EVALUATING THE IMPACT OF CEREBROVASCULAR DISEASE ON COGNITION USING QUANTITATIVE MRI

by

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ABSTRACT

Evaluating the impact of cerebrovascular disease on cognition using quantitative MRI

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Brain atrophy and cerebrovascular disease are associated with cognitive dysfunction, increase in frequency with age and commonly co-occur. However, the relationships between these pathologies and their independent contributions to cognitive function remain unclear. This study quantified brain atrophy and multiple expressions of cerebrovascular disease in 205 individuals, including 34 normal elderly controls, 30 with cognitive impairment and 141 with dementia. Correlations between brain measures were identified and factor analysis was used to generate independent variables that could be used in multiple linear regression models of brain-behavior relationships. The results confirm and extend previous findings suggesting that brain atrophy is the strongest correlate of cognitive impairment. Atrophy was the only relevant factor in those under age 65. Diffuse and strategically located cerebrovascular disease contributed independently to cognitive status in those over age 65. Both the volume and location of cerebrovascular disease (e.g. anterior-medial thalamus) were important determinants of the effects of cerebrovascular disease on cognition. The concept of strategic location of cerebrovascular disease was extended to subcortical white matter pathways and possible specific effects of hyperintensities in acetylcholinergic white matter pathways were identified. Taken together, these *in vivo* studies demonstrate that cerebrovascular disease has small but independent effects on cognitive function and provide impetus to study interventions which might slow or halt the development of cerebrovascular disease with age.

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LIST OF ABBREVIATIONS

AD = Alzheimer's disease
ADDTC = Alzheimer's Disease Diagnostic and Treatment Centers
AMT = Anterior-medial thalamus
ANOVA = Analysis of variance
ANCOVA = Analysis of co-variance
CIND = Cognitive impairment, no dementia
CVLT = California Verbal Learning Test
CSF = Cerebrospinal fluid (see also vCSF and sCSF)
CT = Computed tomography
CVD = Cerebrovascular disease
DRS = Dementia rating scale
DSM = Diagnostic and Statistical Manual of Mental Disorders
FAS = Phonemic fluency (for words beginning with F, A, and S)
FRS = Functional Rating Scale
ICD = International Statistical Classification of Diseases
kNN = k-nearest neighbors
MMSE = Mini-Mental Status Examination
MRI = Magnetic resonance imaging
MTLT = Medial temporal lobe thickness.
NBM = Nucleus basalis of Meynert
NINCDS/ADRDA = National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association
NINDS-AIREN = National Institute of Neurological Disorders and Stroke - Association Internationale pour la Recherche et l'Enseignement en Neurosciences
PLT = Posterior-lateral thalamus.
sCSF = Sulcal cerebrospinal fluid
TIC = Total intra-cranial capacity
VaD = Vascular dementia
VCI = Vascular cognitive impairment
vCSF = Ventricular cerebrospinal fluid
WCST = Wisconsin Card Sort Test

CHAPTER I
THESIS INTRODUCTION

Dementia

Dementia is a cluster of cognitive and functional impairments with multiple possible causes. It is not a single disease. According to the criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM-III-R), dementia is defined as an impairment in memory and at least one other cognitive domain that interferes with social or occupational functioning and represents a decline from a previous level of functioning (American Psychiatric Association, 1994). Often, the cognitive and functional decline occurs insidiously and relentlessly over a period of several years. Dementia is a common disorder in the elderly, affecting one in twelve North Americans over age 65 and approximately one-third of those over 85 (Canadian Study of Health and Aging Working Group., 1994) (Ebly *et al.*, 1994) (Breitner *et al.*, 1999). In European population-based cohorts, the prevalence of dementia in individuals age 65 and older is above 6% (Lobo *et al.*, 2000) (Fratiglioni *et al.*, 2000) (Breteler *et al.*, 1998). Around the world, studies have shown that both prevalence and incidence rates of dementia increase markedly with age (Breteler *et al.*, 1998) (Ott *et al.*, 1995) (Lobo *et al.*, 2000) (Yoshitake *et al.*, 1995). The personal, social and financial costs of dementia are enormous (Ostbye, Crosse, 1994), especially as a larger segment of the population is approaching the at-risk age group (Canadian Study of Health and Aging Working Group., 1994). According to current estimates, one in six men and almost one in three women will suffer from dementia for at least some of their lifetime (Breteler *et al.*, 1998). If these prevalence estimates remain constant, the number of Canadians with dementia (estimated at 252 600 in 1992) will

more than double (to 592 000) by 2021 (Canadian Study of Health and Aging Working Group., 1994).

Causes of cognitive impairment:

Alzheimer's disease (AD) and cerebrovascular disease (CVD)

Alzheimer's disease (AD) is the most common, but not the only, cause of dementia. Occasionally, medical conditions such as infections, thyroid deficiency or B12 deficiency, and autoimmune disorders such as multiple sclerosis, can cause symptoms of dementia that can be improved with treatment. In addition to AD, other degenerative diseases can cause dementia, for example: Lewy Body Disease, Parkinson's disease syndromes, Frontal-Temporal degeneration and Huntington's disease. Cerebrovascular disease (CVD) may also be considered a degenerative condition of the cerebral blood supply which can cause dementia. The term vascular dementia, or VaD refers to dementia as a consequence of CVD. Although there are many potential causes of dementia, AD and CVD are the two most common, and they frequently co-occur. In both European and North American populations, AD is estimated to occur four times more frequently than VaD (Fratiglioni *et al.*, 2000) (Canadian Study of Health and Aging Working Group., 1994) (Ebly *et al.*, 1994) (Breteler *et al.*, 1998) (Breitner *et al.*, 1999) (Ott *et al.*, 1995). In contrast, VaD may be more frequent than AD amongst the elderly in Japan (Yoshitake *et al.*, 1995). Epidemiological studies suggest that individually or in combination, AD and CVD account for roughly eighty percent of all dementias (Canadian Study of Health and Aging Working Group., 1994) (Ebly *et al.*, 1994) (Ott *et al.*, 1995) (Lobo *et al.*, 2000). Recent studies have suggested that AD pathology may be worsened by blood flow deficits. The neuropathology of AD has long been thought to

have a vascular component (Brun, Englund, 1981). Neuropathological studies suggest that the pathogenesis of AD may, at least in part, be due to impaired vascular delivery of nutrients to the brain (for detailed review, see J.C. de la Torre, 1997) (de la Torre, 1997). Recent epidemiological studies also suggest that treatment of vascular risk factors reduces the incidence of dementia (Forette *et al.*, 1998) (Launer *et al.*, 2000) (Kivipelto *et al.*, 2001) (Wolozin *et al.*, 2000) (Jick *et al.*, 2000). Understanding the relationships of these two most common causes of dementia remains a challenge.

What is known about the role of CVD in cognitive impairment

Like AD, CVD becomes increasingly common with age. The risk of having a stroke (the loss or alteration of bodily function that results from a disruption of the blood supply to any part of the brain) doubles with each decade of life after age 55 (Rodriguez *et al.*, 2002). Population studies have shown that increasing age is the strongest correlate of small lacunar strokes (Longstreth *et al.*, 1998) (Longstreth, Jr., 1998) as well as of infarcts without clinical or functional correlates (Lee *et al.*, 2000). A stroke, by definition, involves a change in function, and cognitive domains are commonly affected. Focal lesions affecting critical brain areas can have sudden and severe consequences. Brain structures central to memory processing (medial temporal lobe (Corkin *et al.*, 1997), anterior thalamus and mamillothalamic tract (Tatemichi *et al.*, 1992)) or executive function (pre-frontal (Hildebrandt *et al.*, 1998) and frontal cortex (Leskela *et al.*, 1999) (Smith, Jonides, 1999)) have been identified by correlating pathological changes with behaviors observed in previously healthy individuals with sudden brain injuries. These "strategic" infarcts are thought to be relatively uncommon and are usually reported in individual case studies or small case series. Very few cases of VaD occur without any

coexisting AD (Hulette *et al.*, 1997) (Snowdon *et al.*, 1997) and approximately half of all individuals with probable VaD have significant co-occurring AD neuropathology at autopsy (Victoroff *et al.*, 1995) (Gold *et al.*, 1997) (Kosunen *et al.*, 1996) (Swan *et al.*, 1999).

While rarely the sole cause of cognitive impairment, CVD is present in over 25% of individuals over age 60 (Vermeer *et al.*, 2002) (Longstreth *et al.*, 1998) (Bernick *et al.*, 2001). Many individuals have "silent" infarcts, defined as lesions >3 mm in deep white matter tracts that do not result in impairments in neurological, cognitive or activities of daily living and are identified as incidental findings on a brain scan (Kertesz *et al.*, 1988). These silent lesions occur five times more commonly than symptomatic brain infarcts (Vermeer *et al.*, 2002) and may not be entirely benign.

Cerebrovascular disease is also associated with increased signal intensity, or hyperintensities) on magnetic resonance imaging (MRI) of the brain. Small hyperintensities are commonly seen, even in healthy younger people. Frequently, hyperintense caps are found at the anterior or posterior tips of the lateral ventricles or along the lateral wall of the ventricles. They correlate with slight discontinuity of the ependymal lining (Fazekas *et al.*, 1993a), and are not thought to affect cognitive function (Pantoni *et al.*, 1999). However, with aging, more extensive white matter hyperintensities emerge quite commonly. Microscopic studies of hyperintense lesions have shown mostly demyelination, axonal and glial cell loss, reactive gliosis, and arteriolosclerosis in the small vessels (Brun, Englund, 1986) (Fazekas *et al.*, 1993a) (Marshall *et al.*, 1988) (Braffman *et al.*, 1988) (Englund *et al.*, 1988) (Fazekas *et al.*, 1991b). These changes are proportional to the degree of radiological changes (van Gijn,

1998) and are usually associated with MRI hyperintensities (Munoz *et al.*, 1993). Hyperintensities in deep white matter, basal ganglia and periventricular white matter territories have also been related to small lacunar infarcts, loss of ependymal cell layer, increased periependymal extracellular fluid, decrease in axon number and enlarged perivascular spaces (Fazekas *et al.*, 1993a) (Pantoni *et al.*, 1999). All of these pathological correlates of MR hyperintensities have been shown to occur in experimental animal models of CVD using either chronic hypoperfusion or acute infarction (Pantoni, Garcia, 1997).

It is generally accepted that, while there are multiple causes of MRI hyperintensities, the majority of age-associated white matter hyperintensities are related to vascular disturbances in the broadest sense, including blood vessel wall damage and blood flow abnormalities which lead to ischemia or hypoxia. The evidence supporting this assumption is reviewed in detail elsewhere (Pantoni, Garcia, 1997) (Pantoni *et al.*, 1999) (van Gijn, 1998). The vascular disturbances result in reduced flow of blood through small penetrating vessels. This leads to the demyelination, axonal and glial cell loss and reactive gliosis that manifest as hyperintensities. Certain brain regions, for example the deep white matter near the ventricular system or the deep gray matter nuclei, have the most vulnerable vascular supply. Thus, if a single small perforating vessel becomes occluded or the flow through it is reduced, complete or partial infarction of a small area of deep white matter or deep gray matter can occur.

