Microanatomical Correlates of Cognitive Ability and Decline: Normal Ageing, MCI, and Alzheimer’s Disease

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Few microanatomical measures have been reliably correlated with cognitive measures in aging and Alzheimer’s disease (AD), particularly in the early stages of degeneration, such as mild cognitive impairment (MCI). However, cortical minicolumn organization has been shown to correlate with cognitive ability in aging monkeys, and the present study extends this finding to humans. We have previously reported that minicolumn spacing of cells in human association cortex is selectively reduced in normal aging (minicolumn thinning). The present study found that such measures detected early disease changes in MCI as well as further minicolumn thinning and disruption in AD. Plaques, tangles, and minicolumns were quantified, postmortem, for 20 controls, 10 MCI, and 20 AD subjects. Minicolumn changes were correlated with premortem cognitive scores (mini-mental state examination and verbal fluency). Two regions were studied from each brain: association cortex in the planum temporale (BA22) and primary auditory cortex (BA41). The relationship between minicolumns and cognitive function was strongest in association cortex, whereas in primary auditory cortex, it appeared to be an epiphenomenon of overall brain atrophy. Microanatomical changes reflecting selective regional vulnerability to AD pathology and differential involvement in the cognitive deficit of AD are therefore detectable in the early stage of MCI.

Keywords: aging, Alzheimer’s disease, cognition, mild cognitive impairment, minicolumn

Introduction

A small number of studies have investigated the modular structure of the cerebral cortex at the level of the minicolumn in aging and dementia (Buldyrev et al. 2000; Chance et al. 2006). Although there is much focus on the pathological markers of dementia, residual cognitive function is the product of the remaining intact cerebral architecture. Understanding the loss of neural structure is therefore as important as understanding the gain of pathology (Bussière et al. 2003). The minicolumn microcircuit has been proposed as a fundamental organizing principle of cortical structure and function (Mountcastle 1997). Established early by the radial units that form the cortex during development (Rakic 1995), columnar organization of cell bodies, axons, and dendrite bundles persists in adulthood and is prone to change with age (Chance et al. 2006; Casanova et al. 2007; Di Rosa et al. 2009).

We have previously reported that minicolumn spacing of cells in association cortex is reduced in normal aging (minicolumn thinning without cell loss) (Chance et al. 2006). Regional selectivity of age-associated minicolumn thinning, with greater thinning in association cortex than in primary sensory cortex, appears to reflect differential regional vulnerability to Alzheimer’s disease (AD) tangle pathology. A 2-stage model has been proposed whereby minicolumn thinning in normal aging precedes the minicolumn degeneration that accompanies the onset of dementia (Chance 2006). Since age is the greatest risk factor for AD, early detection will be facilitated by greater understanding of the continuity between age-related change and pathology. Mild cognitive impairment (MCI) offers a window on this transition but MCI is not yet well characterized at a cytoarchitectural level (Saito and Murayama 2007). The most robust finding seems to be intermediate pathology in MCI brains as compared with AD and control brains (Price and Morris 1999; Guillotet et al. 2003; Markesbery et al. 2006).

Synapse loss has shown the most consistent structural relationship with functional deficits in dementia, so far (Terry et al. 1991). Few other microanatomical measures have been reliably correlated with cognitive measures in aging and AD. Cruz et al. (2004) have shown that minicolumn organization correlates with cognitive ability in aging monkeys; as normal monkeys showed increased cognitive impairment due to aging, their minicolumn organization was more disrupted. Furthermore, the relationship is specific to the region of cortex associated with the function, whereas a neighboring region that is not thought to contribute to the function shows no correlation (Cruz et al. 2009). No studies to date have correlated minicolumn measures with cognitive scores in humans, and none have assessed minicolumns in MCI.

The goal of the present study was to perform a similar study in humans relating minicolumn measures to cognitive ability using a rare collection of postmortem tissue from donors who had taken part in longitudinal premortem neuropsychological testing. Two neighboring regions were investigated with different predicted involvement in cognitive function. In a previous study, these regions had shown different effects of normal aging on minicolumn width (Chance et al. 2006). We hypothesized that minicolumn thinning and degeneration would be found in amnestic MCI and AD, with greater minicolumn degeneration in AD than MCI, reflecting a 2-stage process. We expected that association cortex would show more substantial neuropathology than primary auditory cortex and that minicolumn thinning would be correlated with lower cognitive scores. We predicted greater effects in association cortex than primary auditory cortex, with relative sparing of primary auditory cortex in MCI on the basis that this region is only affected late in the disease process.

