Cognitive Decline Strongly Correlates with Cortical Atrophy in Alzheimer’s Dementia

PETER R. MOUTON,* 1 LEE J. MARTIN,*‡ MICHAEL E. CALHOUN,* GLORIA DAL FORNO,† AND DONALD L. PRICE*†‡

Neuropathology Division and Departments of *Pathology, †Neurology, and ‡Neurosciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Received 7 October 1997; Revised 24 July 1998; Accepted 29 July 1998

MOUTON, P. R., L. J. MARTIN, M. E. CALHOUN, G. DAL FORNO, AND D. L. PRICE. Cognitive decline strongly correlates with cortical atrophy in Alzheimer’s dementia. NEUROBIOL. AGING 19(5) 371–377, 1998.—Alzheimer’s disease (AD) is characterized by progressive dementia and distinct neuropathology at autopsy. In order to test the relationship between dementia severity and loss of brain volumes, we prospectively documented the neurological/medical health of 26 male and 26 female controls and AD cases, and evaluated a subset of controls and AD cases using the Mini Mental State Examination (MMSE). At autopsy, Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) criteria confirmed diagnoses in 33 AD cases and 19 controls, and using unbiased stereology we quantified total volumes of cortical gray matter, subcortical grey matter including white matter, and forebrain. For ages of death between 50 to 100 years, controls showed minor cortical atrophy in the absence of cognitive decline. Cortical atrophy in AD cases was 20 to 25% greater than that in controls; AD patients dying at older ages showed less severe cortical atrophy than those dying at younger ages. Across all AD cases there was a strong correlation between cognitive performance on the Mini Mental State Examination and cortical volume loss. These findings confirm fundamental differences in the temporal patterns of cortical volume loss in aging and AD, and support cortical degeneration as the primary basis for cognitive decline in AD. © 1998 Elsevier Science Inc.

ALZHEIMER’S disease (AD) afflicts approximately 5 to 10% of individuals between the ages of 65 and 80 years, and up to 20% of the elderly over age 80. Probable AD manifests as early disorientation and confusion, followed by progressive cognitive decline to severe dementia and end-stage AD (19). Confirmation of definite AD according to Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) guidelines requires the demonstrated presence of high densities of neocortical neuritic plaques (NP) (21), while other histopathological rating systems include neurofibrillary tangles (NFT) (3).

In addition to NPs and NFTs, Alzheimer described severe cortical volume loss (atrophy) as a consistent neuropathological finding in AD over 90 years ago (1). Since then, cortical atrophy in AD cases and nondemented aged controls has been quantified on antemortem neuroimages (9,16,18,30) and at postmortem examination (12,17,20,26–28) using a wide variety of sampling and measurement techniques. Antemortem neuroimaging studies without histopathological confirmation of diagnoses have reported that early AD can be identified on the basis of hippocampal atrophy (9,18), while semi-quantitative neuroimaging studies suggest that there is a strong clinicopathological correlation between cognitive decline and total cortical atrophy in AD (30). Postmortem studies of cortical volume loss in normal aging and AD cases report 10 to 25% reductions in total cortical volume beyond that expected during normal aging (12,17,20,26–28).

Morphometric analysis of degenerative brain changes could provide insight into the structural basis for normal cognitive function in nondemented persons and cognitive decline in AD (22). To date, stereological studies have defined the differences between the relative stability of neuronal populations during normal aging (24,26,28,36) and severe loss in some (28,36), but not all (27), neuronal populations in AD. Although stereological studies have confirmed that synaptophysin-immunoreactive presynaptic boutons occupy a substantial volume of cerebral cortex in rats (6) and nonhuman primates (23), rigorous analysis of synaptic bouton numbers are difficult in autopsied human brains. Semi-quantitative studies of synaptic densities show minor age-related changes in hippocampal formation in nonhuman primates (34,35), while studies in humans show severe reductions in cortical synaptic clefts (11,33,37) and dendritic spines (4,8,13) in AD cases compared to aged controls. A recent study of synaptophysin protein levels indicates that loss of synaptic boutons precedes cognitive decline in early AD (32).

