working memory, and spatial working memory; but not significantly impaired on tests of inhibition, processing speed, or visuospatial skills.

There were no significant correlations between age of seizure onset and cognitive domain scores within the subset of participants with TSC who had a lifetime history of seizures ($N = 16$). There was no significant difference in gray matter volume in deficit areas between the TSC patients with a history of seizures and the TSC patients without seizures ($F_{1,23} = 1.508, P = 0.232$). There was, however, still a significant difference in gray matter volume in deficit areas between healthy comparison subjects ($n = 25$) and subjects with TSC without a history of seizures ($F_{1,32} = 4.29, P < 0.001$).

**Structure-Function Associations**

**Long-Term Memory**

There was a significant positive correlation over all subjects between long-term memory $Z$-score and gray matter volume in bilateral thalamus (Talairach coordinates: 9, -10, 15), bilateral caudate nucleus, and left anterior cingulate cortex (approximate Brodmann area [BA] 32; Fig. 1A). In other words, better long-term memory performance was associated with greater gray matter volume in these regions.

Total gray matter volume estimated for each subject over all these regions of morphometrically defined structure-function association was correlated with long-term memory score ($r = 0.616, df = 49, P < 0.0001$; see Fig. 3A). An ANCOVA model fitted to the long-term memory scores using these anatomical summary data showed no significant effect of group ($F_{1,46} = 2.714, P < 0.106$), a significant effect of gray matter volume ($F_{1,46} = 15.928, P < 0.001$), but no significant group × gray matter interaction ($F_{1,46} = 0.151, P < 0.700$).

**Verbal Working Memory**

There was a significant positive correlation between verbal working memory $Z$-score and gray matter volume in bilateral thalamus (Talairach coordinates: 4, -13, 5), bilateral caudate nucleus, right parahippocampal gyrus (BA 30), and several regions of frontal cortex including medial, middle, and superior frontal gyri (BA 6, 8, and 9; Fig. 1B). In other words, better verbal

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**Table 3**

Regional distributions of tubers and nodules in patients with TSC and regional gray matter volumes in both patients with TSC and healthy volunteers.

<table>
<thead>
<tr>
<th>Anatomical region</th>
<th>Lesion load</th>
<th>Gray matter volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SNs</td>
<td>Tubers</td>
</tr>
<tr>
<td></td>
<td>% of subjects</td>
<td>mean % occupancy</td>
</tr>
<tr>
<td>Frontal</td>
<td>24.0</td>
<td>0.010</td>
</tr>
<tr>
<td>Occipital</td>
<td>0.0</td>
<td>0.000</td>
</tr>
<tr>
<td>Parietal</td>
<td>0.0</td>
<td>0.000</td>
</tr>
<tr>
<td>Temporal cortical</td>
<td>12.0</td>
<td>0.000</td>
</tr>
<tr>
<td>Temporal subcortical</td>
<td>16.0</td>
<td>0.000</td>
</tr>
<tr>
<td>Cingulate</td>
<td>16.0</td>
<td>0.004</td>
</tr>
<tr>
<td>Insula</td>
<td>16.0</td>
<td>0.000</td>
</tr>
<tr>
<td>Basal ganglia</td>
<td>56.0</td>
<td>0.661</td>
</tr>
<tr>
<td>Thalamus</td>
<td>48.0</td>
<td>0.181</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.0</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Note: This table summarizes the lesion load profile, presented in a form to aid the direct comparison of regional gray matter volumes with regional volumes of tubers and nodules. The frequency and distribution of lesions in this sample of patients with TSC is presented in detail elsewhere (Ridler and others 2004). % of subjects, percent of subjects with lesion affecting that structure; mean % occupancy, the mean percent of lesion occupancy (lesion volume in a region/total volume of the region).
workin memory performance was associated with greater gray matter volume in these regions.

Total gray matter volume estimated for each subject over all these regions of morphometrically defined structure-function association was correlated with verbal working memory score \( r = 0.616, df = 39, P < 0.0001; \) see Fig. 3B).

An ANCOVA model fitted to the verbal working memory scores using these anatomical summary data showed a significant effect of group \((F_{1,36} = 6.498, P < 0.015)\), a significant effect of gray matter volume \((F_{1,36} = 69.392, P < 0.001)\), but no significant group \(
\text{X}\) gray matter interaction \((F_{1,36} = 1.733, P < 0.196)\).
There was a significant positive correlation between spatial working memory Z-score and gray matter volume in bilateral thalamus (Talairach coordinates: 2, -2, 13), bilateral caudate nucleus, and left anterior cingulate cortex (Fig. 1C). In other words, better spatial working memory performance was associated with greater gray matter volume in these regions.

