Iron concentrations are elevated in cortical and basal ganglia regions in Alzheimer disease (AD) patients1-3 and indicate a disruption of iron homeostasis in the brain. Higher iron concentrations in the AD brain may increase the possibility of free iron catalyzing lipid peroxidation, which can lead to cell membrane damage and cell death. Improved histochemical stains now reveal iron in attenuated core, clear core, amorphous amyloid plaques, in neurofibrillary tangles, and in cortical neurons in AD brains.4,5 Iron also promotes the aggregation of β-amyloid peptide6 and increases β-amyloid toxicity.7 Although AD is traditionally characterized as a gray matter disease, histopathologic,8 biochemical,9,10 and MR imaging11-16 changes in AD white matter have been observed in several studies. Some early research12 indicated that white matter changes in AD were not purely a secondary phenomenon related to neuronal degeneration, while reports from the past few years17,18 suggest that demyelination may have closer links to AD pathogenesis than previously thought.

Quantitative measurements of the proton transverse relaxation rate (R2) using MR imaging have the potential to indirectly measure brain iron and characterize other features of AD white-matter pathology in vivo. MR imaging probes the net magnetization of protons in the presence of an external magnetic field. R2 is defined as the reciprocal of the proton transverse relaxation time, T2 (ie, R2 = 1/T2). Iron has a large effect on R2, because it has a very large magnetization in the magnetic field of the MR scanner and hence causes local distortions of the magnetic field, which enhance the relaxation rates of diffusing water molecules. In contrast to the effects of iron, the breakdown of myelin in AD17,18 results in increased water content, particularly in white matter,18,19 reducing R2. As a generalization, the greater the concentration of water molecules in brain tissue, the lower the relaxation rate (ie, longer T2 relaxation time), provided other tissue parameters (like iron content) remain constant. Hence, in normal cortical brain tissue, where gray and adjacent white matter have similar iron concentrations,20 the typical R2 of gray matter is smaller (10.9 seconds⁻¹) than the typical water content higher (86%) compared with white matter (mean R2, 13.3 seconds⁻¹; mean water content, 73%).21,22

There are few quantitative in vivo MR imaging studies investigating R2 changes between AD and control subjects,14,23-29 and only one,25 to the our knowledge, that includes participants potentially in the preclinical stage of the disease. Studies14,23,24 comparing the field-dependent R2 increase (FDRI) between AD and healthy patients reported higher R2 values in the basal ganglia and lower R2 values in the frontal white matter of patients compared with controls. One report29 showed no change in hippocampal T2 values in AD patients compared with controls, whereas 3 other studies25,26,28 observed increases in hippocampal T2 times (R2 reduction). Laasko et al25 also examined quantitative T2 changes in a nondemented group with memory impairment (age-associated memory impairment or AAMI) in addition to AD participants. In this study,25 there was no significant difference in T2 observed between the AAMI group and elderly controls for the hippocampus, amygdala, thalamus, and temporal and
parietal white matter. These previous studies were limited, however, in that cortical gray matter regions typically affected in AD (eg, temporal lobe) were not examined,23-29 nor was T₂ always measured in high-iron basal ganglia regions,75-29 in which iron³ and R₂,²³,²⁴ can increase in AD. For other studies no white matter,²⁷-²⁹ or only one white matter region,¹⁴,²⁴-²⁶ was measured, preventing a wider assessment of white matter changes. The MR imaging protocols used in prior studies were also not optimal, which may have impeded the accurate measurement of the R₂ parameter (eg, only 2 spin-echoes used for R₂ calculation),¹⁴,²³,²⁴,²⁸ thick sections increasing partial volume artifacts²⁵,²⁷).

In the present study, we have applied a simple, but sensitive, quantitative proton transverse relaxation rate (R₂) scanning protocol to multiple cortical and subcortical gray and white matter regions in the brains of elderly participants with memory impairment (subjectively and objectively defined) and healthy age- and sex-matched controls without memory problems. The participants with memory problems represented a cognitive continuum, from those with subjective memory complaints with no objectively defined impairment to patients with severe, objective cognitive impairment and likely dementia. This study was so designed to assess changes in R₂ across a broad range of cognitive performance levels and, more specifically, to examine correlations between neuropsychological test scores and R₂ data. We adapted a single-spin-echo MR imaging protocol³⁰ that has been widely used in our laboratory. This protocol has previously been used to quantify hepatic iron concentration in both iron-loaded hemochromatosis and thalassemia patients and noniron-loaded hepatitis patients with iron concentrations as low as 40 μg/g (wet weight). By comparison, the basal ganglia of the normal human brain have iron concentrations ranging from 90 to 210 μg/g (wet weight).²¹

Although memory impairment is not unique to AD, it is one of the main symptoms that can become apparent early in this disease. There is currently no direct evidence that brain iron levels are elevated in nondemented people with memory problems. Oxidative damage, however, is one of the earliest pathologic events in AD,³¹ occurring long before clinical symptoms of the illness are usually observed.²⁴ Furthermore, iron may be an important contributor toward this oxidative damage.²⁷,³² Taken together, these observations suggest that there could well be elevated iron levels in the brains of individuals with age-related memory impairment. There is also some indication that white matter T₂-hyperintensities (low R₂) are associated with subjective cognitive failures³³ and suggestions that white matter atrophy may precede gray matter atrophy in preclinical AD patients.²⁴ Hence, our hypothesis is that, in participants with memory problems, R₂ will show an increase relative to controls in gray matter regions, which reflects iron increases, and show a decrease in white matter regions, which suggests a breakdown in myelin and increasing water content. The hypothesized directionally opposite involvement of R₂ in gray and white matter related to memory loss should also produce contrasting correlations between R₂ and neuropsychological test scores, depending on the brain tissue type.