The working hypothesis for the present study was that progressive cortical atrophy, ostensibly caused by widespread degeneration/disconnectivity of cortical synapses, leads to progressive cognitive impairment in AD. As a first test of this hypothesis, we
used unbiased stereology to estimate cortical, subcortical, and whole forebrain volumes in autopsied brains from a cohort of well-studied AD cases and controls. In a subset of subjects in which performance on the Mini Mental State Examination (MMSE) was assessed, we quantified the correlation between cognitive function and regional brain volumes.

MATERIALS AND METHODS

Subjects

The cases used in this study comprised 26 males and 26 females who were recruited from the Baltimore Longitudinal Study of Aging (BLSA) and the Alzheimer’s Disease Research Center (ADRC) at the Johns Hopkins School of Medicine and the National Institute on Aging (Baltimore, Maryland, USA). All cases had received complete medical and neurological examinations at a minimum of yearly intervals. The cognitive status of a subset of probable AD cases and controls were assessed periodically with the MMSE (14). This subset included 22 of 33 (67%) of the definite AD cases [age range 59 to 92 years (mean 80, SD 8.5)] and 7 of 19 (37%) of the controls [age range 69 to 91 years (mean 79, SD 8.7)]. Clinical evidence of unexplained, severe, and progressive dementia was present in all probable AD cases (final MMSE score < 25; mean 6.7, SD 6.6), while controls showed no evidence of neurological disease (final MMSE scores ≥ 25; mean 28.4, SD 1.5).

Histopathological Diagnosis

The brains were removed and weighed in an unfixed state, with postmortem delays ranging from 2 to 28 h (Table 1). Each forebrain was isolated from cerebellum and brainstem with a coronal cut at the level of the mammillary bodies. The forebrain was sectioned in the midsagittal plane into two hemispheres, and both hemispheres were placed in 10% neutral-buffered formalin for 7–10 days until preparation for stereology as described below. Tissue samples for diagnostic purposes were taken from 33 regions throughout the brainstem, cerebellum, and cerebrum of the right hemisphere. Final diagnoses according to the CERAD guidelines (21) were based on clinical assessments and the semi-quantitative assessment of Hirano silver-stained, dystrophic neurite-containing plaques (NP) in all neocortical regions. Cases defined as “definite AD” corresponded to Braak classification of amyloid stage C and NFT stage III–VI, while normal controls defined by CERAD criteria corresponded to amyloid stages A–B and NFT stages I–IV (3).

Stereology

The formalin-fixed left cerebral hemisphere was cut in the coronal plane into 1.0-cm thick slabs using a tissue slicer. The first cut was placed in a random position in the first 1.0-cm interval from the frontal pole. Subsequent cuts were placed at uniform intervals through the entire hemisphere, as described previously (23). High-contrast digital images of the anterior face of each slice were recorded at a resolution of 300 dots per inch. Figure 1 shows representative images from a control and an AD case. Volumetric analyses using point counting and the Cavalieri principle (7,15) were performed on the neuroimages in serial order from frontal to occipital pole using the STEREOLOGER system (Systems Plan-
ning and Analysis, Inc., Alexandria, VA, USA). The software program automatically positioned a point-grid randomly over each slice, as shown in Fig. 1A. The distance between points (area per point $= 180.5 \text{ mm}^2$) was selected to produce a coefficient of error for individual volume estimates of less than 10%. The accuracy of the point counting/Cavalieri method has been previously verified in relation to Archimedes’ water displacement method (31). A trained individual blind to diagnosis, gender, and clinical history of the cases counted grid-points which hit the reference volumes. These reference volumes were defined as: 1) cerebral cortex ($V_{ctx}$), which included the gray matter of the iso-, paleo-, and archicortices; and 2) forebrain ($V_{brn}$), which included caudate, putamen, basal forebrain, amygdala, thalamus, globus pallidus and white matter, and the structures included in $V_{ctx}$. Subcortical volume ($V_{sub}$) was calculated as the difference between these two reference volumes ($V_{sub} = V_{brn} - V_{ctx}$). Uncorrected volume measurements on a single (left) hemisphere from each brain were doubled to estimate values for whole brain. The total time required to perform volumetric analyses ranged from 0.5 to 1.0 h per case.