Total gray matter volume estimated for each subject over all these regions of morphometrically defined structure–function association was correlated with spatial working memory score ($r = 0.600, df = 49, P < 0.0001$; see Fig. 3C). An ANCOVA model fitted to the spatial working memory scores using these anatomical summary data showed no significant effect of group ($F_{1,46} = 2.268, P < 0.139$), a significant effect of gray matter volume ($F_{1,46} = 10.030, P < 0.003$), and a significant group × gray matter interaction ($F_{1,46} = 4.472, P < 0.040$).

**Structure–Function Associations: Volumetric Results**

We sought to corroborate the results of computational morphometry by testing for association between memory performance and regional volumes estimated by automated parcellation in thalamus and basal ganglia. Gray matter volume in thalamus was positively correlated with verbal working memory ($r = 0.363, df = 39, P < 0.021$), spatial working memory ($r = 0.330, df = 49, P < 0.019$), and long-term memory ($r = 0.271, df = 49, P < 0.057$). Gray matter volume in basal ganglia was positively correlated with verbal working memory ($r = 0.307, df = 39, P < 0.054$), spatial working memory ($r = 0.349, df = 49, P < 0.013$), and long-term memory ($r = 0.273, df = 49, P < 0.055$).

**Structure–Function Associations: Neuroradiological Lesion Load**

The total number of tubers, the total volume of tubers, and the total volume of SENs were estimated for each patient with TSC. There was no significant correlation between any of these global measures of lesion load and cognitive performance summarized by any of the domain-specific Z-scores. There were also no significant correlations between regional measures of lesion load (see Table 3) and cognitive performance summarized by any of the domain-specific Z-scores.

**Discussion**

We have reported 3 main findings: evidence for a bilaterally symmetric, predominantly subcortical pattern of gray matter deficit in patients with TSC; evidence for an uneven profile of memory impairment implicating long-term and working memory in these high-functioning patients compared with IQ-matched volunteers; and evidence that memory performance was positively correlated with gray matter density in thalamic, striatal, and prefrontal brain regions. It therefore seems likely that a key neuroanatomical substrate of selective memory deficit in these patients with TSC is gray matter deficit in subcortical nuclei (thalamus and caudate). In contrast, there was no evidence for a significant association between cognitive impairment and neuroradiological lesion load in patients with TSC.
Extending the Definition of Neuroanatomical Abnormalities in TSC

We have demonstrated extensive and symmetric deficits of gray matter in subcortical structures (cerebellum, thalamus, and basal ganglia) that broadly replicate the bilaterally symmetric deficits of subcortical gray matter that we reported in a previous morphometric analysis of an independent study of adults with TSC (Ridler and others 2001). We have also replicated our previous finding of bilaterally symmetric and extensive deficits of white matter in the territories of major intrahemispheric tracts (Ridler and others 2001). Interestingly, there is significant overlap between deficit regions reported here and subsequent findings identified using a manual measurement technique (Yorns and others 2001; DiMario 2004), despite different samples and methods. Moreover, in the current study, we have coregistered whole-brain maps of gray matter and white matter in a standard space with radiologically defined maps of tubers and nodules. This has both facilitated a quantitative analysis of classical lesion load and allowed us to explore systematically the nature of association between morphometric and radiological markers of neuroanatomical abnormality in patients with TSC.

We have previously reported that the distribution of tubers and nodules is regionally heterogeneous but not significantly lateralized and that the density of tubers and nodules is positively correlated (Ridler and others 2004). There was a minor degree of exact anatomical coincidence between the distributions of tubers and nodules and the distributions of gray and white matter deficit in people with TSC. However, there was only one example (in occipital cortex) of a strong negative correlation between tuber volume and local gray matter volume and only one marginally significant example of a negative correlation between nodule volume and local gray matter volume (in basal ganglia). This suggests that not all parenchymal deficits directly reflect local lesion load. We also tested the related hypothesis that deficits could apparently arise due to local replacement of typical gray matter by atypical tubers or nodules that might be less probably classified as gray matter by our segmentation algorithm. However, the volumes of radiological lesions were generally small compared with the scale of between-group differences in gray matter, and gray matter deficits in cerebellum, thalamus, and basal ganglia remained significant even after correction of regional volumes for the presence of tubers or nodules in the group of patients with TSC.