### Methods

#### Participants

Twelve participants reporting memory loss were examined (Table 1). The participants were recruited by (1) an experienced practicing geriatrician based at a tertiary teaching hospital and a local private hospital and (2) a multidisciplinary clinical team specializing in aging and dementia, based at the Neurosciences Unit of the State Health Department. Eleven age- and sex-matched healthy control participants (Table 1) were recruited from a data base of control volunteers, referred via (1) an AD research unit and (2) private recruitment. The control participants had no reports or indications of memory problems, no family history of AD or other neurodegenerative diseases, and no history of a previous head injury. The ethics committee of the hospital where the MR imaging scanning took place approved the study. Written consent was provided by all participants or by their authorized representative (in the case of any patients whose competence to consent may have been compromised), after appraising an information sheet outlining the MR imaging procedure and study protocol.

#### Memory and Cognitive Assessment

An experienced psychologist administered the California Verbal Learning Test (CVLT) on the day of the MR imaging scan. One participant did not complete the CVLT on the day of scanning but had previously undergone a CVLT examination 11 days before imaging took place, and these CVLT data were therefore used instead. The CVLT was used to assess verbal learning and memory status objectively. Participants were asked to learn and immediately recall a 16-word list across 5 learning trials (immediate recall). They were then asked subsequently to recall these items after both a short and long delay. Participants were further tested on their ability to distinguish between “old” (ie, previously presented items) and “new” items (ie, items that had not been previously presented) in a forced choice “yes/no” response format. During the delay period of the CVLT, the Mini-Mental State Examination (MMSE) and the National Adult Reading Test (NART) were administered to the participants. All volunteers also completed a self-evaluation stress, anxiety, and depression questionnaire (DASS21). To assess how cognition and memory levels might affect R₂, the participants were separated into 2 subgroups (MC1, ie, no cognitive impairment and MC2, ie, mild to severe cognitive impairment) by using a cutoff score of 24 on the MMSE (MC1 ≥ 24, MC2 < 24, see Table 1).

### Table 1: Demographic details and neuropsychological test scores of the participants

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>MC1</th>
<th>MC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of individuals</td>
<td>11</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Age (y)</td>
<td>70.7 (6.9)</td>
<td>69.2 (8.8)</td>
<td>75.5 (8.8)</td>
</tr>
<tr>
<td>Gender % (no. of men)</td>
<td>27 (3)</td>
<td>33 (2)</td>
<td>17 (1)</td>
</tr>
<tr>
<td>Education (y)</td>
<td>11.6 (2.5)</td>
<td>10.3 (3.7)</td>
<td>11.2 (2.6)</td>
</tr>
<tr>
<td>MMSE</td>
<td>28.0 (1.7)</td>
<td>25.5 (1.8)*</td>
<td>18.5 (4.3)*</td>
</tr>
<tr>
<td>NART</td>
<td>110.7 (6.5)</td>
<td>104.9 (4.2)</td>
<td>113.5 (6.0)</td>
</tr>
<tr>
<td>CVLT-IFR</td>
<td>52.46 (9.1)</td>
<td>43.17 (8.4)</td>
<td>18.67 (8.6)*</td>
</tr>
<tr>
<td>CVLT-SDFR</td>
<td>10.00 (2.78)</td>
<td>8.67 (1.97)</td>
<td>0.50 (0.84)*</td>
</tr>
<tr>
<td>CVLT-LDFR</td>
<td>10.64 (2.98)</td>
<td>9.00 (2.97)</td>
<td>0.17 (0.41)*</td>
</tr>
</tbody>
</table>

*Significant difference between controls and MC1 or MC2 subgroups (P < .05, two-tailed test).
**MR Imaging Scanning Procedure**

MR imaging scanning was performed on a 1.5T whole-body scanner (Siemens Magnetom Vision Plus). A head coil was used for examination of the brain tissue. The heads of all participants were secured firmly, but comfortably, in the head coil. Coronal images were acquired in half Fourier mode with a multisection single-spin-echo pulse sequence. Coronal images were used because the hippocampus is more easily identified in this acquisition plane. A pulse repetition time (TR) of 2500 milliseconds and 7 echo times (TE) of 6, 15, 25, 40, 60, 80, and 100 milliseconds were used. An inversion recovery sequence (TIR) was also acquired after the spin-echo protocol to assist in anatomic identification. Coronal image sections 5 mm thick were measured over 192 by 256 (phase encoding direction by frequency encoding direction) or 160 by 256 image reconstruction matrices with a field of view (FOV) of 180 × 240 mm or 150 × 240 mm, giving a pixel resolution of about 1 mm. Twenty-two coronal images (section gap, 1.5–2.5 mm) were acquired for each subject. A 1-L bag of Hartmann solution (compound sodium lactate, Viaflex) was imaged in anatomic identification. Coronal image sections 5 mm thick were measured over 192 by 256 (phase encoding direction by frequency encoding direction) or 160 by 256 image reconstruction matrices with a field of view (FOV) of 180 × 240 mm or 150 × 240 mm, giving a pixel resolution of about 1 mm. Twenty-two coronal images (section gap, 1.5–2.5 mm) were acquired for each subject. A 1-L bag of Hartmann solution (compound sodium lactate, Viaflex) was imaged in situ with the brain tissue to provide a reference signal intensity for the correction of instrumental drift.30