Statistical Analyses

ANOVA and regression analysis were performed with the JMP statistics package (SAS Institute, Cary, NC, USA). For testing inferences, planned comparisons were carried out using diagnosis and age as independent variables, $V_{ctx}$ and MMSE as dependent variables, and age, lag time, and postmortem delay as possible covariants. Step-wise regression analysis was used to evaluate correlations between MMSE scores and $V_{ctx}$ in the AD cases and to assess possible effects of age, lag time, postmortem delay.

Group means and regression variables were considered statistically significant with a minimal probability of Type I error ($p < 0.05$).

RESULTS

As shown in Table 1, the average age at death of the 33 AD cases was 9 years greater [$F(1, 50) = 6.9; p < 0.01$] than that for 19 controls, while the mean brain weight of the AD cases was 15% less than that for controls [$F(1, 42) = 11.6; p < 0.001$]. Compared to controls mean total volumes for AD cases were reduced on average 23% in cortex [$F(1, 50) = 21.9; p < 0.00001$], 22% in forebrain [$F(1, 50) = 21.8; p < 0.00001$], and 21% in subcortical brain regions [$F(1, 50) = 16.0; p < 0.0002$].

Figure 2 shows the area (y axis) of cortical grey matter on each of the 1-cm thick slabs cut along the frontal to the occipital axis of AD (n = 33) and control (n = 19) cases. The differences in total area under each curve in Fig. 2 represents a 23% average reduction in the $V_{ctx}$ for AD cases compared to controls. As shown in this figure, the distribution of cortical volume loss in AD cases extends along the entire frontal-occipital axis.

Figure 3 shows $V_{ctx}$ for 19 controls (solid line) and 33 AD (dashed line) cases. In controls, the regression line shown in the figure [$V_{ctx}$ (all ctrls) = $-2.23\text{Age} + 648.7$] reveals a trend toward reduced $V_{ctx}$ at later ages of death for controls [$F(1, 18) = 3.43; R^2 = 0.18, p = 0.082$]. Evaluation of the regression by gender shows that female controls dying during the fifth to tenth decades have a slightly steeper age-related decline in $V_{ctx}$ than male controls dying during the same age interval. The best-fit regression line for $V_{ctx}$ by age at death for 6 female controls (age 69 to 89 years) is: $V_{ctx}$ (female ctrl) = $-2.24\text{Age} + 603.2$ [$F(1, 5) = 4.32; R^2 = 0.59, p =$
0.13. Using this regression equation to predict $V_{ctx}$ for female controls dying at age 50 and 100 years shows values of 491 cc and 379 cc, respectively, or cortical volume losses of about 22 cc per decade. In comparison, analysis of $V_{ctx}$ for 13 male controls shows relatively minor age-related changes in total cortical volume during the same age interval ($V_{ctx}$ (male ctrl) $= -1.165\text{Age} + 594.7; R^2 = 0.18, p < 0.082$). The regression equation for $V_{ctx}$ as a function of age at death for AD cases (line not shown) is: $V_{ctx} = 1.22\text{Age} + 280$ ($R^2 = 0.01, p = 0.48$).

ANOVA of $V_{ctx}$ as a function of age and gender shows a main effects not significant. Thus, analysis of $V_{ctx}$ changes in female (59 to 100 years) generates $V_{ctx}$ values of about 537 cc and 478 cc, respectively, or an increase in cortical volume of about 24 cc per decade. ANOVA of $V_{ctx}$ for 33 AD cases shows a significant interaction ($F$ (age $\times$ gender) = 7.76, $p < 0.009$), with significant main effects ($F$(gender) = 12.6, $p < 0.001$; $F$(age) = 4.1, $p < 0.05$). Thus, we find that $V_{ctx}$ is significantly greater in AD patients dying at older ages compared to that for AD patients dying at younger ages, with this effect being more pronounced in females than in males.

Figure 3 is a plot of individual $V_{ctx}$ values in cubic centimeters (y axis) as a function of age at death in years (x axis) for 19 controls ($\odot$) and 33 AD ($\odot$) cases. Regression line indicates rate of $V_{ctx}$ decline at increasing ages at death for controls ($V_{ctx}$ = $-2.23\text{Age} + 648.7; R^2 = 0.18, p < 0.082$). Using this regression equation to predict $V_{ctx}$ for female AD patients dying at age 50 and 100 years is: $V_{ctx} = 1.22\text{Age} + 280$ ($R^2 = 0.01, p = 0.48$).