Materials and Methods

Subjects

Formalin-fixed brain tissue was sampled from 50 adults (20 normal controls, 10 amnestic MCI, and 20 confirmed AD) who had died...
between the ages of 62 and 92 years. The healthy controls were free
from neurological or psychiatric diseases. The brains were part of the
Thomas Willis Oxford Brain Collection, drawn from the OPTIMA
cohort—a prospective longitudinal clinicopathological study of aging
and cognitive decline. Subjects underwent clinical testing at several
time points in life. The results from the mini-mental state examination
(MMSE) and semantic fluency test were used in the present study. MCI
subjects were identified as such by clinical assessment in life and did
not fulfill criteria for AD at death. AD patients were confirmed with a
Braak staging of V/VI at postmortem. Cases were selected from the
larger Thomas Willis collection to yield comparable group mean
fixation times and ages at death as far as possible, although pair
matching was not possible. Neuropsychological and demographic
information per group can be found in Table 1.

Demographic details and potentially confounding variables, including
age at death, fixation time, and postmortem interval, were subjected to
statistical analysis (see below) (Table 1). No comorbidity of alcohol or
illicit drug misuse was detected in our sample’s records. The most
common causes of death were bronchopneumonia and cardiac failure.
Incomplete ApoE genotype data indicated that the frequency of E4
alleles fitted with expectations for these groups: typical in controls,
slightly elevated in AD, and less common in MCI. Since the data were
incomplete, ApoE was not studied further. This project was carried out
with approval of the UK National Research Ethics Service, study code
07/H0605/69, and informed consent was obtained from all subjects and
family representatives.

Brains were bisected and assigned a randomized code by a third party
so that measurements could be made blind to diagnosis. Only the left
cerebral hemisphere was available for study, and this was fixed in 10%
formalin. Samples from different brain regions were taken for confirma-
tion of diagnosis according to the criteria of the Consortium to Establish
a Registry for Alzheimer’s Disease and assigned a Braak score. Brains that
showed substantial signs of other pathology, including Creutzfeldt-Jacob
disease, Parkinson’s disease, Lewy body disease, Huntington’s disease,
cerebrovascular disease, and brain tumors, were excluded.

Neuropsychology
Subjects underwent regular neuropsychological testing in life (typically
every 6 months). Two neuropsychological test scores were used to
look for anatomical correlates in the present study. MMSE score was
selected as a standard assessment for overall memory and cognitive
decline in common clinical use, and category fluency (also called
semantic fluency) was selected because it has been shown to
discriminate MCI from other diagnoses in the OPTIMA cohort (de
Jager et al. 2003; de Jager 2004). Test scores were selected from the last
appropriate time point preceding the subject’s death by less than 20 months.
Examination of the previous time points ensured that the selected test was the last one
that was representative of the subject’s cognitive state and disease
course before the potential confounding effects of terminal illness were
apparent. This avoided a “terminal stage bias” in the data. An MMSE
and category fluency score were required from each subject. Two control
subjects had missing scores.

The group of control subjects had a typical range of cognitive scores
that were not significantly correlated with age. This ensured that the
relationship between minicolumn structure and cognitive ability in
normal control subjects could be disentangled from the gross effect of
normal brain aging. It enabled testing whether or not minicolumn
thickness is a nonspecific effect of aging or selectively related to
cognitive ability.

Tissue Sampling
The regions of interest (ROIs) were the planum temporale (PT) and the
primary auditory region of Heschl’s gyrus (HG). Five-millimeter thick
blocks of temporal lobe were cut orthogonal to the long axis of the
lobe, systematically random with respect to the anterior boundary of
HG, sampling exhaustively through HG and PT, as defined below.
Blocks were cut by hand using a calibrated metal guide.

HG was defined as Heschl’s gyrus, bounded by Heschl’s sulcus
posteriorly, the first transverse sulcus anteriorly (Kim et al. 2000) and
laterally by the superolateral margin of the superior temporal gyrus
(Zetzsche et al. 2001) containing cytoarchitectural regions TC and TBC
following the definitions of von Economo and Koskinas (1925). The
lower bank of the Sylvian fissure posterior to HG was measured as PT.
This consisted of the PT bounded anteriorly by Heschl’s sulcus,
including regions TB and TA1, excluding the posterior ascending ramus
that formed the posterior border of the PT.

The distinction between PT and the primary auditory region of HG is
important. Statistical mapping of cytoarchitectonic boundaries has
found only limited overlap between the microscopic definition of
primary auditory cortex and the macroscopic definition of HG
(Rademacher et al. 2001). Therefore, we followed cytoarchitectural
definitions, including the presence of tighter columnarity in the
primary auditory cortex, and the successful discrimination of the
primary auditory region within HG was confirmed by the measure-
ments of minicolumns that were narrow and dense in the primary
auditory region (see Results below).