**R₂ Image Analysis**

Before R₂ computation, the source images were checked for any image co-registration problems. In a few participants there were minor mis-registrations between echo times (at most 2 pixels), and these registration errors were manually corrected in the source images before R₂ processing. Freehand regions of interest, delineating specific brain regions, were drawn on spin-echo images by using in-house software (Fig 1). R₂ values for each region of interest were calculated according to the methodology of St. Pierre et al.30 The technique takes into account factors such as background noise, machine signal intensity level offsets, and gain drift between measurements. Because a single-spin-echo technique was used, magnetization transfer effects were assumed to be minimal.33 The transverse relaxation rate, R₂, was calculated by curve fitting the voxel signal intensity data for all echo times to a monoexponential spin-echo signal intensity equation. Spin density (Io) values for each voxel were also computed by extrapolation of the monoexponential curve to TE = 0 milliseconds. For one subject, only 6 echoes were used to fit the signal intensity decay data because the Hartman solution bag moved during the last echo time (TE = 15 milliseconds). One image analyst defined all anatomic regions and generated the R₂ images. The R₂ analysis was performed blind to the identity of the participants.

To ensure a homogeneous region was defined, and minimize the contribution of CSF, the R₂ and Io histograms of each region were assessed before recording the final R₂ value. CSF has a small R₂ value (long T₂) and hence partial volume contamination of voxels can lead to an underestimation of the average R₂ value within a region of interest. To reduce CSF partial volume effects, Bartzokis et al.23,24 used histogram analysis of the T₂ data within a region of interest to identify and remove pixels that fell above the high-end inflection point of their T₂ histogram. We adopted a similar procedure.

Regions of interest were first delineated on the early echo time images (6 or 15 milliseconds). The R₂ and spin density values for each pixel were then computed, as described above, and displayed as an image and as a histogram distribution. Isolated pixels, typically <8 seconds⁻¹, were removed from the R₂ image by using an interactive graphic editing tool and observing the results on the updated R₂ histogram. These edited pixels were either less than the minimum between the 2 modes observed in the R₂ histogram or less than the low-end inflection point of the R₂ histogram where the CSF and brain tissue components of the histogram were not fully resolved. As an additional check, we also inspected the spin-density histogram for CSF contributions. Although the spin density is proportional to water concentration in brain tissue, the high water content of CSF does not produce high spin-density values. Instead, because the T₁ time of CSF is comparable to the repetition time (2500 milliseconds), the CSF signal intensity saturates and produces a low spin-density value. The spin-density image and histogram can therefore be used to assess CSF contributions (low spin density) to regions of interest in a process similar to the R₂ analysis. After this histogram analysis procedure, the mean R₂ value for the region of interest was recorded. This procedure, together with a visual inspection of late echo-time images (TE 80 milliseconds), ensured that mostly normal-appearing white matter (NAWM) was included in the analysis.

Cortical gray matter and the adjacent white matter, along with small regions such as the substantia nigra and red nucleus, can be difficult to delineate and identify on spin-echo images, increasing the possibility of partial volume effects from the adjacent brain tissue affecting the analysis. For example, in a few participants the image contrast or quality was insufficient to accurately delineate gray from white matter in the frontal and temporal lobes. In other instances, variations in cortical structure meant that it was not always possible to define the same location on both sides of the brain. Hence, for some participants, it was not possible to obtain an analysis in these regions. In such cases, these regions were therefore not included for analysis resulting in the following small reductions in the number of samples. Sample numbers for the controls, MC1, and MC2 subgroups were 11, 6, and 6, respectively, with the following exceptions: red nucleus left, right (controls = 8), temporal white right (controls = 10; MC2 = 4),
temporal gray left (MC1 = 5), frontal white left, right (MC1 = 5; MC2 = 5), frontal gray left (MC1 = 5), and substantia nigra right (MC1 = 5).

Fourteen cortical and subcortical gray and white matter regions from 2 noncontiguous sections were analyzed (Fig 1). The globus pallidus, putamen, caudate nucleus, temporal and frontal cortex (gray and white matter), corpus callosum (body), internal capsule (anterior limb), and subcortical frontal white matter were measured from the anterior section (Fig 1A). The anterior section was selected to maximize the area of the lenticular nucleus and caudate and was positioned approximately through the anterior commissure. All white matter regions were measured from the anterior section. The hippocampus, thalamus, substantia nigra, and red nucleus were measured from the posterior section (Fig 1B), which was typically located 2 sections (about 15 mm) posterior to the first. In 3 control participants, the location of the posterior section did not include the red nucleus. Both hemispheres were measured separately to allow hemispherical comparisons. The globus pallidus, putamen, caudate nucleus, substantia nigra, and red nucleus were evaluated because they have some of the highest iron concentrations in the brain.21 In postmortem examinations of AD patients, increased iron concentrations have previously been measured in the globus pallidus, hippocampus, amygdala, and frontal, motor, parietal, and temporal cortex.1-5 The hippocampus is also strongly affected early in AD pathogenesis, but there are conflicting reports25-29 regarding the significance of T2 changes in this region. Frontal white matter regions have been measured in all of the Bartozzis studies,14,23,24 and these researchers consistently measure lower R2 values in AD compared with controls. In our study, 5 white matter regions from one section were analyzed to explore interregional variations in R2 in this tissue type.