White matter hyperintensities are associated with subtle cognitive deficits in domains associated with frontal lobe functions such as executive processes, attention and speed of processing in both cognitively impaired and healthy elderly individuals (Briley

et al., 1997) (DeCarli *et al.*, 1995). Otherwise healthy individuals with white matter lesions are more likely to report subjective cognitive difficulties than those without white matter lesions, even in the absence of objective impairments (de Groot *et al.*, 2001). In these otherwise healthy elderly, there may be a threshold effect, with only extensive white matter lesions associated with cognitive impairment. Thresholds of 10 cm^3 (Boone *et al.*, 1992), and 0.5% of the intra-cranial capacity (DeCarli *et al.*, 1995) have been found to relate to cognitive deficits in small samples of otherwise healthy elderly. Extensive white matter lesions also have been associated with specific symptoms and signs (including gait disturbances, urinary incontinence and mood or behavioral disorders) (Briley *et al.*, 1997). Gradual cognitive impairment, mimicking the impairments of AD, can occur due to white matter lesions in the absence of AD (Pantoni *et al.*, 1996).

CVD resulting in deep white matter or deep gray matter lesions can worsen the degree of cognitive impairment caused by AD pathology. Autopsy studies suggest that individuals with CVD exhibit cognitive impairment with less AD pathology compared to those without CVD (Snowdon *et al.*, 1997) (Heyman *et al.*, 1998) (Nagy *et al.*, 1997). Neuropathological studies also suggest that vascular disease is common in dementia and approximately half of all individuals with clinical diagnoses of AD have co-occurring CVD pathology at autopsy (Brun, Englund, 1986) (Kalaria, Ballard, 1999).

Reasons for confusion over the role of CVD in cognitive impairment

Despite the large body of research into the role of CVD in cognitive impairment, a detailed understanding of its relevance in the common and complex setting of aging and dementia has not yet emerged.

Diagnostic overlaps

Most studies of the cognitive consequences of cerebrovascular disease use a group comparison approach. The two most common designs involve 1) comparing individuals with clinically diagnosed VaD to those with AD or 2) comparing those with and without imaging changes such as white matter hyperintensities or lacunar infarcts. These group comparison studies have provided many of the initial insights into the potential contribution of CVD to cognitive impairment, yet there remain methodological limitations to these analyses.

There is no single, reliable definition of VaD (Knopman *et al.*, 2001). At least five sets of criteria are commonly used for the diagnosis of VaD (American Psychiatric Association, 1994) (World Health Organization, 1992) (Chui *et al.*, 1992) (Roman *et al.*, 1993) (Hachinski *et al.*, 1975). These criteria have poor agreement (kappa 0.2 to 0.35) (Chui *et al.*, 2000) (Pohjasvaara *et al.*, 2000). In samples of individuals with suspected VaD analyzed with each criteria, 30% to 90% of the samples would be labeled VaD depending on the criteria applied (Pohjasvaara *et al.*, 2000) (Verhey *et al.*, 1996) (Wetterling *et al.*, 1996). The VaD criteria are generally specific, but not sensitive. The ADDTC and NINDS-AIREN criteria for probable VaD have high specificity (91% and 93%, respectively), but poor sensitivity (25% and 20% respectively) (Gold *et al.*, 2002). Criteria that include possible VaD show somewhat better sensitivity (ADDTC 63%; NINDS-AIREN 58%), but with less specificity (64% and 80%) (Gold *et al.*, 1997).

The overlap between AD and VaD is also seen in the opposite direction: 30-60% of individuals with clinically diagnosed AD have some degree of co-occurring vascular pathology at autopsy (Brun, Englund, 1986) (Bowler *et al.*, 1998) (Kalaria, Ballard,

1999) (Victoroff *et al.*, 1995) (Ellis *et al.*, 1996). Community-based autopsy studies have shown that less than 45% of individuals with a clinical diagnosis of AD have only pure AD pathology (Bowler *et al.*, 1998) (Lim *et al.*, 1999).

Thus, the present study is not limited to comparisons between diagnostic categories or sub-groups. The relationships between brain imaging and cognition are evaluated across all subjects in the sample.

Visual rating scales

Studies of large samples often use semi-quantitative rating scales to assess severity of atrophy or hyperintensities. Subjective rating scales are simple to apply to a large number of scans. However, there are many different rating scales in common use (Scheltens *et al.*, 1998) and they are not always reproducible between different centers (Davis *et al.*, 1992). Study results on the same sample may vary depending on the scale applied (Mantyla *et al.*, 1997). This greatly complicates the comparison of findings across studies. Therefore, this study seeks to develop and apply a reliable method for measuring brain compartment volumes on MRI.

Correlated measures

Both semi-quantitative rating scales and quantitative studies are limited by correlations between brain measures. Generally those individuals with greater white matter hyperintensity volumes show increased ventricular size and reduced cortical volumes (Schmidt *et al.*, 1993) (Swan *et al.*, 2000) (La Rue *et al.*, 1995) (DeCarli *et al.*, 1995) (Ylikoski *et al.*, 1993). It is not clear whether the impairments are more closely related to the effects of CVD or brain atrophy. Some studies suggest that after accounting for atrophy, white matter hyperintensities are not related to cognitive

impairment (Mungas *et al.*, 2001) (Hirono *et al.*, 2000), while other studies suggest that both gray matter volume and white matter hyperintensities are independently associated with cognitive impairments (Stout *et al.*, 1996).

An additional complexity involves correlations between different expressions of CVD. Diffuse white matter lesions commonly co-occur with other vascular lesions (large vessel strokes or focal lacunar infarcts to either gray matter structures or white matter pathways). Thus, it is difficult to untangle the extent to which each lesion type independently contributes to cognitive impairment (Pantoni *et al.*, 1999).

Therefore, this project assesses the relationships between brain and lesion volumes and seeks to identify independent measures that can be used for brain-behavior analyses.

Thesis outline and hypotheses

CVD and AD are the leading causes of cognitive neurological disability in the elderly and are increasingly recognized to co-occur and to synergistically besiege the quality of life of our graying population. Understanding the relationship between AD and CVD is especially important as more effective preventative treatments for both pathologies become available. This project seeks to understand the contribution of CVD to cognitive impairment in the complex setting of aging and recognizes the frequent co-occurrence with AD.

Detailed demographic, medical history, neuroimaging and neuropsychological data from cognitively impaired individuals without co-occurring psychiatric or neurological diseases are used in these analyses. In addition to the more typical diagnostic group comparison approach, the risk factors, correlations and cognitive

consequences of brain atrophy and hyperintensities are explored across the entire sample, rather than by comparing average scores between diagnostic groups.

As described above, MRI is highly sensitive to cerebrovascular disease. In addition, MR imaging is a useful tool for evaluating brain atrophy, or tissue loss. Both global and focal atrophy, for example to medial temporal lobe structures, can be detected by MRI. The role of CVD, after accounting for contributions of global and focal brain atrophy has only recently begun to be explored (Fein *et al.*, 2000) (Mungas *et al.*, 2001). Structural magnetic resonance imaging is non-invasive and safe. MRI provides information about brain structure in live participants, with little risk. There are no replicated scientific studies demonstrating health hazards associated with single or cumulative exposures to conventional magnetic fields (Schenck, 2000). Providing patients are appropriately screened, there is a very high degree of patient safety since the magnetic susceptibility of human tissues is small (Schenck, 2000). Demonstrated risks relate to foreign objects in the body that might move under the influence of a strong magnetic field, or to the anxiety felt by some individuals when lying in the tight confines of the scanner. In Chapter Two, a method using MRI to quantify brain atrophy and expressions of CVD is presented. Two hypotheses are tested: 1) that the method can be performed reliably and 2) that both measures of atrophy and CVD contribute independently to models of cognitive impairment in the sample. These validation studies are performed using a relatively small sample (20 participants).

In Chapter Three, this method is applied to structural magnetic resonance imaging (MRI) scans from all eligible volunteers referred to a cognitive neurology clinic and age-matched normal volunteers (total of 205 participants). A large sample was recruited to

enable multivariate statistical analyses. Detailed clinical histories and neuropsychological test scores are also collected. The neuropsychological tests selected assess diverse cognitive domains, across the range of ability from normal to severely impaired. Summaries of the descriptive, imaging and neuropsychological data collected from the sample and used for all subsequent analyses are presented. The data for each of the major clinical diagnostic groups are provided. Three hypotheses are tested in this chapter. First, the role of location of cerebrovascular disease is addressed. It is hypothesized that cerebrovascular lesions which result in a sudden decline in cognitive function will occur only in those brain regions that are primarily affected by the neuropathology of AD. The second hypothesis is that neuroimaging will reveal greater medial temporal lobe atrophy and minimal cerebrovascular disease in probable AD, with the opposite pattern in the VaD group. Third, it is hypothesized that the groups will show different patterns of impairment on neuropsychological tests; specifically, the probable AD group will have greater impairments on verbal memory tasks, with less impairments on measures of executive function compared to those with VaD.

It is not enough, however, to show that the group with greater executive impairments also has greater cerebrovascular disease. The correlations between brain measurements should be carefully considered so that independent contributors to cognition can be identified. In Chapter Four, the relationships between brain measures are explored and independent variables for brain-behavior analysis are identified. In addition, the relationships between cerebrovascular risk factors and brain volumes are evaluated. It is hypothesized that, if the increased risk of cognitive impairment with CVD risk factors is mediated by accelerated Alzheimer's degeneration, then CVD risk

factors would be associated with increased global and medial temporal lobe atrophy in a cognitive neurology clinic sample.

The independent measures identified in Chapter Four are then used in Chapter Five to evaluate the independent contribution of CVD to multiple cognitive and functional outcomes. Based on the findings from group comparison studies, it is hypothesized that the volumes of CVD and atrophy will independently correlate with measures of cognitive impairment. Specifically, MRI hyperintensities reflecting small vessel disease will contribute to frontal-lobe mediated tasks; in contrast, MRI volumes reflecting atrophy will be the strongest predictors of memory and global cognitive function.

In Chapter Six, the relevance of strategic localization is re-visited, with a focus on strategic effects of white matter hyperintensities involving cholinergic white matter pathways. In this chapter, it is hypothesized that hyperintensities in the deep white matter that have been widely considered diffuse and non-specific may be affecting a key pathway for cortical acetylcholinergic innervation. It is predicted that these lesions will result in cognitive impairments linked to cholinergic dysfunction including impaired visuospatial orientation and increased perseverative behaviors, with equivalent memory and global deficits.

Finally, a brief summary and discussion of the major findings of the thesis project are included in the final chapter.

In summary, this project evaluates the impact of cerebrovascular disease on cognition using quantitative MR imaging. To do this, four major objectives are addressed. These are: 1) to develop a method to evaluate cerebrovascular disease and

atrophy quantitatively, 2) to explore the relationships between brain measures and the role of risk factors, 3) to determine the contribution of independent brain variables (including cerebrovascular disease and atrophy measures) to cognitive impairment and 4) to determine the relevance of location of cerebrovascular disease.

CHAPTER II
QUANTITATIVE EVALUATION OF ATROPHY AND HYPERINTENSITIES

INTRODUCTION

Many of the uncertainties surrounding the role of white matter lesions are due to inconsistency among visual scales used to rate severity on MRI (Mantyla *et al.*, 1997). This greatly complicates the comparison of findings across studies. In addition, diffuse white matter lesions commonly co-occur with other vascular lesions (large vessel strokes or focal lacunar infarcts to either gray matter structures or white matter pathways). Thus, it is difficult to separate the independent contribution of each lesion to cognitive impairment (Pantoni *et al.*, 1999).