In addition to these results indicating that gray matter deficits could not simply be explained by the local density of classical lesions, we also found a number of negative correlations between tuber density and gray matter volume in remote cortical or subcortical structures and a negative correlation between tuber density and right frontal white matter. This finding of morphometric abnormalities correlated with a higher lesion load (whether measured by lesion volume, as in the current study, or lesion number as in some previous studies) has some support from the literature. For example, there are prior reports of a relationship between lesion number and both increased ventricular size (DiMario 2004) and decreased subcortical gray matter volume (Ridler and others 2001). Prior data show that radial migration of tubers to cortex disrupts subjacent white matter and leaves a trail of dysplastic neuronal precursor cells (Braffman and others 1992). These architectural and cellular abnormalities of white matter in the path of tuber migration might be expected to interfere with normal axonal guidance mechanisms that are important in orienting axonal projection from normal cortex in the vicinity of the tuber to distant cortical and subcortical targets. In short, radial migration of tubers to frontal cortex may, for example, disrupt later developmental processes important for securing anatomical afferentation of remote cortical and subcortical regions by frontal cortex; and relative absence of afferentation is known to have a negative effect on the growth of cortical and subcortical structures (Kolb and others 1983, 1998). This mechanistic hypothesis could be tested in future studies using diffusion tensor imaging to quantify more precisely white matter projections between cortical regions of high tuber density and distantly interconnected cortical and subcortical regions that show negatively correlated reductions in gray matter volume.

Memory Function and Its Neuroanatomical Correlates in TSC

The cognitive profile was uneven for patients with TSC, with patients performing as well as IQ-matched controls on most tasks and disproportionately badly on tasks within several domains. There were significant group differences on all measures of cued or free recall for both verbal and visual modalities. This was specific to immediate recall of information, and there was not an increased rate of forgetting with delay. There were also no differences in any of the measures of recognition memory, despite the fact that verbal recognition scores showed no ceiling effect (a problem that often complicates interpretation of recognition performance). There were significant differences in both the spatial and verbal working memory tasks, which involved executive processes. However, there were no differences on either the verbal or spatial Sternberg tasks, which involved maintenance of information in working memory but no manipulation. There were no salient group differences in any measures of either inhibition or speed of information processing. Therefore, these were not considered to be the underlying cause of TSC group deficits for long-term memory retrieval or executively demanding working memory.

At first glance, this profile seems more “frontal” than might be expected in a classical amnesic syndrome due to medial temporal lobe or diencephalic damage. For example, impairment of recall in the context of spared recognition, no exacerbation of recall as a function of increasing delay, and intact forward digit span but impaired backward digit span are all compatible with a frontal profile of memory impairment (Lezak 1983; Kopelman 2002). There were, however, also differences between the profile of TSC deficits and the classical profile of frontal lobe patients. In fact, the profile of deficit in the TSC group was consistent with neither a classical amnesic syndrome nor a classical frontal syndrome. It is interesting to note that the profiles of both cognitive and brain structural abnormalities share some similarities with the patterns found in normal aging (Rubin 1999).

Results, however, showed clearest similarities to the neuropsychological profile produced by basal ganglia and thalamic abnormalities that has sometimes been termed “frontal-like” or fronto-striatal in nature (Owen and others 1990). The TSC long-term memory profile was similar to that of patients with early Huntington’s disease (a basal ganglia disorder) (Lawrence and others 2000) and patients with thalamic lesions (Van der Werf and others 2003; Zoppelt and others 2003). Both of these subcortical disorders cause specific deficits in long-term
memory recall, without an increased rate of forgetting, while recognition is usually spared. In relation to working memory, there were also subtle differences between the pattern of test results observed in these subcortical disorders and in frontal lobe lesions. For example, the pattern of SWM deficit in both TSC and early subcortical dementia (Huntington's disease and Parkinson's disease) spared strategy and within-search errors while between-search errors were increased (Owen and others 1990, 1992, 1993, 1997, 1998; Lawrence and others 1998, 2000); whereas patients with frontal lobe lesions typically show an increase in both between- and within-search errors and impairment in the use of an effective strategy (Owen and others 1990; Robbins and others 1999). These long-term and working memory deficits in basal ganglia and thalamic disorders are possibly caused by interference with a critical memory circuit including diencephalic structures, cingulate cortex, temporal lobes, and frontal cortex (Kopelman 2002). One generalization emerging from these and other data is that aspects of both long-term and working memory function may depend on integrated function of similar fronto-striato-thalamic circuits (Alexander and others 1986; Robbins and others 1999; Watkins and others 2000; Green and others 2002; LaBerge 2002). In this context, it is not surprising that subcortical gray matter deficits in TSC are associated with impaired long-term recall and manipulation in working memory (although the dependence of memory in TSC on fronto-striatal circuits also damaged by neurodegenerative disorders should not, of course, be taken to mean that TSC is a progressively dementing neurodegenerative disorder).