The plot of individual $V_{ctx}$ values for AD cases dying between ages 59 to 94 years shows more severe cortical volume loss than predicted by normal aging alone (Fig. 3). The regression equation for $V_{ctx}$ in 33 AD cases as a function of age at death is $V_{ctx}$ (all AD) = $1.22\text{Age} + 280$ [$F(1, 31) = 0.62; R^2 = 0.01, p = 0.63$]). The slightly positive slope of this regression equation (line not shown) indicates a different pattern from that observed in normal controls (i.e., lower $V_{ctx}$ values at later ages of death). Furthermore, evaluation of regression equations by gender reveals differences in the temporal pattern of cortical volume loss. Regression analysis of 20 female AD cases shows a significant increase in $V_{ctx}$ at older ages of death [$F(1, 19) = 4.11; R^2 = 0.19, p < 0.05$] with the best-fit line showing a strongly positive slope [$V_{ctx}$ (AD female) = $4.34\text{Age} - 9.33$]. Using this regression equation to predict $V_{ctx}$ for female AD patients dying at age 50 and 100 years gives values of 208 cc and 425 cc, respectively, or an increase in cortical volume of about 43 cc per decade. In comparison, regression analysis of $V_{ctx}$ for 13 male AD cases shows relatively minor changes in $V_{ctx}$ for patients dying at earlier versus later ages ($V_{ctx}$ (male ctrl) = $-1.165\text{Age} + 594.7; R^2 = 0.18, p < 0.082$).


discussion

Most studies to identify structural correlates to cognitive function in aging and AD have used semi-quantitative methods to assess NP and NFT densities (2,3). Although modern stereological techniques offer relatively rigorous approaches for quantifying these and other structural brain changes associated with aging and neurodegenerative disease (5,6,23–28,31,36), these studies remain problematic for several reasons, including high biological variability of AD-type neuropathology, the practical difficulties associated

FIG. 4. Plot of final MMSE score (range 1 to 30 possible correct responses) as a function of $V_{ctx}$ in cubic centimeters for 22 AD cases ($\odot$) and 7 controls ($\odot$). The regression equation for the line shown for 22 AD cases (minus covariance for age) is: MMSE = $0.06V_{ctx} - 16.28$ ($R^2 = 0.34; p < 0.007$).
CORTICAL ATROPHY IN AD

with sampling the human cerebral cortex, and the relative scarcity of autopsied brains from well-studied control subjects. As a result, the clinicopathological associations between neuropathology and indices of cognitive performance in aging and AD remain unclear.

Previous stereological studies of autopsied brains across the human lifespan show relative stability in the total numbers of neuron in the noradrenergic locus coeruleus (24), the cerebral cortex (26), and the majority of hippocampal regions (28,36) during normal aging. Consistent with this view, we now report in 19 well-studied controls, including 7 BLSA cases, that total cortical, subcortical, and forebrain volumes are relatively stable in the absence of cognitive decline. In the clinically well-studied BLSA subjects we find no evidence that these minor age-related losses of cortical volume are associated with cognitive decline as measured by the MMSE (Fig. 4; Table 2). In contrast to normal aging, AD cases in the present study showed significant 20 to 23% reductions in total cortical volume (Vctx), total forebrain volume (Vbrn) and total subcortical volume (Vsub) compared to controls. Furthermore, we report that a strong clinicopathological correlation exists between cognitive impairment and cortical volume loss in AD.

Our analysis focused primarily on cortical volume loss, rather than whole brain or subcortical volume loss, because cortex is the brain region most closely associated with global cognitive performance. Our study emphasized Vctx changes during the fifth to the tenth decades because the most significant changes in brain volumes and brain weights occur during these ages (10,16,17,20,22). Among the possible factors which could confound our results are that controls had shorter lag times (number of months between last MMSE test and death) than AD cases (Table 1). This difference arose because the control group included a substantial number (n = 7) of BLSA subjects, and these subjects received more frequent MMSE testing than AD cases. However, step-wise regression analyses including lag time, age, and postmortem delay as covariants confirmed no appreciable impact of these covariants on group differences or correlations involving Vctx.