For sectioning, the blocks were cryoprotected by immersion in a 30%
sucrose solution, periodically refreshed, for 4 weeks, then frozen, and
stored at -80 °C. A cryotome was used to cut 30-μm thick frozen
sections for slide mounting. For the analysis of minicolumns, two 30-μm
thick sections were selected within each ROI, spaced to preserve the
systematic random nature of the sample so that the entire ROI had a
d Chance of being sampled. The sections were Cresyl violet Nissl stained
(for illustration, see Fig. 1). Each ROI from each hemisphere was
therefore analyzed on 2 slides. Serial sections neighboring each
minicolumn section were also taken to quantify the extent of AD-type
pathology. One of these sections was methenamine silver stained
for senile plaques, and the other section was immunostained for
neurofibrillary tangles.

Tissue Staining and Immunohistochemistry
To demonstrate senile plaques in the PT and HG, a methenamine silver
stain was applied to 30-μm thick sections heated in solution in the oven
for 90 min at 60 °C. Cresyl Violet (0.1%) was used for counterstaining.
To assess tangle pathology in both the PT and HG, 30-μm thick
sections were reacted with phosphorylation-dependent antitau mono-
clonal mouse antibody AT8 obtained from Innogenetics. A primary
antibody concentration of 1:1,500 was incubated for 60 min at room
temperature. HRP rabbit/mouse secondary antibody was applied for 45
min, and staining was visualized using nickel-enhanced diaminobenzi-
dine with Hematoxylin counterstain.

Image Analysis
Plaque Assessment
Plaque load was assessed on 4 digital photomicrographs captured at
random coordinates in each ROI using a microscope with ×4 objective
lens. A grid was superimposed on these photos, and grid points were
counted where they were overlying the plaques. Any regions of tissue
loss on the slide were also quantified by grid point counting in order to
derive the intact tissue reference area. Plaque load was calculated as
the percentage of intact tissue covered by plaques. This assessment of
the area of coverage was chosen since it has been found to be a more
accurate reflection of disease severity than plaque number (Nagy et al.
1995).

### Table 1

<table>
<thead>
<tr>
<th>Diagnosis Group</th>
<th>MMSE Score</th>
<th>Category Fluency</th>
<th>Age (years)</th>
<th>Fixation Time (months)</th>
<th>Postmortem Interval (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, N = 20a</td>
<td>29.0 ± 1.1</td>
<td>21.3 ± 5.2</td>
<td>80.9 ± 6.3</td>
<td>102.6 ± 42.6</td>
<td>52.8 ± 28.8</td>
</tr>
<tr>
<td>MCI, N = 10</td>
<td>23.9 ± 4.8</td>
<td>12.4 ± 4.9</td>
<td>84.1 ± 8.7</td>
<td>112.8 ± 59.1</td>
<td>59.7 ± 30.3</td>
</tr>
<tr>
<td>AD, N = 20</td>
<td>11.6 ± 6.5</td>
<td>6.0 ± 5.6</td>
<td>73.9 ± 7.0</td>
<td>109.2 ± 40.4</td>
<td>64.3 ± 23.4</td>
</tr>
</tbody>
</table>

*aFull neuropsychology information not available for 2 cases.
*bFull postmortem interval information not available for 5 cases.
**Minicolumn Measurements**

Minicolumn width, neuron density, and number of microsegments were quantified using semiautomated image analysis. Microsegments contain too few cells (fewer than 10) to constitute a minicolumn, due, in controls, to incomplete columns that fall partially outside the plane of focus but also due to minicolumn breakdown or disruption in disease.

Minicolumns were quantified using semiautomated image analysis. The method has been reported in detail with validation and discussion of assumptions by Casanova and Switala (2005). For measurements, 2 sections from each ROI provided an adequate sample. The sections were a systematically spaced subset that preserved randomization with respect to the boundaries of the ROIs. Minicolumns are clearest in lamina III, so minicolumn detection was optimized for lamina III. In summary, lamina III was photographed through a ×4 objective lens, normal to the cortical surface, and the photomicrographs were digitized at 0.4 μm/pixel resolution. Three micrographs were captured from each ROI, each micrograph comprising a region about 1 mm² in area. Fields were selected randomly excluding regions of high cortical curvature such as the fundi of sulci or the apices of gyri since, although minicolumns are still clearly visible, high curvature affects cell distribution (Chance et al. 2004).

A minicolumn is composed of the cell dense core, and the cell sparse periphery of the cell column where local circuits, synapses, and dendritic branches predominate. Minicolumn width is calculated from the combined width of the dense core region plus the sum of the 2 halves of the peripheral neuropil on either side.