Currently, the most widely applied strategies to explore the relationships between atrophy, hyperintensities and clinical status include: 1) semi-quantitative rating scales, 2) intensity-based tracings and 3) segmentation. Each strategy has limitations. First, large research studies often use semi-quantitative rating scales to assess severity of atrophy or hyperintensities efficiently and there are many different rating scales in common use (Scheltens *et al.*, 1998). Subjective rating scales are simple to apply to a large number of scans. However, they are not always reproducible between different centers (Davis *et al.*, 1992) and study results on the same sample may vary depending on the scale applied (Mantyla *et al.*, 1997). There is a lack of consensus regarding basic issues, including which features are the most important to measure (Fazekas *et al.*, 2002).

A second approach uses computer tracings based on intensity-thresholds to delineate individual lesions. This approach is frequently used in multiple sclerosis research to generate total hyperintensity volumes, without separately considering lesions in different locations. Often a single observer can demonstrate high reliability; however,

variability between observers can occur due to the subjective decisions required in setting the thresholds (Filippi *et al.*, 1996). Further, the many small hyperintensities commonly seen in aging, cognitive impairment and dementia render such an approach extremely laborious.

Finally, tissue classification techniques, also called segmentation, attempt to improve the reliability between raters by automation. The spectrum of strategies includes the k-Nearest Neighbors (kNN) approach (Kikinis *et al.*, 1992) (Clarke *et al.*, 1993) (Byrum *et al.*, 1996) (Vinitski *et al.*, 1999), artificial neural networks (Pachai *et al.*, 1998), maximum likelihood (Rajapakse *et al.*, 1996), and fuzzy clustering (Miki *et al.*, 1997). The goal of these approaches is to generate reliable, quantitative information from the MR data efficiently. Segmentation generates brain, cerebrospinal fluid (CSF) and hyperintensity volumes. The segmentation of hyperintense lesion load, however, is complicated by partial volume effects and image inhomogeneities (Bedell *et al.*, 1997). This can lead either to an underestimation of lesion volume or an overestimation through misclassification of some amount of normal tissue as lesion (Grimaud *et al.*, 1996). Additionally, as with the tracing approach, most automated segmentation approaches do not distinguish between anatomically, and possibly functionally, distinct lesions. Only a few quantitative studies have examined both atrophy and regional hyperintensities (Fein *et al.*, 2000) (DeCarli *et al.*, 1995); further, periventricular, deep white matter and deep gray matter changes are rarely quantified separately. This is important in dementia, where large volumes of white matter hyperintensities have been shown to have subtle effects on cognitive status in both dementia and normal aging (DeCarli *et al.*, 1996) (Boone *et al.*, 1992). Even small lesions to the thalamus and basal ganglia may

significantly affect cognitive status in dementia (Castaigne *et al.*, 1981) (Ott, Saver, 1993).

Thus, the goal of this study was to improve a quantitative segmentation method and to determine the utility of simultaneously measuring brain, CSF and hyperintensity volumes for understanding brain-behavior relationships.

METHODS

Participants

Twenty participants were selected from a university dementia study which included voluntary participants from the cognitive neurology clinic, as well as community volunteers. This group was selected randomly from the larger database of eligible participants. Five normal elderly control participants, twelve participants with clinically diagnosed AD using NINCDS/ADRDS criteria (McKhann *et al.*, 1984) and three individuals with clinically diagnosed pure vascular dementia by the NINDS-AIREN criteria (Roman *et al.*, 1993) were included. This distribution reflects the distribution of normal control, AD and VaD within the larger database and is thus likely to be representative of the range of hyperintense lesion and atrophy seen throughout the sample. All individuals with dementia were in the mild to moderate range (average Mini-Mental Status Exam score (MMSE, maximum=30) (Folstein *et al.*, 1975) was 22.1, range: 14-28). Each individual had an MRI scan within ten weeks of a detailed neuropsychological assessment. Out of a larger neuropsychological battery, three test scores were chosen *a priori* to assess the impact of lesion load on cognitive domains commonly impaired in dementia. The Dementia Rating Scale (DRS) (Mattis, 1976) total score was used as a measure of general cognitive function and the five subscores.

reflecting attention, conceptualization, construction, memory and initiation, were used to examine individual cognitive domains. In addition, the acquisition score from the California Verbal Learning Test (CVLT) (Delis *et al.*, 1987) was used as a measure of episodic memory and the perseveration score from the Wisconsin Card Sorting Test (WCST) was taken as a measure of executive function (Heaton, 1981) (Stuss *et al.*, 2000).

Data acquisition protocol

All brain images were acquired using a 1.5-T Signa MR imager (GE Medical Systems, Milwaukee, WI). A standard interleaved spin-echo acquisition was performed in the axial plane covering the whole brain including cerebellum. T2- (figure 1a) and proton density-weighted (PD; figure 1b) MR images were acquired without gaps using 3mm thick slices (TE = 30, 80 msec; TR = 3000 msec, 0.5 excitations, field of view 20 x 20 cm, matrix 256 x 192). Images were transferred to a Sun workstation (Sun Microsystems Inc., Mountain View, CA), and all analyses were performed blind to patient demographics, clinical and neuropsychological data.

Data processing

Segmentation

The initial segmentation was performed based on previously published approaches (Kikinis *et al.*, 1992) (Byrum *et al.*, 1996) using a two dimensional kNN with the addition of hyperintensities as an extra tissue class (figure 1c). A structured "seeding" protocol was applied over 3 slices to select a total of 30 training points (10 per slice) for each of gray matter, white matter and CSF. Slices were determined with reference to neuroanatomic landmarks as described by Byrum et al. (1996) (Byrum *et al.*,

1996). Lesion voxels were selected on the same 3 slices by a standardized protocol with one seed for each periventricular cap or rim in each hemisphere and one seed for each white matter hyperintensity, to a maximum of 10 points per slice. Segmented images were generated by kNN extrapolation from the seeded points to classify all voxels in the image (figure 1d).

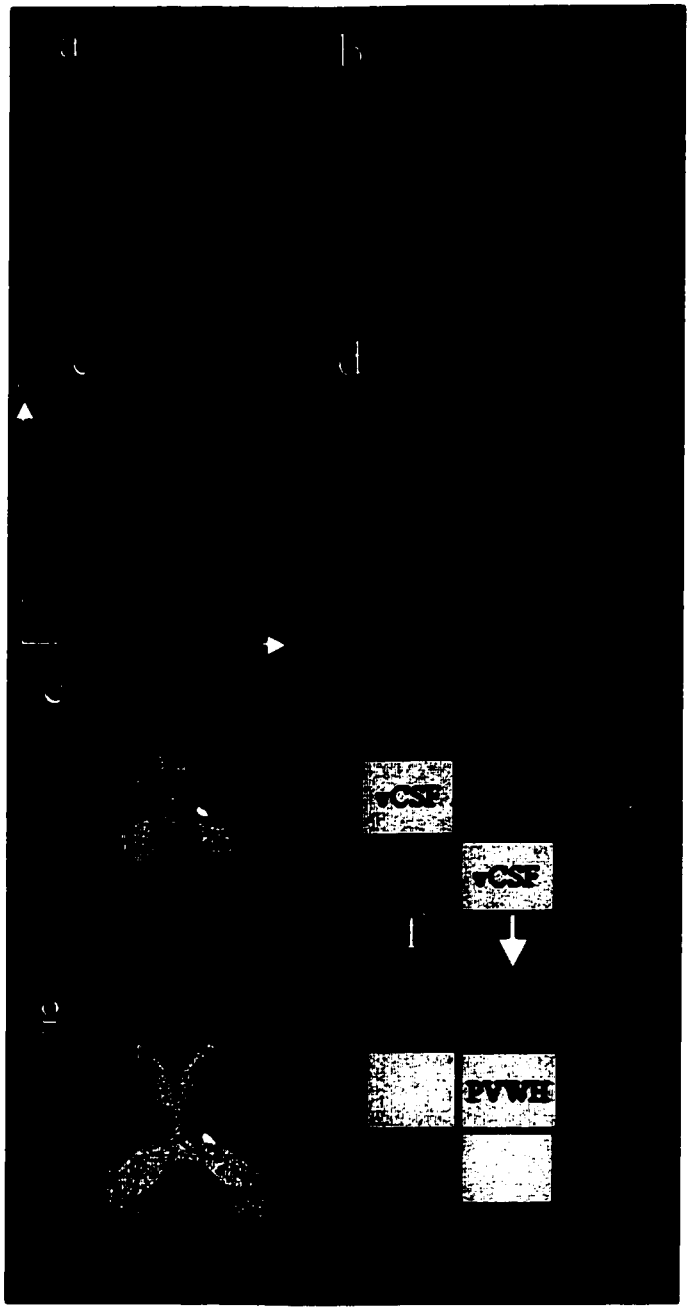

Figure 1: Segmentation and post-processing method The T2- (a) and proton density-weighted (b) MR images are used to select representative points which were on the intensities of the sample voxels yielded an initial segmented image with brain (gray), CSF (dark blue) and lesion (red) tissue types (d). Note voxels in the sulci erroneously classified as lesion. After removal of non-brain tissue, vCSF reclassification (light blue) and delineation of lesions within the thalamus (yellow) and basal ganglia (green) were performed manually (e). Automated reclassification of remaining lesion occurred based on the distribution of voxels sharing edges with a lesion cluster in three dimensions (f). After a last manual review, the final reclassified image without misclassified voxels was used to generate volumes for each tissue type (g).

Manual post-processing

This image was saved as a stack of 2-D slices, imported into in-house software and processed to separate brain from non-brain tissue. To define the ventricles and to sub-classify hyperintense lesion within the basal ganglia and thalamus into separate compartments, large regions of interest were outlined and all voxels of a specified type were converted to a different type within the region using a flood-fill tool. In this manner, CSF within the ventricles was labeled ventricular CSF (vCSF) and deep gray matter lesions were labeled as basal ganglia or thalamic (figure 1e). In order to label deep gray matter hyperintensities in the dementia sample, regions of interest were drawn using anatomic landmarks to delimit the basal ganglia region and the thalamus.

The inferior extent of the thalamus was defined by the suprasellar cistern, an area of CSF that lies just below the thalamic nuclei, and is surrounded by the medial temporal lobes. At this level, the brainstem becomes continuous with the cerebral tissue (there is no CSF surrounding the brainstem structures). From the slice above the suprasellar cistern to the most superior slice including the thalamus, any hyperintense lesion or CSF voxels within the thalamus were defined as thalamic lesion. The medial border of the thalamus was defined by the third ventricle and the lateral border defined by the internal capsule. Similarly, all hyperintense lesion and CSF voxels within basal ganglia structures (specifically the caudate and lentiform nucleus, including the putamen and the globus pallidus; see figure 2) were outlined and reclassified as basal ganglia lesion. The medial border of the lentiform nucleus was defined by the internal capsule and the lateral border was defined by the external capsule. The caudate was limited medially by the wall of the lateral ventricles and laterally by the internal capsule. Basal ganglia lesions were

identified on all slices containing the deep gray matter nuclei. Thus, lesions in the caudate were identified on higher slices than those within the putamen or globus pallidus.