There is a trend in our data for older controls to show lower cortical volumes than younger controls. This age-effect of approximately 20% Vctx reduction during the fifth to tenth decades in controls is somewhat greater than the 12.3% age-effect reported by Pakkenberg and Gundersen (26) using the same stereological methods. However, two important differences between that study and ours support the confluence of these studies. First, our 20% age-effect refers to combined volume of neocortex and archicortex; the 12.3% age-effect reported by Pakkenberg and Gundersen refers to volume of neocortex only. Combining Vctx values for archicortex and neocortex in the study by Pakkenberg and Gundersen increases that study’s age-effect to 15%. The second difference between these studies is that we focused exclusively on controls dying after age 50; the study by Pakkenberg and Gundersen included a large number of controls dying between the ages of 20 and 50 years of age. Taking these two factors into account shows that these studies are in close agreement concerning reductions in total cortical brain volume of approximately 15 to 20% during normal aging.

With regard to possible gender differences, males in our study were found to have larger brain volumes on average than females, as expected because larger brains are, on average, required by males to support proportionally larger bodies (29). This gender-effect was comparable for controls (18%) and AD (15%) cases, indicating that gender per se does not affect Vctx in normal aging or AD. Evaluation of the regression equations generated from our control data showed that the change in Vctx in female and male subjects during normal aging is roughly 22 cc and 12 cc per decade, respectively. Thus, females were slightly more likely to show age-related reductions in total cortical volumes. This gender difference in age-related loss of total cortical volume was supported by a significant age by gender interaction during the fifth through tenth decades. This finding is consistent with the results from a recent neuroimaging study showing that non-demented aged males experience a gradual onset of total brain atrophy beginning around the start of the fifth decade, followed by a steady decline into old age. Females in the same age groups exhibited brain atrophy beginning later, i.e., during the fifth to sixth decade, and showed a steeper decline than males through the seventh and eighth decades (16). The results from that antemortem neuroimaging study combined with our findings at postmortem examination support the view that during normal aging there is a minor but consistent reduction in total cortical volume, with females experiencing a slightly more rapid decline in total cortical volume than males.

In addition to cortical NPs and NFTs, Alzheimer described severe cortical and brain atrophy in his original study of a 51-year-old female with progressive dementia (1). Previous stereological studies of brains from AD patients show severe reductions in total numbers of neurons in hippocampus (28,36) and locus coeruleus (5), and the absence of global losses of cortical neurons in AD compared to aged-matched controls (27). Our stereological analysis of brain volumes in histopathologically confirmed, clinically well-studied AD cases demonstrates that male and female cases experience comparable, severe (20 to 23%) loss of cortical and brain volumes beyond those expected from normal aging. On this point there is remarkable consistency between our findings and the stereological estimates of brain atrophy in AD from the study by Regeur et al. (27). Both studies used the same methods and materials, i.e., the Cavalieri/point-counting stereological approach, to analyze volumetric changes in systematically sampled reference spaces in brain. Although the subjects in the study by Regeur et al. were not as closely monitored for neuropsychological performance as BLSA and ADRC cases in our study, the cases included in the previous study were diagnosed according to CERAD criteria. Our estimate of a 17% reduction in mean total cortical volume for 20 female AD cases compared to 6 female controls is only slightly higher than the 14% reduction in total cortical volume reported in the previous study for 11 female AD cases in comparison to 10 age- and gender-matched controls (27). Our study included 13 male AD cases which on average showed reductions in mean Vctx of about 20% in comparison to 13 male controls. In summary, these studies using unbiased stereological methods in histopathologically confirmed AD cases show approximately 15 to 20% greater reductions in total cortical and forebrainbrain volumes than expected during normal aging.

Significant differences appear to be present in the temporal

| TABLE 2 Correlations Between MMSE and Vctx for 29 AD and Control Cases with Covariance for Age |
|---------------------------------|--------|--------|--------|--------|
| Groups                        | df     | F      | R²     | p      |
| All (C + AD)                  | 27     | 39.2   | 0.59   | 0.00001|
| C only                        | 5      | 0.94   | 0.26   | 0.78   |
| AD (total)                    | 20     | 27.93  | 0.58   | 0.00001|
| AD (male)                     | 7      | 13.12  | 0.65   | 0.008  |
| AD (female)                   | 11     | 7.73   | 0.41   | 0.020  |