Statistical Analysis

To corroborate the validity of the R2 technique for assessing brain iron concentrations, the average R2 values for several brain regions from the control participants were plotted against the postmortem iron concentrations of normal adults published by Hallgren and Sourander.21 The Hallgren and Sourander21 data provide reference iron concentrations for many of the brain regions analyzed in this study, thereby allowing us to validate our MR imaging protocol for assessing brain iron in vivo.21 The correlation between R2 and iron concentration was thereby established, an analysis of variance was used to determine which of these group means significantly differed (t test, as directional changes in R2 were hypothesized. The right and left sides were treated independently, because the effects of cognitive impairment may not be symmetric. Iron levels tend to increase with age, but typically reach a plateau in the elderly population.21 There are also indications that R2 values in white matter correlate strongly with age.25 Therefore, to eliminate the effect of age itself on iron levels or white matter R2 values in comparisons between groups, an analysis of covariance (with age as the covariate) was also used to assess R2 differences.

To investigate correlations between R2 values and cognitive status for the memory-complaint participants, the data for the 2 groups were first pooled (MC1 and MC2) to cover the widest range of scores.
**R₂ Differences between the Groups**

After ANCOVA adjustment for age (where applicable), the least cognitively impaired memory-complaint subgroup, MC1, had significantly higher R₂ values in the right temporal cortex (Fig 3A) and significantly lower R₂ values in the left internal capsule (Fig 3B) compared with the controls. In the most cognitively impaired subgroup, MC2, R₂ values were significantly higher in the right temporal cortex (Fig 3A) and significantly lower in the left internal capsule (Fig 3B), frontal and temporal (Fig 3C) white matter, compared with the controls. There was a significant difference in R₂ values between the MC1 and MC2 subgroups (MC2 lower) for the right subcortical frontal white matter (mean difference = 0.92 seconds⁻¹; t = 1.977; P = .038), and both sides of the frontal white matter, (left mean difference = 1.34 seconds⁻¹; t = 2.175; P = .031; right mean difference = 1.56 seconds⁻¹; t = 3.303; P = .005) after ANCOVA adjustment for age (where applicable).

A plot of differences in R₂ between the memory-complaint subgroups and the control group graphically demonstrates the major trends in R₂ for all gray and white matter regions (Fig 4) and some interesting results for individual regions. For example, the right hippocampus of the MC1 subgroup had one of the numerically largest rises in R₂ (Figs 3D and 4A), and this increase approached statistical significance (P = .0514; one-tailed t test); however, hippocampal R₂ values in the most impaired subgroup (MC2) were essentially unchanged compared with the controls (Fig 4B). The red nucleus and caudate nucleus were the only other gray matter regions, apart from the temporal cortex, to show a consistent trend toward increasing R₂ values in both hemispheres for the MC2 subgroup (Fig 4B). In both memory-complaint subgroups, there was a consistent, but statistically insignificant, trend toward lower R₂ values in the putamen and substantia nigra (Fig 4). The most consistent trend observed, however, was decreasing R₂ values for all white matter regions of both hemispheres in the MC2 subgroup compared with the controls (Fig 4B).

**R₂ Correlations with Neuropsychological Data**

Two gray matter regions from the combined memory-complaint group (MC1 + MC2), the thalamus and particularly the red nucleus, showed moderate-to-strong statistically significant correlations with neuropsychological measures (Fig 5). Some interesting results for individual regions include a significant correlation between R₂ values in the red nucleus and neuropsychological measures (Fig 5A). In both memory-complaint subgroups, there was a consistent, but statistically insignificant, trend toward lower R₂ values in the putamen and substantia nigra (Fig 5B). The most consistent trend observed, however, was decreasing R₂ values for all white matter regions of both hemispheres in the MC2 subgroup compared with the controls (Fig 5B).
significant correlations \( r = -0.62 \) to \(-0.77; P < .04 \) between immediate, short-delay, and long-delay free recall scores from the CVLT and \( R^2 \) values. \( R^2 \) measurements from both sides of the red nucleus also correlated strongly with the MMSE score \( r = -0.69 \) to \(-0.74; P < .02 \). Furthermore, the partial Spearman rank-order correlation coefficients between \( R^2 \) values and neuropsychological test scores were nearly all negative for gray matter regions and mostly positive for the white matter regions (Table 2). Exceptions to these trends were found for the internal capsule and the right temporal white matter, which generally had negative correlation coefficients with test scores (Table 2), and the left hippocampus, which had positive correlation coefficients with most of these scores (Table 2). \( \chi^2 \) tests indicated that negative correlation coefficients were more frequently associated with gray matter regions for the IFR \((89\%; \chi^2 = 10.89; P = .001)\), SDFR \((94\%; \chi^2 = 14.22; P = .0002)\), and LDFFR \((94\%; \chi^2 = 14.22; P = .0002)\) tests (Table 2). The number of positive correlation coefficients associated with the white matter regions was not significant, though the frequency of positive correlation coefficients approached statistical significance for the IFR parameter \((80\%, \chi^2 = 3.6, P = .0578)\).