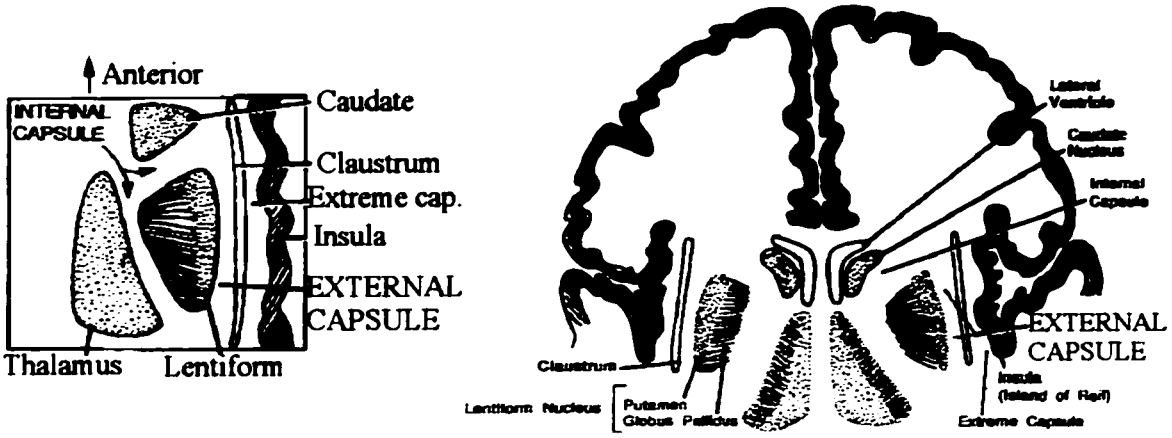

Figure 2: Deep gray matter nuclei as seen with axial (left) and coronal (right) views (modified, with permission, from lecture notes of "Structure and Function", by Dr. I. Taylor, University of Toronto Faculty of Medicine, 1996). Note that on the MR images used in this project, the external capsule, the claustrum and the extreme capsule cannot be distinguished (shown separately in this diagram). These are seen collectively as a thin band of white matter lateral to the putamen and medial to the insular cortex.

Automatic post-processing

A 3-D classification algorithm was applied to separate deep white matter from periventricular lesions and to correct voxels misclassified due to partial volume effects. The program searched for hyperintense voxels not specifically designated in the previous steps and connected all lesion voxels sharing an edge in x, y or z directions into one group. Grouping lesions based on diagonal contacts was explored, but resulted in the aggregation of anatomically separate regions and required considerably greater processing time. The distribution of tissue surrounding each lesion group was used to make reclassification decisions (figure 1f). All lesion groups that were connected to vCSF voxels were, by definition, assigned to the periventricular category. All lesion groups that arose due to partial volume effects were connected to both CSF and either

gray matter or the background region outside the brain. These were labeled "misclassified". Hyperintense areas within the ventricles (e.g. choroid plexus, or whorls of CSF near foramen that are often segmented as lesion) were automatically reclassified as "intra-ventricular hyperintensity" and this volume was assigned back to the vCSF compartment to provide a total ventricular volume. Lesion groups not labeled as basal ganglia, thalamic, periventricular, intra-ventricular or "misclassified" were assigned to the deep white matter lesion compartment. Finally, voxels labeled misclassified were reassigned to either brain or CSF based on their T2 and PD intensities.

Final assessment

A final, manual inspection was performed to correct any areas where the program defaults were inappropriate. For example, inhomogeneity or tissue necrosis can cause some voxels within large lesions to have lower intensity values and thus be segmented as CSF (see Figure 1d, the right occipital PVWH). The medial ends of sulci often contained a small number voxels that remained segmented as lesion, surrounded entirely by gray matter due to partial volume effects. Since these voxels were not connected to both gray matter and CSF voxels, the program would not have labeled these misclassified. Where these events occurred, the areas were manually labeled using the outline and floodfill tool described above. Finally, all voxels labeled misclassified were re-categorized as CSF or brain, based on their location in T2-PD intensity feature space (see feature map - figure 1c).

After these final edits, the volume of each classified tissue compartment was calculated from the final image (figure 1g, including periventricular hyperintensities, deep white matter hyperintensities, basal ganglia hyperintensities and thalamic

hyperintensities, gray matter, white matter, vCSF and sCSF). The PD-T2 images did not provide adequate contrast between gray and white matter, the two compartment volumes were combined to generate a brain parenchyma volume. These data were exported to a statistical software package for analysis.

In order to account for differences in head size between subjects, all volumes were expressed as a percentage of the total intra-cranial capacity (TIC). TIC was calculated by summing the volume of all brain, CSF and hyperintensity volumes for each individual. Thus, for example, the deep white matter hyperintensity volume for each individual was divided by their TIC to provide a head-size corrected volume of deep white matter hyperintensities.

Statistics

Two statistical analyses were performed on this data. First, the reliability of the semi-automated approach was determined using two trained raters. Segmented scans of the twenty participants were processed twice by one rater (RS) and once by a second independent rater (CR). Raters were trained using an independent set of five MR scans from individuals with probable AD (with or without CVD) and these data were discarded. Repeated segmentations for the intra-rater analysis were performed more than one month apart and independently blinded and randomized for each session. Intra- and inter-rater statistics were generated using the intraclass correlation coefficient of reliability (Shrout, Fleiss, 1979), (Fleiss, 1986). Second, the volumes generated by the semi-automated approach were used to explore the utility of collecting these different volumes for examining brain-behavior relationships. The question of which brain measures are relevant for predicting neuropsychological test performance was asked using lesion and

atrophy measures as predictive variables in two-block linear regression analyses. Multi-block regression designs allow different groups of variables to be entered into the regression equation under different conditions. For each neuropsychological measure, the first block consisted of demographic variables (age and education) that were forced to enter the equation. The second block consisted of the volumetric measures (parenchyma, vCSF, sCSF, PVWH, deep white, thalamic and basal ganglia hyperintensities) which were entered into the model only if significantly altering the equation as assessed by the comparing differences in equation R^2 (F-test). Criteria for stepwise entrance into the equation and overall equation significance levels were adjusted for multiple comparisons using the Bonferroni correction.

For all significant equations, secondary regression analyses were performed using total lesion volume in place of the sub-classified volumes to determine the utility of sub-classification.

RESULTS

The average age of the twenty participants was 72 years (± standard deviation 8.6) and the average years of education was 14 (± 3.8). The average MMSE score was 24 (± 4.3, out of a maximum of 30), and the average DRS total score was 121 ± 17 (out of a maximum of 144). The range of scores reflected the diversity expected of a sample including normal controls and individuals with mild to moderate dementia.

The post-segmentation reclassification approach applied to the dementia sample proved to be highly reliable (table 1). Intra-class correlation coefficients above 0.4 are generally considered adequate, and over 0.7 are considered good (Shrout, Fleiss, 1979) (Fleiss, 1986). Total lesion volume, after accounting for misclassification, had excellent

intra- and inter-rater reliability (>0.97). The lesion sub-classification was also very reliable (table 1). Thalamic and basal ganglia hyperintensity volumes were relatively small (<0.12% of total intracranial capacity). The average parenchymal, vCSF, sCSF and total lesion volumes (table 1) were consistent with those reported elsewhere for dementia (Kennedy *et al.*, 1989) (DeCarli *et al.*, 1992) (Kikinis *et al.*, 1992) (Jackson *et al.*, 1994).

Table 1: Reliability of semi-automated segmentation and post-processing.

Compartment	Intra-rater reliability (1 rater, 2 trials)	Inter-rater reliability (2 raters, 1 trial)	Average volume (cm^3)	Average volume (%TIC)	Range (min-max; % TIC)
TIC	.99	.99	1.413	100	n/a
Brain parenchyma	.99	.99	911	65	57 – 76
Sulcal CSF	.98	.98	146	10	5.2 –16
Ventricular CSF	.99	.99	52	3.7	0.8 – 9.9
Periventricular HI	.99	.99	32	2.3	0.2 – 15
Deep white HI	.99	.83	0.90	$6.0x10^{-2}$	0 – 0.28
Basal ganglia HI	.99	.90	0.40	$2.6x10^{-2}$	0 – 0.12
Thalamic HI	.98	.86	0.04	$3.5x10^{-3}$	0 – 0.02

TIC = Total intra-cranial capacity
HI = Hyperintensities

The average misclassified compartment in this sample was 84 cm^3 or 6% of TIC. Misclassified volumes were not correlated with any other measure of lesion volume, including total (correctly classified) lesion volumes or the number of lesion training points selected. All lesion measures except for periventricular hyperintensity volume were significantly correlated with age ($r=0.47-0.59$; $p<0.05$). Thus, all regression models in the brain-behavior analysis accounted for the effects of age, in addition to years of education, since education is known to affect neuropsychological test performance (Anstey *et al.*, 2000).

In the first model, parenchymal (R^2 change = 0.55) and thalamic lesion volumes (R^2 change = 0.17) predicted the DRS total score (model R^2=0.75; p<0.0005) (Figure 3). This finding did not simply reflect differences between the normal controls and participants with dementia: thalamic lesions were highly correlated with DRS total score in the dementia sub-group (n=15, Pearson correlation coefficient = -0.72; p=0.002).

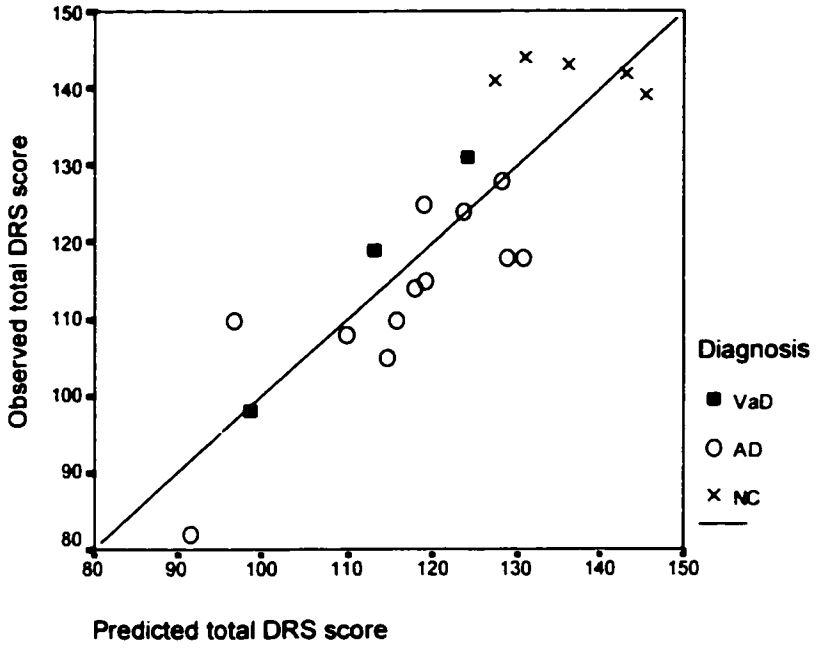

Figure 3: Results of the regression analysis predicting DRS total score
The predicted DRS score was calculated from the regression equation which included weighted contributions of age, parenchymal volume and thalamic hyperintensity volume and is shown on the x-axis as "Predicted total DRS score". This was highly correlated with the observed DRS score (y-axis), indicating a strong regression model (model R^2=0.75; p<0.0005). Individual cases are plotted by diagnosis.