We found that volumetric changes in radiologically normal gray matter—most consistently, thalamus, and caudate nucleus—predicted cognitive performance in these domains. The Talairach coordinates of the thalamic locus most consistently or strongly correlated with long-term memory deficit suggest that deficits in the dorsomedial nucleus, implying disruption of the prefronto-caudate-thalamic circuit, are especially prejudicial to recall from long-term memory. The strong correlation of bilateral caudate and thalamic regions and performance in both working memory domains also fits well with other evidence of subcortical involvement in executive components of working memory via fronto-striatal circuits. Furthermore, there appears to be an overlap between the fronto-striatal circuits involved in the executive or attentional control aspects of both working memory and the retrieval aspects of long-term memory tasks that was disrupted on several tests in TSC.

Memory performance was generally dependent on some of the areas identified as abnormal by morphometric case-control comparison rather than on the volume of radiological lesions. There was, in fact, no relationship between global or regional lesion volume and neuropsychological performance. However, the percentage of brain volume affected by lesions in this sample was relatively small, and lesions are known to develop early in brain development, providing ample opportunity for compensatory cortical reorganization.

**Molecular Mechanisms of Memory Impairment in TSC**

We know that TSC is a neuronal migration disorder with focal and widespread anatomic and histological abnormalities identifiable within the brains of patients with TSC (DiMario 2004). These migrational disturbances are a direct result of disturbed cellular control owing to a loss of the functional interaction of the hamartin-tuberin complex with its downstream nuclear signaling components (DiMario 2004). As a consequence, regional morphometric brain disturbances have been described both in conjunction with and separate from migration lesions (Ridler and others 2001; DiMario 2004).

It is interesting to note that memory deficits were specifically implicated in our study of TSC when we recall that one of the downstream targets of hamartin and tuberin is molecular target of rapamycin (mTOR) (Au and others 2004). mTOR is implicated in the cellular mechanisms of memory (Kelleher and others 2004) and, although present in the classical brain lesions, tuberin and hamartin are unable to regulate the activity of downstream targets like mTOR in patients with TSC (Han and others 2004). It will be important in future studies to integrate molecular and system-level markers relevant to memory dysfunction in TSC and, indeed, to consider a possible therapeutic role for rapamycin in prevention or remediation of cognitive deficits associated with this disorder.

**Inhibition and Information-Processing Speed**

An important question in the neuropsychological profiles of individuals with TSC is the extent to which findings such as memory deficits are specific or secondary to other factors such as general intellectual ability, response inhibition, or processing speed. Here we studied IQ-matched samples, thus controlling experimentally for effects of general ability. We also reported that there were no significant group differences on tasks of response inhibition or processing speed. It is important to note that the discussion so far has concentrated on group differences. However, there was an increased variability of performance rates in the TSC group on all measures of both inhibition and processing speed, and, in both domains, there were some individuals who were significantly impaired, with scores at or below the 5th percentile of performance by the healthy comparison subjects. In short, findings suggest that the profile of working memory deficits and impaired recall but intact recognition is not attributable to these general factors. However, it seems likely that, for a subset of subjects, inhibition or information processing deficits may be a factor in poor performance on memory measures.

**Methodological Issues**

Some methodological issues deserve comment. First, we note that several of our patients had a history of epilepsy, which may be associated with brain structural changes in its own right. However, our results indicate that gray matter deficits could not be solely attributed either to the effects of seizures or to antiepileptic medication as patients with TSC and no history of seizures still showed significant gray matter deficits in the same subcortical regions as those with a history of epilepsy and antiepileptic medication. Second, concerns regarding the validity of between-group differences defined by computational morphometry and their possible sensitivity to imperfect registration of regional boundaries (Bookstein 2001) have been addressed by the complementary analysis of gray matter differences at a regional level of analysis, using a prior anatomically parcellated template image (Schmahmann and others 1999; Tzourio-Mazoyer and others 2002). These volumetric results substantially corroborated those obtained by computational morphometry in terms of key deficits in thalamus, basal ganglia, and cerebellum. Third, we acknowledge that the sample size is modest (N = 50 for between-group comparisons) and is reduced...
further for some analyses, for example, the within-group correlational analyses of the relationship between regional gray matter volumes and classical lesion load in the patient group (N = 25). Under these circumstances, we can expect some degree of type 2 error; largely for this reason, we have highlighted some trend correlations (0.05 < P < 0.1) that might be significant in a larger study. Fourth, we have studied a group of individuals with TSC who have normal intellectual abilities (mean IQ = 117). About 55% of individuals with TSC are known to have normal global abilities, and our results are likely to be fairly representative of this group. Extending this approach to include the remaining 45% of individuals may help to shed some light on possible structural determinants of global abilities in TSC. Fifth, our group of patients was not genotyped and may have been heterogeneous in terms of underlying genetic mutations. It is an interesting question for future research to explore possible endophenotypic differences in brain structure between individuals with mutations of TSC1 and TSC2 genes.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

Notes
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