**Discussion**

The strong linear correlation between mean \( R^2 \) values in our control group and reference postmortem iron concentrations for 10 brain regions (8 gray matter) in healthy adults (Fig 2) demonstrates the validity of our MR imaging protocol for assessing gray matter brain iron changes in vivo. The slope, intercept, and strength of the correlation (see Fig 2) obtained in this research were comparable to other in vivo studies\(^{36,37}\) on brain iron relaxometry at 1.5T.

At a higher field strength (3T), Gelman et al\(^{38}\) also reported a strong correlation \( r = 0.92 \) between \( R^2 \) and postmortem iron concentrations and a similar intercept to our study, but a slope nearly 3 times larger. Those researchers,\(^{38}\) however, suggest \( R^2 \) may be a more specific measure of iron in the brain because cortical white and gray matter regions with similar iron concentrations have similar \( R^2 \) values, but significantly different \( R^2 \) values. In the introduction, we acknowledged that \( R^2 \) would differ in gray and white matter of similar iron concentration because of the significant difference in water content (about 13\%,\(^{29}\)); however, although other factors besides tissue iron concentrations contribute to \( R^2 \) (eg, water content), in the case of gray matter, where water concentrations are similar,\(^{20}\) iron appears to be the dominant factor determining \( R^2 \).

The \( R^2 \) parameter may be insensitive to tissue water concentrations, but differences in diamagnetic susceptibility, most notably at air-tissue interfaces, can produce global mag-

---

**Table 2: Correlation coefficient signs between \( R^2 \) values and neuropsychological test scores for the 12 subjects with memory complaints**

<table>
<thead>
<tr>
<th>Region</th>
<th>Hemisphere</th>
<th>MMSE</th>
<th>CVLT</th>
<th>SDFR</th>
<th>LDFFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gray matter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>L</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Putamen</td>
<td>R</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>R</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Temporal cortex</td>
<td>L</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>R</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td>L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Red nucleus</td>
<td>L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>R</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>White matter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corpus callosum</td>
<td>L</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Subcortical frontal white</td>
<td>R</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Internal capsule</td>
<td>L</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Frontal white</td>
<td>L</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Temporal white</td>
<td>R</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

**Note.**—CVLT indicates California Verbal Learning Test; IFR, immediate free recall; LDFFR, long delay free recall; MMSE, Mini Mental State Examination; SDFR, short delay free recall. Data are displayed as the relevant Spearman’s rank-order partial correlation coefficient sign (+, positive; −, negative) for each brain region and test score. Note the overall frequency of negative correlation coefficients for gray matter regions and positive correlation coefficients for white matter regions.
netic inhomogeneity artifacts on R' 2 images. Both Hikita et al.\textsuperscript{2} and Gelman et al.\textsuperscript{38} noted areas of hyperintensity in their R' 2 images. Despite these artifacts, Gelman et al.\textsuperscript{38} reported a strong correlation (r = 0.90) between R' 2 and postmortem iron concentrations in gray and white matter. By using a similar MR imaging sequence at 1.5T, however, Hikita et al.\textsuperscript{37} did not observe a strong correlation (r = 0.33). Although global magnetic heterogeneity artifacts may be avoided by careful region of interest selection or corrected through the application of additional pulse sequences, these procedures may not always be practical or completely successful, because of the complex geometry within the brain and around the skull.

As discussed above, when regions of similar water content are considered, the iron-related specificity of R 2 is enhanced. Hence, increases in gray matter R 2 values are thought to reflect iron increases in this brain tissue type. One could speculate that iron increases in white matter should also increase R 2 values, but currently there are insufficient quantitative data relating R 2 values and iron concentrations in white matter to confidently extend this interpretation to white matter brain regions. There are, however, postmortem studies\textsuperscript{3,20} supporting the strong inverse relationship (water increase, R 2 decrease) between R 2 and gravimetrically determined water content in white matter (r = -0.99; P = 0.0115\textsuperscript{10}; r = -0.87; P = 0.0219\textsuperscript{20}). Hence, in brain regions where iron decreases can be ruled out, lower R 2 values are most likely the result of increases in tissue water content.