Parenchymal volume was a significant predictor of memory function as assessed using the memory subscore of the DRS (model R^2=0.58; p=0.003) and the CVLT (model R^2=0.57; p=0.003). Thalamic lesions and parenchyma were significant predictors of the conceptualization subscore of the DRS (model R^2=0.79; p<0.0005) and thalamic lesion alone predicted the attention subscore (model R^2=0.54; p=0.005). Basal ganglia

hyperintensity volume was a significant predictor of the construction subscore of the DRS (model R^2=0.56; p=0.004), although this was driven by the dementia subgroup: there was no correlation between DRS construction and basal ganglia hyperintensity in the five normal control subjects. Parenchymal volume was the only predictor of the initiation subscore of the DRS (model R^2=0.62; p=0.001), and of perseveration to previous response on the WCST (model R^2=0.72; p<0.0005). The model for WCST perseveration to previous category was not significant after accounting for multiple comparisons. Brain, thalamic and basal ganglia hyperintensity volumes were important predictors of cognitive impairment in dementia.

To assess the utility of dividing lesion into subtypes, the above regression models were re-applied using total lesion volume (the sum of all hyperintensity volumes) in place of the sub-classified volumes. Brain parenchyma volume remained a significant predictor of total DRS, CVLT and the memory and initiation subscores of the DRS (all R^2 = 0.57 - 0.62; all p<0.003). However, the total lesion volume was not a significant predictor of any of the neuropsychological variables (DRS total, and the conceptualization, attention and construction subscores) that were predicted above by lesion sub-compartments.

DISCUSSION

The reliability of the semi-automated method developed in this study was high. This approach is less arduous to complete than manual tracing of lesions, and has the advantage of providing brain, sulcal and ventricular CSF volumes in addition to the hyperintensity measures. The kNN approach was selected for this work because it is a simple and robust method for segmenting data from MR scans with short acquisition

times (Clarke *et al.*, 1993). The acquisition of additional MR images for a higher-dimensional kNN has potential to offer further information (Vinitski *et al.*, 1999). However, co-registration is required for the segmentation of multiple, independently acquired scans and this may introduce more variability than the segmentation itself (Guttmann *et al.*, 1999). One of the strengths of the approach described here is the ability to apply it to any segmented image that can define white matter hyperintensities. Higher dimensional segmentations, fuzzy clustering and fully automated approaches all result in a segmented image which can be used as input for this approach. Additionally, while this study specifically examined hyperintensities in deep white matter, periventricular white matter, basal ganglia and thalamic regions, any region of interest can be similarly measured, provided the area can be reliably demarcated.

This study demonstrates the importance of progressing beyond estimates of total lesion volume. Regression models using total lesion volume masked important predictors of neuropsychological status. In dementia, thalamic and basal ganglia lesions were important contributors to cognitive impairment. Despite their relatively small volume and the limited sample size in this study, thalamic lesions emerged as a significant independent predictor of overall cognitive impairment, conceptualization and attention, in people with both VaD and clinically diagnosed AD. This underlines the importance of strategic location, whereby even small lesions, if critically situated, can negatively affect cognition. Indeed, small infarcts in the thalamus have been shown to cause dementia even in the absence of AD neuropathology (Castaigne *et al.*, 1981). Basal ganglia hyperintensities were also related to cognitive dysfunction. These findings form in vivo support of a finding from the Nun study (Snowdon *et al.*, 1997), in which individuals

with AD neuropathology and thalamic, basal ganglia or deep white matter infarcts were more likely to express dementia than those with only AD neuropathology. This relationship has also been found in other autopsy series (Hulette *et al.*, 1997), but has not been previously demonstrated in *in vivo* samples. However, given the limited sample, these results should be considered preliminary. Application of this method to a larger sample would be important to determine how common these strategic hyperintensities may be, and whether the relationships with cognitive impairment apply across the range of severity and age typically seen in clinical settings.

The role of white matter hyperintensities in dementia has been controversial. Individuals with AD and extensive white matter changes have different patterns of cognitive and functional imaging impairments than those with AD alone (DeCarli *et al.*, 1996). Further, healthy elderly individuals with white matter hyperintensities have been shown to have subtle cognitive impairments compared with age-matched elderly individuals without white matter hyperintensities (DeCarli *et al.*, 1995). In contrast, a study of younger healthy subjects showed that community volunteers with periventricular white matter hyperintensities perform similarly to those volunteers without white matter changes (Rao *et al.*, 1989). Recent findings from the Nun study have also suggested that periventricular white matter hyperintensities may have no deleterious consequences (Smith *et al.*, 2000). However, none of these studies have examined hyperintensities in other anatomical locations. According to our findings, lesions in other key brain areas might be correlated with PVWH and have a more significant impact on cognitive function in older individuals. A larger study would be useful to examine the role of periventricular hyperintensities in cognition, after accounting for the contributions to

cognitive impairment of atrophy and other focal hyperintensities. This will be undertaken in Chapter Five.

It is important to note that focal lesions do not tell the whole story. Atrophy was also a consistent, significant predictor of both general cognitive function and memory in this dementia sample. Measures of atrophy have been repeatedly shown to correlate with cognitive impairments in dementia (Forstl *et al.*, 1995) (Swan *et al.*, 2000) (Fox, Freeborough, 1997).

In summary, the semi-automated image post-processing protocol developed in this study advanced upon previous methods by separately quantifying lesions in different anatomical areas for analysis, which permitted a finer dissection of brain-behavior relationships. Further, the contributions to cognition of separate lesion locations could be discerned, which were masked when total lesion volume alone was used, as in many previous studies. The simultaneous quantification of brain volume, CSF volumes and anatomically distinct hyperintensity volumes has potential utility for studying the effects of emerging treatments and for improving understanding of the cognitive impairments in dementia.

CHAPTER III
APPLICATION TO A LARGE COGNITIVE NEUROLOGY CLINIC SAMPLE

INTRODUCTION

The questions raised in this project were addressed using a tertiary cognitive neurology clinic sample and normal volunteers from the community. This chapter describes the sample selection and diagnostic criteria for participants, presents the core methods and summarizes the demographic, volumetric and neuropsychological data. To facilitate interpretation and comparison with other studies, data are provided by major diagnostic groups within this sample.

Three hypotheses are tested in this chapter. First, the role of location of cerebrovascular disease is addressed by observing which lesion locations are associated with sudden changes in cognitive status defined by each patient's clinical history.

The neuropathology of AD affects the brain in stages (Braak, Braak, 1991) (Braak, Braak, 1995) (Brun, Englund, 1981). The structures of the limbic system are affected earliest and most severely by AD, including the transentorhinal cortex (Braak and Braak stage I), the entorhinal cortex and hippocampus (stage II), the nucleus basalis of Meynert and hypothalamus (stage III), the posterior cingulate and temporal lobe cortex (stage IV), and the anterior-medial thalamus (stage V). These areas, and the white matter tracts that connect them (mamillothalamic tract, fornix), are commonly affected in published case studies of focal brain injury causing sudden onset of new cognitive impairment (Tatemichi *et al.*, 1995) (D'Esposito *et al.*, 1995) (Duyckaerts *et al.*, 1985) (Katayama *et al.*, 1999). Based on these observations, it is hypothesized that cerebrovascular lesions which result in a sudden decline in cognitive function will occur in a limited number of subcortical brain structures. Specifically, sudden cognitive decline

will occur after lesions involving limbic structures, including the structures of the medial temporal lobe (the hippocampal formation, entorhinal and transentorhinal cortex and amygdala), the mamillary bodies, the anterior-medial thalamus or the posterior cingulate. In addition, extensive damage to isocortical association areas due to large vessel strokes may result in sudden cognitive impairment comparable in severity to the later stages of AD that involve these cortical regions.

The remaining analyses focus on differences between diagnostic groups on neuropsychological and neuroimaging measures. Groups of patients with VaD tend to show less impairment in verbal long-term memory and greater impairment on frontal executive functions, compared with AD patients (Looi, Sachdev, 1999). It is hypothesized that this trend would also be observed in the present sample.

As mentioned above, the medial temporal lobe is affected early and severely by AD pathology. Several studies have found greater medial temporal lobe atrophy in AD compared to normal aging (O'Brien *et al.*, 1997) (Killiany *et al.*, 1993) (Jack *et al.*, 1997), but few have directly examined AD compared to VaD. Since the diagnostic criteria for VaD used in this analysis are good at excluding co-occurring AD (Gold *et al.*, 2002), and since AD preferentially affects the medial temporal lobe, it is hypothesized that those with AD will show greater medial temporal lobe atrophy compared to individuals with VaD. Since those with VaD have cognitive impairment without neurodegeneration of the medial temporal lobe, this measure should be close to normal (in the unaffected side, if a stroke has damaged the medial temporal lobe). In contrast, it is hypothesized that the VaD group, by definition, will have more subcortical white matter hyperintensities than

all other groups, while the AD and CIND groups will show relatively less white matter disease, but still greater than the volumes found in the normal control population.

METHODS

Participants

Participants were volunteers of the Sunnybrook Dementia study, who comprised consecutive eligible patients from a university cognitive neurology clinic, as well as community volunteers. All patients received a standard neurological examination and routine biochemical screening and all participants underwent detailed cognitive testing and magnetic resonance imaging (MRI). Individuals were excluded if the MRI and neuropsychological testing were separated by more than ten weeks, or if MR scans were technically inadequate for volumetric analysis. All subjects were between the ages of 37 and 90 and had to be fluent in English to be included; two individuals with early-onset familial AD were included at ages 37 and 38. Possible secondary causes of dementia (other than vascular disease) and concomitant neurological or psychiatric illnesses were exclusionary. In addition to clinical history, a depression scale and behavioural questionnaire, commonly used in dementia populations, were applied to screen for concomitant psychiatric illnesses. This protocol was reviewed and approved by the institutional Research Ethics Board, and written informed consent was obtained from all subjects or their legal guardians.

Diagnoses were made by an experienced clinician based on standard research criteria. Dementia was defined using the DSM-IV criteria (American Psychiatric Association, 1994). For descriptive purposes, individuals with dementia were categorized by clinical diagnosis as either possible or probable AD (McKhann *et al.*,

1984), or probable VaD (Roman *et al.*, 1993) (Chui *et al.*, 1992). The NINCDS-AIREN criteria, which emphasize neuroradiological data and a temporal relationship between stroke and dementia, identify the fewest cases of VaD, but have high specificity (93%) (Gold *et al.*, 2002) (Verhey *et al.*, 1996) (Wetterling *et al.*, 1996) (Chui *et al.*, 2000). To minimize the likelihood of underlying AD in the VaD subgroup, the NINCDS-AIREN criteria were used.