Cresyl violet staining is not sensitive to formalin fixation, and all the tissue in this study had been stored in formalin for longer than the 3 weeks required before stabilizing (Quester and Schroder 1997). Furthermore, fixation did not differ between groups (see below).

**Statistical Analysis**

Statistical analyses were conducted using SPSS software (version 17.0). For each measured variable, tests of both ROIs were initially combined in a repeated measures analysis of variance (rmANOVA) with diagnosis as a between-subject factor and ROI as the within-subject factor. Any significant effects were followed up with univariate ANOVAs of each ROI separately to identify which ROI was primarily affected. Potential covariates were identified as age at death, postmortem interval, fixation time, and total brain weight. Of these, age at death differed between groups ($F = 8.4, df = 2, 49, P < 0.01$) and brain weight differed between groups ($F = 8.0, degrees of freedom \[df\] = 2, 49, $P < 0.01$). Therefore, the influence of these potential covariates was tested by incorporating them into the ANOVAs as reported below. Postmortem interval did not differ between groups ($F = 0.8, df = 2, 43, P = 0.48$) and fixation time did not differ ($F = 0.2, df = 2, 49, P = 0.82$) so these were not incorporated into ANOVAs. Post hoc tests were also used to further clarify the differences between the 3 diagnostic groups.

Pearson’s correlation analysis was applied to examine the relationship between pathological markers, minicolumn width data, and neuropsychological test scores. The relationship between brain weight and minicolumn width data was also investigated by correlation analysis.

For minicolumn data (width and microsegment number), all groups passed Kolmogorov-Smirnov tests, indicating a normal distribution and Box’s M-tests for equality of variance. The semiautomated measures of neuron density passed the Kolmogorov-Smirnov test but the measures from the PT region did not pass Box’s M-test due to the difference between the small variance of the MCI group and the large variances of the control and AD groups. Plaque load and tangle density did not pass Kolmogorov-Smirnov tests or Box’s M-tests in all groups, due to the floor effect, particularly in control subjects, of a large number of zero values. Consequently, nonparametric Kruskal-Wallis tests for multiple independent samples (3-way: controls, MCI, AD) and Mann-Whitney U tests (2-tailed) were applied to the plaque and tangle data and the PT neuron density measures. Levene’s tests of equality of variance were also performed.

To avoid the problems of multiple testing, data were initially compressed into single tests (i.e., rmANOVA) wherever possible. Details of the results have been reported as F-statistics, t-statistics, chi-squared, U-statistics, or $r^2$ coefficients as appropriate. In order to improve intelligibility, statistical details are confined to positive results and negative results, which are borderline or demonstrate an important negative.
Results

Plaques
In region PT, a 3-way Kruskal--Wallis test revealed a diagnosis effect with lowest plaque load in controls and highest load in AD ($X^2 = 27.1$, df = 2, $P < 0.01$). Post hoc Mann--Whitney $U$ tests demonstrated this effect was due primarily to higher plaque load in AD compared with both controls ($U = 24.0$, $Z = -4.8$, $P < 0.01$) and MCI ($U = 25.0$, $Z = -3.3$, $P < 0.01$) but there was also a higher plaque load in MCI compared with controls ($U = 58.0$, $Z = -1.9$, $P = 0.05$) (Table 2).

In HG, there was a similar diagnosis effect ($X^2 = 26.9$, df = 2, $P < 0.01$) and as with the PT, this was due to higher plaque load in AD compared with both controls ($U = 15.0$, $Z = -5.0$, $P < 0.01$) and MCI ($U = 40.0$, $Z = -2.6$, $P < 0.01$) and a higher plaque load in MCI than controls ($U = 52.5$, $Z = -2.1$, $P < 0.05$).

Notwithstanding the failure of the plaque data to satisfy the optimal assumptions for parametric testing, it may be noted that univariate ANOVAs of both PT and HG data, enabling inclusion of covariates, showed that the diagnosis effects persisted with the inclusion of both age and brain weight (PT: $F = 7.4$, df = 2, 49, $P < 0.01$; HG: $F = 8.9$, df = 2, 49, $P < 0.01$).

Tangles
In region PT, a 3-way Kruskal--Wallis test revealed a diagnosis effect with lowest tangle density in controls and highest density in AD ($X^2 = 26.7$, df = 2, $P < 0.01$). Post hoc Mann--Whitney $U$ tests demonstrated the effect was due primarily to higher tangle density in AD compared with both controls ($U = 52.5$, $Z = -4.5$, $P < 0.01$) and MCI ($U = 27.5$, $Z = -3.4$, $P < 0.01$) with no significant difference between controls and MCI ($U = 94.5$, $Z = -0.6$, $P = 0.58$).