C = control; AD = definite AD; R² = squared pearson product coefficient; n = number of cases; ns = non-significant; MMSE = Mini Mental State Examination; Vctx = total cortical volume (cc).
pattern of cortical volume loss during normal aging and AD. As shown in Fig. 3, controls dying at later ages show more severe cortical volume loss than those dying at earlier ages, while AD patients dying at later ages show less severe cortical volume loss than AD patients dying at earlier ages. Our finding of a reduction in the severity of cortical atrophy in older AD cases confirms a semi-quantitative finding on postmortem material from two decades ago showing that older AD patients manifest less severe total and regional brain atrophy than younger AD cases (17). In both normal aging and AD we found evidence to suggest that gender differences may affect the temporal pattern of cortical volume loss during the fifth to tenth decades. Across the same chronological aging continuum, cortical volume loss in female controls showed a steeper negative slope (22 cc per decade) than male controls (12 cc per decade), while cortical volume loss in female AD patients showed a steeper positive slope (43 cc per decade) than males (24 cc per decade). In support of these observations, in both control and AD groups there were statistically significant age-gender interactions. The underlying mechanisms for this apparent gender-effect on cortical volume loss during aging and AD could provide insight into possible gender differences in the incidence and prevalence of AD in elderly populations.

At present, the underlying neurobiological substrate(s) responsible for the age-, disease- and gender-related differences in total cortical volumes reported in this study are unknown. Given that previous stereological studies have shown no global losses of cortical neurons in AD (27), loss of synaptic connectivity in the cortical neuropil provides a likely explanation for both the group differences in total cortical volumes and the clinicopathological correlation between cognitive decline and cortical atrophy in AD. Evidence in support for this hypothesis can be found in semi-quantitative studies showing significant loss of synapses (11,33,37) and dendritic spines (4,8,13) in cerebral cortices of AD cases compared to controls. Our recent examination of clinically well-characterized ADRC and BLSA cases using quantitative immunoblot demonstrates that cortical synapse loss precedes clinical evidence of dementia in AD (32). Furthermore, our recent studies of cortical volume loss in non-AD dementias provide some preliminary evidence in support of the view that the clinicopathological correlation between cognitive decline and cortical atrophy reported here may be related to presently unknown pathogenic mechanisms which may be unique to AD-type dementia. Postmortem examinations of HIV-dementia and Huntington’s disease (HD) cases show severities of total cortical atrophy comparable to those reported here for AD relative to controls (i.e., 20 to 23%), yet in neither HIV-dementia nor HD is cognitive decline related to the severity of cortical atrophy (25,31). Further studies in other neurological conditions involving cortical degeneration (e.g., idiopathic Parkinson’s disease, Lewy-body variant of AD) will provide information on the disease specificity of correlations between cognitive decline and cortical volume loss at autopsy.

In summary, using unbiased stereology applied to well-characterized, histopathologically confirmed AD cases and controls, we report the relative stability of total cortical volume during normal aging in the absence of cognitive decline. Second, we find that the severity of cortical and brain atrophy in AD ranges from 20 to 25% beyond that attributable to normal aging. Third, we confirm earlier studies showing that compared to total cortical volume loss at younger ages of death, cortical volume loss in normal aging increases at later ages of death and in AD decreases at later ages of death. Finally, there is a strong correlation between the severity of cognitive impairment and cortical atrophy in AD but not normal aging. These findings support our overall conclusions that non-demented aged controls and AD patients experience differential rates of change and show temporal patterns of cortical volume loss from age 50 to 100 years. We believe that the presently undefined mechanisms which lead to severe cortical atrophy in AD may play an important etiological role in the progression of dementia in this disease.

ACKNOWLEDGEMENTS

This work was supported by grants from the U.S. Public Health Service (AG05146, NS07179, AG08325), the Alzheimer’s Association, LEAD (AG07914) and Javits Neuroscience Investigator (NS 10580) awards, and the Develbiss Foundation.

The authors acknowledge assistance by Fellows and Residents in the Neuropathology Laboratory and the Autopsy Service in the Pathology Department, Dr. Claudia Kawas and her staff at the ADRC for collection of clinical data, and Dr. Juan Troncoso for providing neuropathological diagnoses in this study. The authors also thank Dr. Mark West for helpful suggestions on the manuscript.

REFERENCES