**R 2 Differences: Gray Matter**

An important and new finding of this research is that in vivo quantitative R 2 values showed a significant increase in the middle to superior temporal gray matter of participants reporting memory problems, but with no objective cognitive impairment (MC1 subgroup), and in participants with mild to severe cognitive impairment (MC2 subgroup). These results suggest iron has increased in the temporal region of both our subgroups (MC1, MC2) compared with the controls. In AD patients, quantitative in vivo R 2 values in the temporal cortex have not been reported, but there is one biochemical report\textsuperscript{4} of higher iron concentrations in the temporal pole in AD postmortem tissue. Other researchers, however, have observed no significant changes in iron concentrations for the superior and middle temporal cortex of AD participants.\textsuperscript{1,3,39}

Hypothetically, if our MC1 subgroup did represent early-stage or mild AD, one might expect R 2 to be elevated in the gray matter of those brain regions that are typically affected early in the pathogenesis of AD. On the basis of the distribution of paired helical filaments (PHF)-tau, used to assess the progression of AD, the entorhinal cortex and hippocampus (stages 1–3)\textsuperscript{20} are the first affected regions in the progression of AD. In the current study, there were no significant changes in hippocampal R 2 values for either memory-complaint subgroup (Figs 3D and 4), consistent with Campeau et al.\textsuperscript{29} The right hippocampus in the MC1 subgroup, however, showed a trend toward higher R 2 values (P = 0.0514; Fig 4A), consistent with biochemical\textsuperscript{3,23,25} and histochemical\textsuperscript{7} examinations that have reported higher iron concentrations (14%–33%) in AD postmortem hippocampal tissue. One 7T MR imaging microscopy study\textsuperscript{41} has also reported a nonsignificant reduction in T 2 times (R 2 increase) in AD hippocampal tissue. The interesting hippocampal result from our current study, combined with the significant R 2 increase in the right temporal cortex in the MC1 subgroup, could be highly significant. If subsequent research on a larger population of incipient AD patients also shows a similar increase in hippocampal and temporal R 2 values, it suggests R 2 measurements could potentially be useful as a biomarker in the earliest stages of AD.

Furthermore, the trend of decreasing R 2 values between the MC1 and MC2 subgroups in the right hippocampus (Figs 3D and 4), may be tentative evidence of disease progression as neuronal degeneration accelerates and the hippocampus atrophies. Atrophy of brain tissue may increase the amount of CSF,\textsuperscript{28} thus reducing R 2 and counteracting any iron increases. It is interesting to note that some researchers have reported R 2 reductions in the hippocampus of AD patients.\textsuperscript{25,26,28} Nevertheless, the reported absence of an association between hip- pocampal volume and T 2 times in AD,\textsuperscript{27,41} suggests atrophy is not the sole mechanism driving R 2 reduction in this brain region. The hippocampus is rich in myelin\textsuperscript{13} relative to other gray matter regions. A loss of myelin in AD hippocampi has been inferred from a decrease in 2'3'-cyclic nucleotide-3'-phosphodiesterase (CNPase) activity,\textsuperscript{7} which suggests a possible mechanism for reducing hippocampal R 2 values in AD, analogous to the pathologic processes reducing R 2 in white matter.

We did not record any significant increases in R 2 for the globus pallidus, caudate nucleus, or putamen, but there was a trend toward elevated R 2 values in the caudate nucleus of both memory-complaint subgroups (Fig 4), as well as an indication of higher R 2 values in the globus pallidus of the MC2 subgroup (Fig 4B). Some researchers, however, have observed significant R 2 increases in the globus pallidus,\textsuperscript{3} putamen, and cau- date nucleus\textsuperscript{24} of AD patients. Furthermore, these researchers also reported significant increases in the FDRI parameter for the globus pallidus,\textsuperscript{23} putamen,\textsuperscript{24} and caudate nucleus\textsuperscript{23,24} regions in AD patients compared with controls. The FDRI parameter is considered to be specifically associated with the total iron contained in the iron-storage protein ferritin,\textsuperscript{24} which comprises as much as 80% of the nonheme iron in gray matter.\textsuperscript{43} There are very limited biochemical data available concerning basal ganglia iron in AD, and those results that are available are equivocal. Whereas Loeffler et al.\textsuperscript{44} reported a significant increase in iron for the globus pallidus in AD, Griffiths and Crossman\textsuperscript{39} did not, and no significant changes in the iron concentrations of the putamen or caudate nucleus have been reported.\textsuperscript{4,39} The preceding discussion suggests further biochemical and MR imaging studies are required to resolve whether and how iron concentrations change in the basal ganglia of AD patients.

There was a trend of lower R 2 values in 2 high iron concentration brain regions in both memory-complaint subgroups—namely, the putamen and substantia nigra (Fig 4). This finding is unexpected and suggests either lower iron concentrations, increased water content, or both. As iron concentrations do not appear to be reduced in the putamen or substantia nigra in AD,\textsuperscript{3,24,39} it seems the origin of the lower R 2 values in these 2 regions may relate to changes in water content or distribution. Furthermore, focal basal ganglia T 2-hyperin- tensities (ie, high T 2, low R 2) are more prevalent in AD participants (21% of cases) compared with controls (12%), and they
are very common in vascular dementia (VaD, 68%). As in white matter, these hyperintensities are probably related to ischemic tissue injury, which results in increased water content, and possibly a structurally related shift in the amount of free (rather than bound) water. As the analysis process removed anomalously low R₂ values typical of focal T₂-hyperintensities, the reduced R₂ values in the putamen and substantia nigra observed in the present study are thought to reflect more diffuse processes related to the nonfocal changes in gray matter, which are potentially of a vascular origin.

There are no postmortem iron data for AD red nuclei. One report, however, measured significantly lower T₂-weighted signal intensities (ie, higher R₂) in the red nuclei of AD patients compared with controls, which suggests that R₂ had increased in the AD patients. In the present study, the trend of higher R₂ values in the red nuclei of our most impaired participants (MC2, Fig 4B) would appear to support the observations of Parsey and Krishnan. It is interesting that, in the current study, R₂ values in the red nucleus had the strongest correlations (of all the regions analyzed) with MMSE and memory scores. The potential significance of these correlations is discussed below.