In HG, there was also a diagnosis effect ($X^2 = 39.7$, df = 2, $P < 0.01$), and this was due to higher tangle density in AD compared with both controls ($U = 10.0$, $Z = -5.6$, $P < 0.01$) and MCI ($U = 60.0$, $Z = -4.2$, $P < 0.01$), and furthermore, unlike region PT, there was a higher tangle density in MCI compared with controls ($U = 80$, $Z = -2.0$, $P < 0.05$).

Although the tangle data also did not satisfy the assumptions for parametric testing, univariate ANOVAs of both PT and HG data enabled consideration of covariates and showed that the diagnosis effects persisted with the inclusion of both age and brain weight as covariates (PT: $F = 7.9$, df = 2, 49, $P < 0.01$; HG: $F = 5.6$, df = 2, 49, $P < 0.01$).

Minicolumn Width
A rmANOVA revealed a difference between diagnostic groups with widest minicolumns in controls ($F = 5.0$, df = 2, 47, $P < 0.05$) and an overall difference between regions—wider minicolumns in PT than HG ($F = 15.4$, df = 1, 47, $P < 0.01$). Univariate ANOVA for region PT found the diagnosis effect ($F = 3.5$, df = 2, 49, $P < 0.05$) remained with the inclusion of age as a covariate ($F = 3.1$, df = 2, 49, $P = 0.05$) and post hoc $t$-tests showed that minicolumns were thinner in MCI ($t = 2.1$, df = 28, $P < 0.05$) and AD ($t = -2.4$, df = 34.4, $P < 0.05$) than in controls, with no clear difference between MCI and AD ($t = -0.3$, df = 25.9, $P = 0.78$).

For HG, there was a clear trend for a diagnosis difference (univariate ANOVA: $F = 3.0$, df = 2, 49, $P = 0.06$) that was significant when including age as a covariate ($F = 3.4$, df = 2, 49, $P < 0.05$). The difference was driven by the more narrow minicolumns in AD compared with controls ($t = -2.3$, df = 38, $P < 0.05$), whereas there was no clear difference between controls and MCI ($t = 1.3$, df = 27.9, $P = 0.20$) or MCI and AD ($t = -1.1$, df = 28, $P = 0.29$) (see Fig. 2A).

Microsegment Number (Minicolumn “Disruption”)
All cases, including controls, contain a number of microsegments due to a proportion of minicolumns falling partly outside the plane of focus. Across all subjects, the number of microsegments was greater in HG than PT ($F = 13.4$, df = 1, 47, $P < 0.01$) reflecting the higher overall density of minicolumns and cells in the HG. Compared with controls, the number of microsegments was increased in both regions in patients (rmANOVA: $F = 6.9$, df = 2, 47, $P < 0.01$) indicating disruption. In PT, there was a clear effect of diagnosis with fewer microsegments in controls and the greatest number of microsegments in AD (univariate ANOVA: $F = 5.7$, df = 2, 49, $P < 0.01$). This effect remained when controlling for age as a covariate ($F = 3.9$, df = 2, 49, $P < 0.05$). The main effect identified by post hoc $t$-tests was an increased number of microsegments in AD compared with controls ($t = -3.5$, $P = 32.4$, $P < 0.01$). There were no significant differences between controls and MCI, MCI, and AD.

Univariate ANOVA indicated a weaker effect in HG; a trend for a diagnosis difference ($F = 2.7$, df = 2, 49, $P = 0.08$) which was obscured further by the inclusion of age as a covariate ($F = 2.4$, df = 2, 49, $P = 0.10$). Nonetheless, post hoc $t$-tests found more microsegments in AD than controls ($t = -2.1$, df = 38, $P < 0.05$) and a trend for more microsegments in AD than MCI ($t = -1.8$, df = 28, $P = 0.08$), although no difference between controls and MCI (see Fig. 2B).

Neuron Density
Neuron density was increased in the MCI group compared with the other groups in both PT and HG (rmANOVA overall diagnosis effect: $F = 3.9$, df = 2, 47, $P < 0.05$). The PT was retested separately with a 3-way Kruskal--Wallis test, due to the different variance between groups (revealed by Box’s M-test, as described above). There was a trend for a similar effect of increased density in MCI and little difference between controls and AD ($X^2 = 5.6$, df = 2, $P = 0.06$). Mann--Whitney $U$ tests confirmed that the MCI group PT neuron density was higher than controls ($U = 43$, $Z = -2.5$, $P < 0.05$) and that AD neuron density in PT was somewhat lower than MCI ($U = 60$, $Z = -1.8$, $P = 0.08$) but not lower than controls ($U = 200$, $Z = 0.0$, $P = 1.0$).