Dementia was not a requirement for participation in this study. The most common criteria for dementia (DSM, ICD) are based on the pattern of impairment typically seen with Alzheimer's disease. This definition serves to select a sub-set of individuals with cognitive impairment who closely resemble the typical AD pattern and severity of cognitive impairment. It fails to include many cases in which memory loss is not the prominent symptom (Bowler *et al.*, 1999) (Roman, Royall, 1999) (Desmond *et al.*, 1999). Some individuals with vascular disease often have cognitive impairment that does not include memory loss. These individuals would be excluded from analyses focused on dementia. Thus, the concept of Vascular Cognitive Impairment (VCI) has been proposed, as a way of including those who meet criteria for dementia (VaD) and those with vascular disease but not meeting dementia criteria (Bowler *et al.*, 1999). Similarly, many individuals with isolated memory loss fail to meet criteria for dementia but may represent a pre-dementia state. New definitions of "Mild Cognitive Impairment" have been proposed in order to identify those at risk for developing AD so that potential therapeutic interventions may be explored (Petersen *et al.*, 2001). Rather than attempting to differentiate mild cognitive impairment from vascular cognitive impairment, likely an even more difficult task than separating AD and VaD, the Canadian study of Health and

Aging defined a category of individuals with "cognitive impairment, no dementia" (CIND) (Graham *et al.*, 1997). The present analysis follows the lead of the Canadian Study of Health and Aging and applies the CIND concept. Individuals with subjective and objective cognitive impairments who did not meet criteria for dementia were categorized as cognitive impairment, no dementia (CIND) (Graham *et al.*, 1997). This category included both those with impairments due to vascular disease and those with isolated memory impairment, thought to be prodromal AD.

Elderly normal controls were community-dwelling individuals with no history of psychiatric or neurological diseases and without subjective or objective cognitive impairment. The presence or absence of white matter hyperintensities on MR imaging was not used as selection criteria for the normal control group.

Demographic information was collected on all participants including age at time of MR scan, number or years of education, sex, duration of cognitive impairment and the number of cerebrovascular risk factors (hypertension, hypercholesterolemia, diabetes, history of coronary or peripheral vascular disease and history of previous stroke or transient ischemic attack). The pattern of cognitive decline in each participant was categorized as either slowly progressive or step-wise. Two types of step-wise decline were identified: 1) a sudden onset of cognitive decline, temporally related to a new stroke event (required for the diagnosis of VaD, also seen in individuals with CIND from vascular causes), 2) a sudden drop in cognitive or functional abilities in individuals with pre-existing cognitive decline (in individuals with pre-existing probable AD or CIND in whom a new stroke occurred).

Neuropsychological testing

The neuropsychological test battery was selected as part of a large, longitudinal study of aging and dementia. Specific tests were chosen with several priorities: a) to provide assessments of general cognitive and functional ability, b) to index impairments in specific cognitive functions known to be related to particular brain regions, c) to have a sufficient range of difficulty and skills to measure the heterogeneity of AD across the range of severity of disease from mild to severe stages, and d) to require a reasonably short time period to administer. The Functional Rating Scale (FRS), was used to assess functional independence for all participants (Feldman *et al.*, 1995). There are eight domains including memory, social/community/occupational, home/hobbies, personal care, language skills, problem solving/reasoning, affect/behavior and orientation. Each domain is rated from 1 (healthy) to 5 (severe). Thus, a normal (minimal) score would be 8 and the most severe (maximal) score is 40. Two common tests of global cognitive function were used: the Folstein Mini-Mental State Examination (MMSE), which ranges from 0-30 (Folstein *et al.*, 1975) and the Mattis Dementia Rating Scale (DRS) which ranges from 0-144 (Mattis, 1976). In addition the following specific cognitive tests were administered: the California Verbal Learning Test (CVLT) (Delis *et al.*, 1987) as a test of learning and short-term memory, the Forward and Backward Digit Span tests from the Wechsler Memory Scale-Revised (WMS-R), (Wechsler, 1987) as tests of working memory, the FAS fluency (Lezack, 1983) and Wisconsin Card Sorting Tests (WCST) (Heaton, 1981) as measures of executive function, the Benton Line Orientation (Benton *et al.*, 1983) task as a test of visuospatial orientation and attention, and the Boston Naming Test (Kaplan *et al.*, 1978) as a language measure.

MR imaging

All brain images were acquired using a 1.5-T Signa MR imager (GE Medical Systems, Milwaukee, WI). A twelve-minute, standard interleaved spin-echo acquisition was performed in the axial plane covering the whole brain including cerebellum. T2- and proton-density-weighted MR images were acquired without gaps using 3mm thick slices (TE = 30, 80 msec; TR = 3000 msec, 0.5 excitations, field of view 20 x 20 cm, matrix 256 x 192). In addition, a ten-minute, 3-D, axially acquired T1-weighted MR scan (TE/TR = 5/35 msec; flip angle = 35°; 1 excitation; voxel dimensions = 0.86 x 0.86 x 1.3; field of view = 20 x 20 cm; matrix 256 x 192) was used for analysis of the medial temporal region. Images were transferred to a Sun workstation (Sun Microsystems Inc., Mountain View, CA), for post-processing protocols.

MRI post-processing

Volumetric brain measures were extracted from the T2-PD MR images using a reliable protocol of segmentation followed by manual and automated post-processing described previously (see last chapter), with three additions. First, lesions within the anterior one-third or medial one-half of the thalamus were further classified as anterior-medial thalamic hyperintensities and lesions in the lateral one-half and posterior two-thirds of the thalamus were classified as posterior-lateral thalamic hyperintensities (inter-class correlation coefficients for both intra- and inter- rater reliability, >0.87). Second, brain regions involved in large cerebral artery strokes were manually defined by a research neuroradiologist (F.Q.G.), who was blinded to all demographic, clinical and neuropsychological data. Third, the medial temporal lobe thickness was measured by a standardized protocol (Gao *et al.*, 2002), described below.

This linear width measurement was collected as a rapid estimate of medial temporal lobe atrophy, using T1-weighted MR images from all participants using a technique modified for MRI from a CT-based measure (Nagy *et al.*, 1996) (O'Brien *et al.*, 2000). Any rotation of the subject's head was corrected. Axial images were then reconstructed parallel to the long axis of the hippocampus at the level of the inter-collicular sulcus of the midbrain for each individual. Medial temporal lobe thickness was measured as the thinnest width of the medial temporal lobe between the anterior-posterior boundaries of the midbrain on this axial slice. This approach has high inter- and intra-rater reliability (intra-class correlation coefficients > 0.98) (Gao *et al.*, 2002). For individuals who had suffered posterior cerebral artery strokes that involved the medial temporal lobe, the thinnest point of the contra-lateral, unaffected medial temporal lobe was used. Re-alignments and measurements were performed in random order and blind to all demographic, clinical, neuropsychological and other quantitative imaging data.

The volumes of all classified tissues were calculated, including hyperintensities in the periventricular, deep white, basal ganglia, anterior-medial thalamic, and posterior-lateral thalamic regions, large vessel strokes, brain parenchyma, ventricular and sulcal CSF compartments. These data, along with the medial temporal lobe width measurements were imported to a statistical software package for further analyses.

All volumes were individually normalized to total intra-cranial capacity (TIC). The TIC consisted of the sum of all brain, CSF and hyperintensity volumes for that individual. The distributions of all brain imaging measures across the sample were explored and, when necessary, transformations were performed to meet the assumption of normality.

RESULTS

Descriptive statistics

205 individuals met criteria for this study, including 34 normal elderly, 30 participants with cognitive impairment but not dementia (CIND), 126 individuals with possible/probable AD (including 109 individuals with probable AD and 17 participants with possible VaD in combination with possible or probable AD) and 15 individuals with probable VaD. Demographic data for the diagnostic groups are summarized in Table 2. The VaD group was significantly older than all the other groups; the AD group was significantly older than the NC group, and the CIND group was intermediate between the AD and NC groups (model $p<0.0005$). All further analyses accounted for the effects of age. The groups did not differ significantly in sex distribution or number of years of education. Not surprisingly, the VaD group had significantly more cerebrovascular disease risk factors than those with AD, CIND or normal elderly. Normal elderly participants and individuals with possible or probable AD had relatively few cerebrovascular disease risk factors, while individuals with CIND were intermediate. This reflects the two types of individuals with CIND; those with CIND related to cerebrovascular injury, and those with mild cognitive deterioration possibly due to prodromal Alzheimer's disease. 12.6% of the sample (25 individuals with possible/probable AD and 1 individual with CIND) were taking acetylcholinesterase inhibitors at the time of participation. An additional 14.6% took one or more of vitamin E, hormone replacement therapy or non-steroidal anti-inflammatory drugs (but not acetylcholinesterase inhibitors).

Cortical infarcts were common in the VaD group: ten of fifteen (66%) individuals with VaD had cortical strokes. Three of these ten people had multiple cortical strokes involving the association cortex in the watershed territories of the middle cerebral artery. In all three of these patients, there were additional infarcts (basal ganglia, thalamic or other cortical). Three people had posterior cerebral artery infarcts which involved the medial temporal lobe. Four people with cortical strokes had additional infarcts involving the anterior-medial thalamic area. Five individuals with VaD had no cortical lesions and would fit into the emerging concept of subcortical ischemic vasculopathy (Erkinjuntti *et al.*, 2000) (Chui, 2000). This concept "incorporates small vessel disease as a primary vascular etiology, lacunar infarcts and ischemic white matter lesions as primary types of brain lesions, and subcortical location as the primary location of lesions" that cause dementia (Erkinjuntti *et al.*, 2000). Two of these five individuals had only diffuse subcortical white matter changes and the other three had white matter changes plus anterior-medial thalamic infarcts.

Cortical and anterior-medial thalamic infarcts were also found in individuals with CIND after a vascular event. Four people with CIND had anterior-medial thalamic hyperintensities, one person had both a PCA stroke involving the medial temporal lobe and anterior-medial thalamic hyperintensities, and one person with CIND had only a PCA stroke involving the medial temporal lobe. The rest of the CIND group had no strategically located cerebrovascular disease, and a range of subcortical white matter changes similar to those found in AD (see Table 2).

Two individuals who met criteria for probable AD later had co-occurring large vessel cortical strokes. There was pre-existing cognitive and functional decline meeting

criteria for probable AD and then subsequently both participants suffered their strokes. Neither stroke involved the medial temporal region and both individuals had medial temporal lobe thickness below the normal cutoff (Gao *et al.*, 2002). Seven people who met criteria for probable AD had anterior-medial thalamic hyperintensities.

Information concerning the pattern of onset or progression of cognitive impairment was collected via patient and family histories. Twenty-three individuals had either a sudden onset of their cognitive impairment or a sudden drop in function superimposed on existing cognitive impairment. In twenty-one of these twenty-three individuals, the sudden change in cognitive status was related in time to a new hyperintensity or stroke visible on MR imaging. In two cases, large volumes of isocortical association cortex were affected (figure 4c). In all other individuals, lesions occurred in one or more of the hypothesized limbic structures: the anterior-medial thalamus, the medial temporal lobe or the cingulate gyrus (figure 4).