R₂ Differences: White Matter

The most dramatic and consistent changes in R₂ were in the white matter of our most impaired subgroup (MC2), where R₂ was significantly lower compared with controls in 3 regions (frontal and temporal white matter, internal capsule [Fig 4B]), and significantly lower than the MC1 subgroup in 2 regions (frontal and subcortical frontal white matter). Furthermore, there was a clear trend of lower R₂ values in the MC2 subgroup compared with controls in all 5 white matter regions (Fig 4B). To the best of our knowledge, this is the first report of decreased R₂ levels in multiple white matter regions in a memory-complaint group and it is consistent with MR imaging studies reporting significantly lower R₂ values in the frontal14,23,24 and parahippocampal20 white matter of AD patients. Biochemical studies suggest that higher water content,10,12,13,20 associated with decreasing protein and lipid levels12,13,18 is the main factor underlying R₂ reduction in AD white matter; however, a reduction in iron concentration cannot be entirely disregarded as a contributor to lower R₂ values in AD white matter. One postmortem report1 has measured a significant decrease in iron concentrations in the occipital white matter of AD brain tissue compared with controls, though the white matter from the superior temporal and motor cortex regions in this study showed no difference in iron concentrations between AD patients and controls. As oligodendrocytes, the main component of white matter, stain for iron in histochemistry studies, a loss of these cells in AD could potentially be reflected in lower iron concentrations, and hence lower R₂ values, in white matter. Regardless of the cause of the R₂-reduction process in white matter, we interpret these decreases in R₂ values in multiple brain regions observed in this study as evidence of widespread white matter pathology in our memory-complaint participants (MC2 subgroup), principally related to a loss of myelin.

It is noteworthy that the R₂ changes in this study were observed in largely NAWM, as any focal lesions were avoided in the measurements reported here. Although white matter T₂-hyperintensities (ie, high T₂-low R₂, focal to confluent) have been the subject of numerous studies related to dementia and cognition, there has been less attention given to NAWM. In the past few years, however, neuropathological studies investigating AD and other dementias have confirmed that NAWM on MR imaging can manifest abnormal myelin pathology, which indicates an intermediate loss of myelin compared with distinct lesions. Sjoberg and Englund reported that the nonfocal white matter pathology in AD patients showed a significant decrease in oligodendrocytes and an increase in astrocytes, compared with controls, and was characterized by “nonfocal ischemic demyelination with glial changes.” These regions of incomplete white matter infarction have previously been associated with decreased R₂ values, protein and lipid levels and increased water content in AD brain tissue. The lack of correlation between the grade of Alzheimer encephalopathy and the severity of white matter changes in AD suggests that white matter disease is not purely a secondary phenomenon related to neuronal degeneration. The significant correlation between Braak stage and white matter protein levels, however, indicates that secondary degeneration probably contributes to white matter loss in AD.

The observation of decreased R₂ values in several white matter regions of our memory-complaint participants when compared with our controls is an important finding, but not in itself unique. Qualitative studies have identified greater numbers and severity of white matter lesions (ie, focal to confluent T₂-signal intensity hyperintensity, low R₂) in AD, VaD, frontotemporal dementia, dementia with Lewy bodies, and Huntington disease (HD), compared with controls. In quantitative MR imaging, significantly lower white matter R₂ values have been reported in AD, MS, and HD, but not in multi-infarct dementia or multiple system atrophy. Of particular interest to this study is the observation that, in multiple sclerosis (MS) patients, T₂ is prolonged (R₂ reduced) in 5 NAWM regions. Similar to MS, our current findings indicate that there are also subtle, diffuse processes affecting several white matter regions in our memory-complaint participants. MS patients also show evidence of basal ganglia T₂-hypointensity (low T₂, high R₂), suggesting that a build up of iron in these structures could be related to the white matter changes that appear to characterize this disease.

Correlations with Neuropsychological Scores

Overall, the negative gray matter and positive white matter correlations (Table 2) between R₂ and cognition/memory scores observed in the current study are consistent with our general hypothesis that, in participants with memory problems, R₂ tends to increase in gray matter and decrease in white matter. There are, however, some exceptions to these general trends. The internal capsule showed mainly weak negative correlations more typical of gray matter, perhaps reflecting the influence of interdigitating gray-matter bridges between the caudate and putamen. R₂ values in the left hippocampus were positively correlated (weakly) with some memory scores and the MMSE results. As discussed above, the high myelin content of the hippocampus, relative to other gray matter regions, could make this structure vulnerable to R₂-reducing processes that are more pronounced in white matter, and which ulti-
timately obscure correlations with memory scores. Laasko et al found no correlation between T2 and memory test scores in their AD patients, but a significant negative correlation was observed in these patients between hippocampal T2 values and MMSE scores. This observation is equivalent to a positive correlation between R2 and MMSE score, which is consistent with our own findings regarding the left hippocampus (Table 2).