Table 2
Summary of measured variables (means and standard deviations)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Minicolumn width (μm)</th>
<th>% Plaque area</th>
<th>Tangles/mm²</th>
<th>Cell density/mm²</th>
<th>Microsegment number (per photomicrograph)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PT</td>
<td>HG</td>
<td>PT</td>
<td>HG</td>
<td>PT</td>
</tr>
<tr>
<td>Control, N = 20</td>
<td>33.88 ± 2.75</td>
<td>30.98 ± 1.67</td>
<td>0.81 ± 1.47</td>
<td>0.96 ± 1.16</td>
<td>0.05 ± 0.21</td>
</tr>
<tr>
<td>MCI, N = 10</td>
<td>31.66 ± 2.49</td>
<td>30.38 ± 0.77</td>
<td>1.47 ± 1.26</td>
<td>2.83 ± 2.90</td>
<td>0.73 ± 2.31</td>
</tr>
<tr>
<td>AD, N = 20</td>
<td>31.33 ± 3.85</td>
<td>29.80 ± 1.61</td>
<td>7.45 ± 5.67</td>
<td>6.65 ± 3.72</td>
<td>36.65 ± 28.42</td>
</tr>
</tbody>
</table>

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quantified in the minicolumn measures will show a closer relationship to cortical atrophy. As a result, the effects of covarying for fixed brain weight and correlations with minicolumn organization are reported here.

Brain weight was not a significant covariate of minicolumn width in PT ($F = 0.7$, df = 1, 49, $P = 0.39$). By contrast, brain weight was a significant covariate of HG minicolumn width ($F = 14.3$, df = 1, 49, $P < 0.01$), and consequently, its inclusion with age in a univariate ANOVA removed the effect of diagnosis ($F = 0.3$, df = 2, 49, $P = 0.78$). For microsegment number in the PT, brain weight was also not a significant covariate ($F = 0.6$, df = 1, 49, $P = 0.45$). However, again for HG, brain weight was a significant covariate of microsegment number ($F = 10.0$, df = 1,49, $P < 0.01$), and its inclusion with age in a univariate ANOVA resulted in no diagnosis effect ($F = 0.67$, df = 2, 49, $P = 0.52$). For neuron density, brain weight was not a significant covariate for HG ($F = 0.0$, df = 1, 49, $P = 0.93$). Brain weight was not correlated with neuron density either across all subjects or within diagnostic groups.

To investigate the relationship between brain weight, neuropsychology and minicolumn measures in the normal control population, a further subset of correlations, revealed that heavier brains were positively associated with wider minicolumns in HG ($r^2 = 0.54$, $P < 0.05$) but not with minicolumn width in PT ($r^2 = 0.04$, $P = 0.87$) (see Fig. 3) and not with microsegment number in either HG or PT. Brain weight in controls was not associated with semantic fluency scores ($r^2 = 0.29$, $P = 0.24$) or MMSE scores ($r^2 = 0.28$, $P = 0.25$).

**Correlations between Pathology and Neural Architecture**

In the full data set of 50 subjects, higher tangle density was correlated with more microsegments (more minicolumn disruption) across diagnoses. This relationship was found in PT ($r^2 = 0.28$, $P < 0.05$) and was a trend in HG ($r^2 = 0.27$, $P = 0.06$). Higher tangle density was also correlated with thinner minicolumns in HG ($r^2 = 0.29$, $P < 0.05$) but not in PT ($r^2 = -0.6$, $P = 0.68$). Higher tangle density was correlated with reduced neuron density in region PT ($r^2 = -0.3$, $P < 0.05$) and in HG ($r^2 = -0.31$, $P < 0.05$). No correlations were found between minicolumn measures and plaque load or between neuron density and plaque load. Within diagnostic groups, there were no significant relationships between minicolumns and pathology (plaques or tangles) in either PT or HG. For neuron density within diagnostic groups, only PT tangle density was negatively correlated with neuron density in AD ($r^2 = -0.5$, $P < 0.05$); no other relationships were identified between neuron density and plaques or tangles.

For the interaction between minicolumn width and neuron density across all 50 subjects, there was no relationship in HG; however, in PT, reduced minicolumn width was correlated with increased neuron density ($r^2 = -0.8$, $P < 0.01$). This relationship in PT held for each of the 3 diagnostic groups tested separately, among which the regression line was lower in AD compared with controls and MCI.