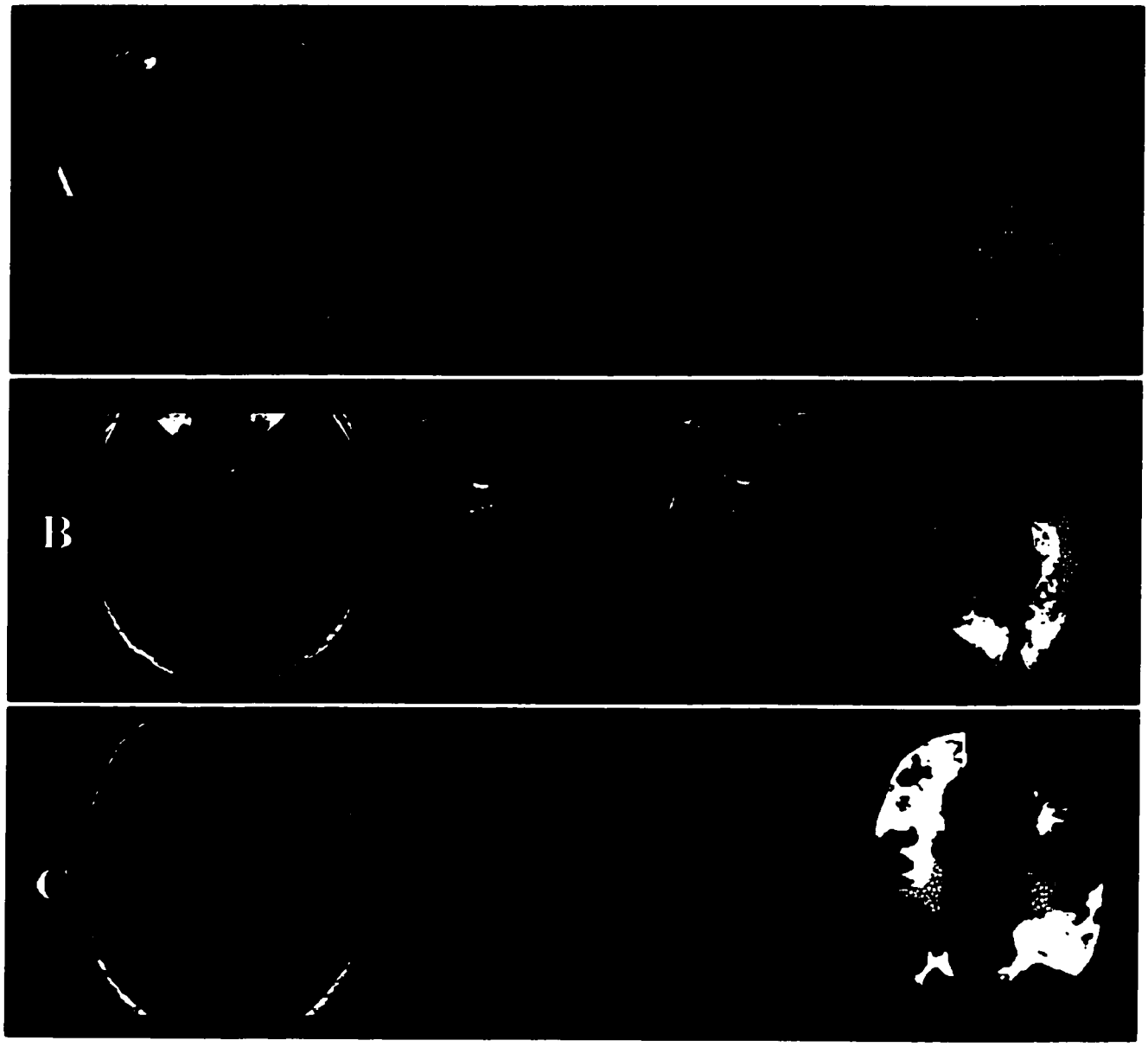

Figure 4: Three individuals with sudden, step-wise decline in cognitive function
From left to right are T1-, T2-, PD-weighted MR and segmented images of a single slice. Sudden onset of impairment occurred after: A) an anterior-medial thalamic small vessel infarct in an 84 year old female with probable VaD (1 year); MMSE = 25; MTLT = 15mm; B) a posterior-cerebral artery (large vessel) infarct affecting the medial temporal lobe in a 59 year old man with probable VaD (2 years); MMSE = 20; MTLT = 13; C) multiple large vessel isocortical association area strokes in a 64 year old male with probable VaD (21 years); MMSE = 23; MTLT = 13.

Anterior-medial thalamic hyperintensities were especially common. Anterior-medial thalamic hyperintensities greater than 5 mm^3 did not occur in any normal controls, and occurred in 29/171 (17%) of those with cognitive impairment. The size of anterior-medial thalamic hyperintensities correlated well with the expression of noticeable change in cognitive status: 10/11 with hyperintensities larger than 60 mm^3 had step-wise decline; in contrast, only 4/18 with hyperintensities smaller than 60 mm^3 had step-wise decline. In addition, those with smaller anterior-medial thalamic hyperintensities and slow decline had greater medial temporal lobe atrophy (figure 5).

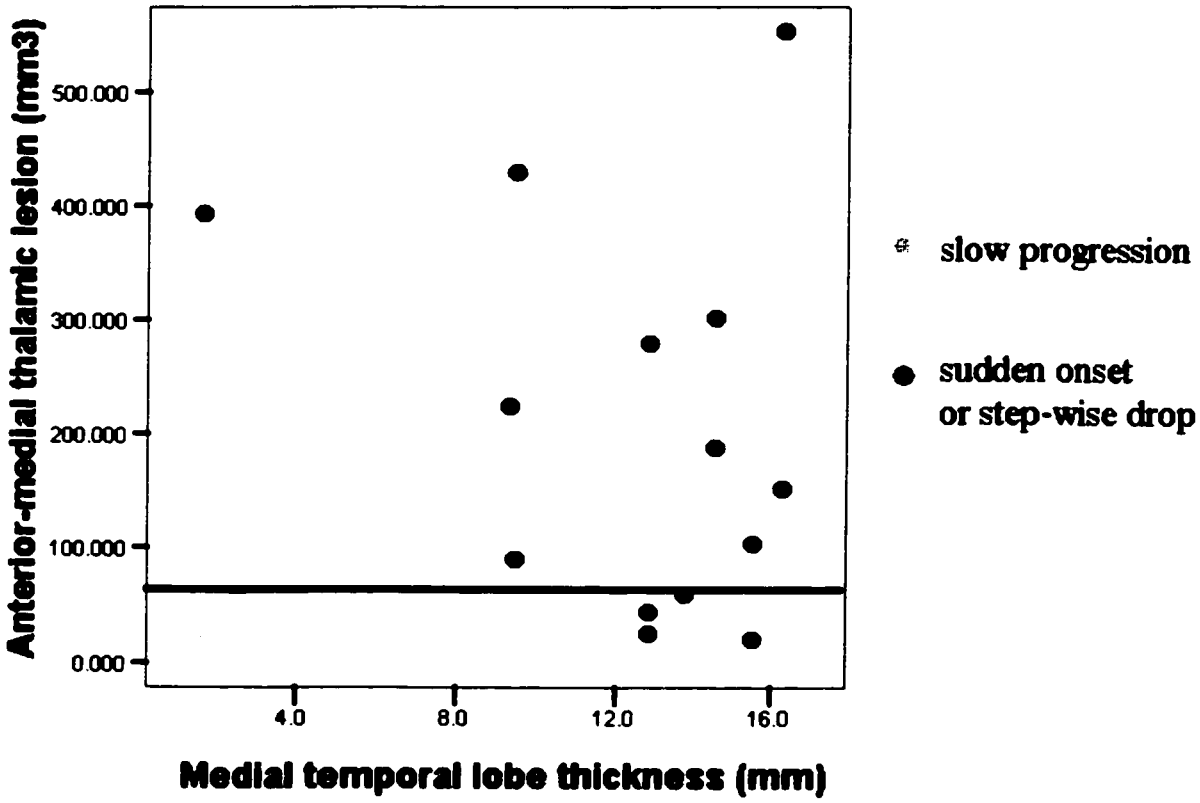

Figure 5: Cognitive decline and medial temporal lobe thickness for all individuals with anterior-medial hyperintensities ≥ 4 voxels. Anterior-medial thalamic hyperintensities over 60 mm^3 (horizontal line), generally resulted in a sudden onset or drop in cognitive function. Smaller hyperintensities that occurred against a backdrop of medial temporal atrophy did not result in an acute change in cognitive function.

Radiological MRI reports identified thalamic infarcts in only eight of the nineteen people. Five of the eight people with VaD who had anterior-medial thalamic hyperintensities were recognized as having thalamic involvement, but only one of seven people with clinical diagnoses of AD, and only two of five with CIND, were identified as having thalamic infarcts.

Brain imaging distributions

All variables except sulcal CSF and medial temporal lobe thickness required transformations to meet the assumption of normality. The volume of cortical stroke, of ventricular CSF and of all hyperintensities were markedly skewed to the right. Logarithmic transformations were performed. Since volumes of zero were possible for one or more measurement, and since the logarithm of zero is not solvable, a constant was added to each variable transformed. The resulting distributions were evaluated for improvement using the Kolmogorov-Smirnov test of normality and using comparison of skewness and kurtosis values for each distribution before and after transformation. For posterior-lateral thalamic and anterior-medial thalamic hyperintensity volumes, a majority of participants had scores of zero, so complete normalization was not possible. For these measures the transformation improved normality (skewness and kurtosis both approaching zero) and all other measures met assumptions of normality after transformation. Therefore, the transformed volumes of ventricular CSF, cortical stroke and all hyperintensities were used for analyses presented in subsequent chapters.

Neuroimaging

Analysis of co-variance (ANCOVA) was performed to explore differences between the diagnostic groups, after accounting for the effects of age. Transformed

variables were used in the analysis for all non-normally distributed measures; however, to facilitate interpretation and comparison, table 2 provides the means and standard deviation of all brain volumes for each diagnostic group. After accounting for the effects of age, diagnostic groups differed on all brain volumes except total intra-cranial capacity (ANCOVA total model $p<0.0005$). Pairwise comparisons between the diagnostic groups (groups with significantly different averages are indicated in table 2 by different letters) showed that the AD and VaD groups had significantly more atrophy (larger ventricular and sulcal CSF and smaller brain parenchyma volumes) compared with the CIND and normal control groups. A subgroup of eleven individuals with CIND had either a single stroke event or co-occurring cerebrovascular disease noted on radiological reports. This group did not differ from VaD in the volume of hyperintensities in the periventricular white matter, subcortical white matter, posterior-lateral thalamic or basal ganglia regions. However, there was less brain parenchyma (VaD average 62% ± 5; vascular CIND average 68% ± 5) and greater sulcal CSF volumes (VaD average 16% ± 3; vascular CIND average 12% ± 3) in the VaD group. Additionally, as expected, those with VaD had the largest volumes of cortical and subcortical hyperintensities. The VaD group had significantly greater volumes of all cerebrovascular disease measures except white matter hyperintensities. There was trend towards increased volumes of deep white matter hyperintensities in the VaD group (VaD average was twice the volume of the CIND and AD groups); however, there was large variability in the VaD group and the group differences were not statistically significant. The AD group had significantly smaller medial temporal lobe thickness than either the normal control, CIND, or VaD groups. The medial temporal lobe thickness was not significantly different between those with

pure AD (n=109; average 7.2 ± 4.3) and those with probable AD plus extensive co-occurring CVD (based on the radiologist scan reports; n=17; average 6.2 ± 4.0). Both these AD subgroups had significantly smaller medial temporal lobe thickness than normal controls (13 ± 2.1) or individuals with VaD (11 ± 1.5) or CIND (11 ± 3.6). In addition, the AD and CIND groups had significantly larger volumes of subcortical white matter hyperintensities (periventricular and deep white matter) compared with the normal control group. This finding remained significant even after excluding the seventeen individuals with probable AD plus co-occurring cerebrovascular disease. The AD, CIND and normal control groups did not differ in the amount of anterior-medial thalamic hyperintensities, while the VaD group showed significantly larger anterior-medial thalamic volumes.