Figure 4 shows a major change in R2 values in the red nucleus between the MC1 and MC2 subgroups, consistent with the strong correlation observed between R2 in the red nucleus and neuropsychological scores; however, the neuropsychological basis behind this correlation in people with memory problems is unclear. Functionally, the red nucleus is associated with motor control and receives aff erent fi bers from the cerebrocortex, the cerebellum, the lentiform nucleus, the subthalamic and hypothalamic nuclei, the substantia nigra, and the spinal cord. In experiments in rats, chronic cerebrovascular ischemia was induced to model the decreased cerebrovascular blood fl ow seen as a risk factor in the development of AD. One of the regions affected was the red nucleus, which was associated with motor coordination de fi cits in the rats. An electron microscopy study observed morphologic and morphometric changes to mitochondria in several brain regions in AD patients (including the red nucleus), but noted that there was no coexisting amyloid accumulation or cys tokeletal pathology in these regions. Evidence of mitochondrial alterations may be an indication of oxidative damage to the neurons in AD.

The thalamus is also a region not typically associated with memory problems in AD, but which, in the present study, manifested signifi cant correlations with memory scores, despite very little change in R2 values between the memory-complaint participants and the controls (Fig 4). Age was strongly correlated with thalamus R2 values (data not shown). Removing age as a covariate appeared to strengthen the association between R2 and memory scores, supporting our observation of the left hippocampus (Table 2).

Figure 4 shows a major change in R2 values in the red nucleus between the MC1 and MC2 subgroups, consistent with the strong correlation observed between R2 in the red nucleus and neuropsychological scores; however, the neuropsychological basis behind this correlation in people with memory problems is unclear. Functionally, the red nucleus is associated with motor control and receives aff erent fi bers from the cerebrocortex, the cerebellum, the lentiform nucleus, the subthalamic and hypothalamic nuclei, the substantia nigra, and the spinal cord. In experiments in rats, chronic cerebrovascular ischemia was induced to model the decreased cerebrovascular blood fl ow seen as a risk factor in the development of AD. One of the regions affected was the red nucleus, which was associated with motor coordination de fi cits in the rats. An electron microscopy study observed morphologic and morphometric changes to mitochondria in several brain regions in AD patients (including the red nucleus), but noted that there was no coexisting amyloid accumulation or cys tokeletal pathology in these regions. Evidence of mitochondrial alterations may be an indication of oxidative damage to the neurons in AD.

The thalamus is also a region not typically associated with memory problems in AD, but which, in the present study, manifested signifi cant correlations with memory scores, despite very little change in R2 values between the memory-complaint participants and the controls (Fig 4). Age was strongly correlated with thalamus R2 values (data not shown). Removing age as a covariate appeared to strengthen the association between R2 and memory scores in this region. An example of thalamus-related memory loss is Korsakoff syndrome. In this syndrome (resulting from thiamine de fi ciency and commonly seen in alcoholics) a number of thalamic nuclei have been implicated in the neuropathology of this disease, but damage to the anterior principal thalamic nucleus seems critical in the development of amnesia. It is interesting that AD pathology has been observed in most thalamic nuclei, and the thalamus does exhibit mitochondrial changes in AD, which suggests oxidative damage.

Conclusion

This study has demonstrated that in vivo quantitative proton transverse relaxation rate (R2) measurements are capable of characterizing gray and white matter pathology associated with age-related cognitive impairment. In general, R2 values from gray and white matter regions showed opposite correlations with cognition and memory scores, supporting our overall hypothesis that opposing R2-modifying processes (increasing iron, increasing water content) were dominant in gray and white matter, respectively. More specifically, the signifi cant increase in temporal cortex R2 values in our MC1 and MC2 subgroups, and the trend toward higher R2 values in the hippocampus of our MC1 subgroup, suggested an increase in iron concentrations in these regions, consistent with incipient AD pathogenesis and biochemical iron data. The signifi cantly lower R2 values in several white matter regions of our MC2 subgroup that were observed are also consistent with AD pathology, and appear to refl ect a widespread loss of myelin in cortical and subcortical regions. The combination of demyelination and atrophy in the hippocampus is thought to contribute to R2-reduction processes (increasing water content) counteracting iron-related R2 increases in our most severely impaired participants. The change in hippocampal R2 values between our least impaired and most impaired participants suggested that quantitative R2 measurements in the hippocampus might off er useful tools for the early diagnosis and monitoring of AD.

Acknowledgments

We thank Dr. Carmela Connor, of the Neurosciences Unit of the Health Department of Western Australia, and Athena Paton, from the McCusker Unit for Alzheimer Disease Research at Hollywood Private Hospital, for their assistance in referring and recruiting volunteers. We are grateful to Dr. Jay Ives and Jodie Ricci, of SKG Radiology, Perth, for facilitating access to the MR imaging scanner and for providing radiographic services.

References

18. Roher AE, Weiss N, Kojohn TA, et al. Increased A beta peptides and reduced lower R2 values in several white matter regions of our MC2 subgroup that were observed are also consistent with AD pathology, and appear to reflect a widespread loss of myelin in cortical and subcortical regions. The combination of demyelination and atrophy in the hippocampus is thought to contribute to R2-reduction processes (increasing water content) counteracting iron-related R2 increases in our most severely impaired participants. The change in hippocampal R2 values between our least impaired and most impaired participants suggested that quantitative R2 measurements in the hippocampus might offer useful tools for the early diagnosis and monitoring of AD.