**Microanatomical Correlates of Cognitive Impairment in Disease**

Across all 48 subjects with complete neuropsychological data, all microanatomical measures of pathology and minicolumn organization showed clear correlations in the expected directions. For both PT and HG, wider minicolumns were
Microanatomical Correlates of Cognitive Ability in Controls

Across the 18 normal aged control subjects with complete neuropsychological data, region PT minicolumn measures correlated with MMSE score (see Fig. 3). Wider minicolumns in PT were associated with higher MMSE score ($r^2 = 0.48$, $P < 0.05$), and more microsegments were associated with lower MMSE scores ($r^2 = -0.62$, $P < 0.01$). In contrast, HG showed no relationship with MMSE score (microsegment number: $r^2 = -0.06$, $P = 0.81$; minicolumn width: $r^2 = 0.36$, $P = 0.15$). Neuron density did not correlate with MMSE score in either HG or PT. No measures in either region were significantly correlated with semantic fluency score.

Discussion

Three key issues emerge from this study: 1) Minicolumn measures correlate with cognitive ability in humans and are remarkably sensitive to regional differences and pathological change, 2) the data provide evidence for a 2-stage process in which minicolumn thinning precedes minicolumn disruption, and 3) changes in the early stage of illness (MCI) offer insight into whether different regional effects are due to a pervasive neuropathology with different intrinsic regional rates of progression or a spreading region-to-region neuropathological distribution.

Minicolumns Relate to Cognitive Ability

A relationship between minicolumn width and cognition has been implied previously by evidence from other disorders, including dyslexia and autism, both of which present with reduced width or spacing between minicolumns (Williams and Casanova 2010). Furthermore, a left hemisphere expansion of minicolumn width has been found in human language cortex that is not present in other primates (Buxhoeveden et al. 2001) and is thought to relate to left hemisphere language specialization. However, the only previous direct correlation with cognitive function was tested in macaque monkeys using a novel measure of minicolumn degeneration. Here, we have demonstrated the first minicolumn structural correlations with cognitive function in humans. We show that minicolumn thinning is associated with greater AD-type pathology, is correlated with individual cognitive scores, and indicates continuity with the normal age-associated thinning identified previously. We also replicate and extend Buldyrev et al.'s (2000) finding of minicolumn disruption in AD.

It was considered possible that minicolumn thinning may be a nonspecific marker of disease and cognitive decline, associated with gross brain atrophy. To offer insight into cognitive changes during aging and disease, it was necessary to determine 1) if the minicolumn thinning was simply an effect of overall brain weight, 2) if the effect in association cortex (represented in this study by PT) was more sensitive to MCI than in primary sensory cortex (represented here by HG), and 3) if the relationship with cognition was selective to association cortex. Furthermore, if meaningful, the relationship between minicolumn width and cognition should be detectable in control subjects as a separate group.

Minicolumn width and minicolumn disruption in the primary auditory region appeared to be more closely tied to whole brain atrophy than they were in the association cortex. This was indicated since brain weight was a significant covariate in the statistical analyses of minicolumn width and microsegment number in HG but not PT. It was also true that in normal controls (i.e., subjects unaffected by disease-related atrophy) minicolumn width in HG was correlated with total brain weight, whereas in PT, it was not.

A different story was found for the relationship with cognitive ability. Although all measures of minicolumn width...
and disruption in both regions were correlated with cognitive decline across the diagnostic groups, only PT minicolumn width was correlated with cognitive ability (MMSE) in control subjects unaffected by disease. Furthermore, reduced brain weight was associated with greater cognitive impairment and disease severity across all subjects, however, as may be expected in the normal population, brain weight did not correlate with cognition in controls. Therefore, it appears that minicolumn structure in primary auditory cortex is indicative of overall brain weight and general atrophy associated with worsening disease, whereas minicolumn width in association cortex (PT) varies independently of total brain weight and relates instead to cognitive ability, in healthy aging and disease.

This is the first demonstration in humans that the minicolumn relationship with cognitive function is selective to association cortex and is not just an epiphenomenon of overall brain atrophy (in contrast to primary auditory cortex). It is similar to the regionally selective structural minicolumn correlation with cognitive ability in aged monkeys (Cruz et al. 2009), which related to minicolumn disruption. The relationship in the present study was with minicolumn width. The structure-function correspondence in humans was limited to the tissue and neuropsychological tests that were available. MMSE score depends on several aspects of speech processing and comprehension likely to involve the PT, including repetition of named prompts, phrase repetition, and following complex verbal commands. The selectivity to association cortex suggested that this region may be more sensitive to change in MCI. Indeed, while the minicolumn measures overall revealed that values in MCI tended to lie between controls and AD, the only clear difference between controls and MCI was found in PT minicolumn width—minicolumns were thinner in MCI.

**A 2-Stage Process**

MCI has not previously been investigated in this manner. Given that cognitive scores in MCI are intermediate between healthy aging and AD, the structural correlations are consistent with the concept that MCI is neuropathologically intermediate (Riley et al. 2002; Van Der Flier et al. 2002; Bennett et al. 2005; Petersen et al. 2006). Furthermore, the differences between minicolumn thinning and minicolumn disruption (microsegment number) support the notion of a 2-stage process (Chance 2006). If MCI is assumed to be an early stage of illness, it appears that minicolumn thinning is detectable sooner than minicolumn disruption: minicolumn thinning was apparent in MCI in the PT, whereas minicolumn disruption was not yet apparent in MCI. The effect of AD on minicolumn width was also more widespread, including both PT and HG, whereas minicolumn disruption in AD only clearly affected the PT and was not yet significant in HG (for an impression of tissue differences between subjects, see Fig. 1)

Minicolumn thinning in the PT was correlated with increased neuron density. This is consistent with the expectation that minicolumn thinning is caused initially by neuropil loss. Yet, neuron density in PT, and HG in particular, did not recapitulate all the diagnostic effects seen on the minicolumns. Neuron density was increased in MCI, as might be expected if cell loss is limited at this stage and the primary effect is on synapse loss and reduced neuropil. At this stage, minicolumn width was reduced only in region PT. In AD, neuron density was reduced in comparison with MCI, suggesting an additional effect of cell loss. However, the cell loss appeared to be in proportion to neuropil reduction, since the neuron density was not significantly less than that of control subjects. By contrast, minicolumn thinning in AD was even greater than MCI and controls, and minicolumn disruption occurred, indicating a combined effect of neuropil loss and cell loss. Consequently, minicolumn measurement detects different effects from those of neuron density. Moreover, neuron density was not clearly correlated with cognitive scores in patients or controls, unlike the minicolumn measures.

The relationship between cytoarchitecture and tangle accumulation was confirmed by the positive correlations between tangle density and minicolumn disruption in both regions PT and HG. This replicates the relationship reported by Buldyrev et al. (2000). We did not replicate our previous finding of a relationship between plaque load and minicolumn thinning in normal aging (Chance et al. 2006). Based on prior evidence that tangles are more closely related to the severity of illness, it is perhaps not surprising that tangles show the more consistent relationship to pathological changes in neural architecture.

**Models of Region Vulnerability: Pervasive or Spreading?**

PT minicolumn width discriminates between controls and MCI, whereas HG measures do not, reflecting the greater sensitivity of association cortex in the early disease process and the lesser vulnerability of primary sensory cortex at this stage.

Pearson et al. (1985) proposed that tangle pathology spreads in accordance with connection patterns in the brain and Braak H and Braak E (1985, 1991) defined a staging method based on chronological differences in regional spread. Many studies over the years have confirmed this pattern of pathology (Price et al. 1991; Esiri and Chance 2006). The neuroplasticity hypothesis offers a further mechanism for differential regional vulnerability (Arendt 2003; Esiri and Chance 2006). This allows for a neuropathological distribution not solely dependent on connectivity in which regional differences in progression are due to different intrinsic rates of neuropathological change, relating to regional differences in neuroplasticity.

A model incorporating intrinsic vulnerability (e.g., Mesulam 1999) offers a clearer link to the changes seen in normal aging. For example, we have shown previously that minicolumns are initially wider in PT and undergo thinning in normal aging, whereas minicolumns are initially narrow in HG and do not alter with age (Chance et al. 2006). The wider minicolumns and higher level of change in association cortex indicate higher plasticity and suggest greater susceptibility to pathology in accordance with the differences between PT and HG described here: a faster rate of tangle accumulation in PT, a greater effect on the neural architecture (minicolumn width) in PT during the MCI stage, and a closer relationship to cognitive ability in PT.

**Conclusions**

On routine examination, the laminar organization of the cortex tends to be more prominent than the radial dimension and, historically, laminar displacements of neurons have been more readily identified than radial alterations (Rakic 2007). Although variation in radial organization can be observed (see Fig. 1), it is the advent of computerized image analysis that has enabled accurate, quantitative assessment of minicolumn structure. Consequently, relationships between minicolumn structure...
and function are only recently being elucidated. Radial abnormalities in several disorders (Casanova et al. 2002; Buxhoeveden et al. 2006; Chance et al. 2008) are beginning to suggest that this form of disruption may occur as frequently as displacements in the laminar dimension.

Although the functional role of minicolumns is uncertain (Horton and Adams 2005), the present results support Cruz et al.’s (2009) assertion of the functional significance of minicolumn integrity—in particular, a relationship with cognition that is specific to the minicolumn measures and is not found for neuron density. Minicolumn thinning appears to precede minicolumn disruption and amnestic MCI looks neuropathologically intermediate between controls and AD. Overall, the data support an initial influence of AD on synapses and neuropil, with a later additional effect on neuron number.

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**References**

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