Table 2: Data summary – demographic and brain volumes. Averages and standard deviations are included for each diagnostic group.

	Normal	CIND	possible/probable AD	VaD	p **
N	34	30	126	15	
Age (yr)	67 ± 8.7 [a]	69 ± 7.7 [a,b]	73 ± 9.8 [b]	78 ± 8.1 [c]	**< 0.001**
Sex (% female)	41	57	50	60	0.54
Education (yr)	15 ± 2.7	14 ± 3.4	14 ± 3.7	14 ± 4.1	0.50
# CVD risk factors	0.29 ± 0.58 [a]	1.4 ± 1.2 [b]	0.56 ± 0.80 [a]	2.3 ± 1.1 [c]	**< 0.001**
Duration (yr)	n/a	2.8 ± 1.9 [a]	4.2 ± 2.8 [b]	5.1 ± 5.6 [b]	**0.014**
MMSE	29 ± 1.3 [a]	27 ± 2.2 [a]	22 ± 4.8 [b]	22 ± 3.8 [b]	**< 0.001**
TIC [+]	1 387 ± 106	1 372 ± 167	1 415 ± 164	1 427 ± 171	0.39
Brain parenchyma *	73 ± 3.1 [a]	70 ± 4.7 [b]	67 ± 4.4 [c]	62 ± 3.7 [d]	**< 0.001**
Sulcal CSF *	11 ± 2.9 [a]	13 ± 3.1 [a]	15 ± 3.5 [b]	16 ± 2.9 [b]	**< 0.001**
Ventricular CSF *	2.2 ± 0.9 [a]	3.2 ± 1.7 [b]	4.1 ± 1.8 [c]	5.3 ± 2.5 [c]	**< 0.001**
medial temporal lobe thickness * (mm)	0.00095 ± 0.00015 (13 ± 2.1) [a]	0.00079 ± 0.00026 (11 ± 3.6) [a]	0.00050 ± 0.00031 (7.0 ± 4.3) [b]	0.00072 ± 0.00039 (11 ± 5.0) [a]	**< 0.001**
Periventricular HI's *	0.24 ± .20 [a]	1.1 ± 1.6 [b]	1.0 ± 1.0 [b]	2.1 ± 2.1 [c]	**< 0.001**
Deep white matter HI's *	0.015 ± 0.020 [a]	0.057 ± 0.076 [b]	0.053 ± 0.075 [b]	0.11 ± 0.11 [b]	**0.003**
Anterior-medial thalamic HI's *	0.000034 ± 0.00011 [a]	0.0016 ± 0.0046 [a]	0.00064 ± 0.0035 [a]	0.010 ± 0.015 [b]	**0.002**
Posterior-lateral thalamic HI's *	0.00043 ± 0.0018 [a]	0.0042 ± 0.010 [b]	0.0026 ± 0.0093 [a,b]	0.0080 ± 0.0093 [c]	**< 0.001**
Basal ganglia HI *	0.0046 ± 0.0070 [a]	0.032 ± 0.051 [c]	0.022 ± 0.042 [b]	0.065 ± 0.13 [c]	**< 0.001**
Cortical stroke *	0.000 [a]	0.15 ± 0.49 [b]	0.027 ± 0.29 [a,b]	1.3 ± 1.5 [c]	**< 0.001**

**Age-adjusted univariate comparisons were performed for all brain imaging measures (covariate entered in the model: age = 71.69).
[+] TIC = Total intra-cranial capacity in cm^3 (including cerebellum) * Volumes expressed as a percentage of TIC HI=Hyperintensity
For variables with significant differences, pairwise comparisons were performed; groups with different letters were significantly different from one another.

Table 2b: Averages and standard deviations of the demographic and brain measures for CIND with and without vascular disease.

	mCIND	**vCIND**	**p ****
N	19	11	
Age (yr)	69.7 ± 6.7	69.0 ± 9.5	0.81
Sex (% female)	53	64	0.57
Education (yr)	14.0 ± 3.1	12.9 ± 4.1	0.41
# CVD risk factors	0.9 ± 0.9	2.2 ± 1.3	**0.003**
Duration (yr)	2.4 ± 1.8	3.3 ± 2.0	0.22
MMSE	27.9 ± 1.9	25.9 ± 2.2	**0.01**
TIC +	1354 ± 189	1402 ± 121	0.49
Brain parenchyma*	70.7 ± 4.1	67.6 ± 5.1	0.05
Sulcal CSF *	12.9 ± 3.0	12.1 ± 3.3	0.49
Ventricular CSF *	2.3 ± 1.0	4.6 ± 1.6	**< 0.0005**
medial temporal lobe thickness * (mm)	.0008 ± .0002 (10.8 ± 2.9)	.0008 ± .0004 (10.9 ± 4.8)	0.79
Periventricular HI's *	0.54 ± .67	**2.2 ± 2.3**	**0.005**
Deep white matter HI's *	.03 ± .06	**.10 ± .08**	**0.001**
Anterior-medial thalamic HI's *	.0000	**.0043 ± .0070**	**< 0.0005**
Posterior-lateral thalamic HI's *	.0003 ± .0006	**.0110 ± .0151**	**< 0.0005**
Basal ganglia HI *	.013 ± .019	**.064 ± .071**	**0.01**
Cortical stroke *	.000	**.401 ± .763**	**0.02**

** Age-adjusted univariate comparisons were performed for brain imaging measures

+ TIC = Total intracranial capacity in cm^3 (including cerebellum)

* HI=Hyperintensity, volumes expressed as a percentage of TIC

Neuropsychology

The entire neuropsychological test battery was completed by 190 of the 205 individuals. Seven normal controls had incomplete batteries (5 missing WCST scores, 1 missing digit span and 1 missing the Boston naming test). The seven normal controls with missing data did not differ (ANOVA model p=0.57) from those with complete neuropsychological testing in age, years of education, sex distribution, functional status (FRS) or global cognitive impairment (DRS total score and MMSE). Of the 171 individuals with cognitive impairment, eight individuals either refused or were unable to complete one or more of the Boston naming (3), Benton line orientation (5), FAS fluency (2) and digit span (3) tests. Six of these individuals had probable AD and two had probable VaD. Individuals with AD who did not complete the entire battery were significantly younger (average age: 62 versus 73 years; p=0.004) and more severely impaired overall (average DRS: 96 versus 115, p=0.001; MMSE: 17 versus 22, p=0.004) compared to those who completed the entire neuropsychological test battery. The two individuals with VaD who did not complete the entire battery had greater functional impairment (average FRS: 31 versus 21, p=0.027) and a trend towards greater overall impairment (average DRS: 96 versus 112, p=0.06). The average scores and group differences shown in table 3 were performed excluding individuals with any missing data.

Table 3: Data summary – neuropsychological test scores. Averages and standard deviations are included for each diagnostic group.

Neuropsychological measure	*Diagnostic Group*				
	Normal control n=27	***CIND*** n=30	***Possible/probable AD*** n=120	***VaD*** n=13	$p_{(age\ adjusted)}$*
FRS	8.0 [a]	12 ± 3.0 [b]	19 ± 4.3 [c]	21 ± 4.6 [c]	**<0.001**
MMSE	28 ± 1.4 [a]	27 ± 2.2 [a]	22 ± 4.7 [b]	22 ± 3.2 [b]	**<0.001**
DRS total	140 ± 2.8 [a]	135 ± 6.7 [a]	115 ± 13.7 [b]	112 ± 8.9 [b]	**<0.001**
DRS attention	36 ± 1.0 [a]	36 ± 1.3 [a]	35 ± 2.3 [b]	33 ± 3.9 [c]	**<0.001**
DRS initiation	37 ± 0.88 [a]	34 ± 5.8 [a]	28 ± 5.9 [b]	27 ± 5.1 [b]	**<0.001**
DRS construction	5.8 ± 0.64 [a]	5.9 ± 0.43 [a]	5.4 ± 1.1 [b]	5.5 ± 0.88 [a,b]	**0.015**
DRS conceptualization	37 ± 1.9 [a]	36 ± 2.9 [a]	33 ± 4.9 [b]	32 ± 3.0 [b]	**<0.001**
DRS memory	25 ± 0.75 [a]	22 ± 3.8 [b]	15 ± 4.3 [c]	15 ± 3.5 [c]	**<0.001**
CVLT – acquisition	49 ± 7.4 [a]	36 ± 10 [b]	19 ± 9.3 [c]	18 ± 8.0 [c]	**<0.001**
CVLT – short delay free recall	9.9 ± 2.7 [a]	5.3 ± 3.4 [b]	1.6 ± 2.1 [c]	0.85 ± 1.3 [c]	**<0.001**
CVLT – short delay cued recall	11 ± 2.5 [a]	7.3 ± 3.3 [b]	3.2 ± 2.5 [c]	2.4 ± 1.4 [c]	**<0.001**
WCST – # categories	3.8 ± 0.92 [a]	2.2 ± 1.5 [b]	1.1 ± 1.3 [c]	0.69 ± 0.75 [c]	**<0.001**
WCST – # correct	47 ± 13 [a]	43 ± 11 [a]	34 ± 10 [b]	30 ± 8.0 [b]	**<0.001**
WCST – non-perseverative errors	7.9 ± 13 [a]	13 ± 15 [a,b]	20 ± 15 [c]	23 ± 17 [b,c]	**0.002**
WCST – perseveration to previous category	7.9 ± 4.3	7.9 ± 4.9	9.0 ± 8.9	11 ± 12	0.54
WCST – perseveration to previous response	1.5 ± 1.9 [a]	4.7 ± 5.6 [b]	7.4 ± 5.3 [c]	11 ± 5.4 [d]	**<0.001**
Boston naming	28 ± 1.5 [a]	26 ± 3.5 [a]	20 ± 6.5 [b]	18 ± 5.9 [b]	**<0.001**
Phonemic fluency (FAS)	46 ± 15 [a]	38 ± 12 [b]	26 ± 13 [c]	19 ± 9.8 [d]	**<0.001**
Benton line orientation	25 ± 4.0 [a]	23 ± 5.2 [a]	16 ± 9.8 [b]	12 ± 11 [b]	**<0.001**
Forward digit span	8.9 ± 2.2 [a]	9.3 ± 2.0 [a]	7.8 ± 2.3 [b]	8.2 ± 1.7 [a,b]	**0.004**
Backward digit span	7.2 ± 2.4 [a]	6.7 ± 2.0 [a]	4.8 ± 2.2 [b]	4.8 ± 1.4 [b]	**<0.001**

* Age-adjusted univariate comparisons (covariate entered in the model: age = 71.69).
For variables with significant differences, pairwise comparisons were performed; groups with different letters were significantly different from one another.

By definition, there were large differences in functional status and global cognitive impairment between normal controls and those with CIND and between the CIND and dementia groups. The CIND group had only very mild impairments in functional status and in global cognition, despite having significant impairments in memory (DRS memory, CVLT) and language (phonemic fluency) functions compared with normal controls. Note that these abnormalities appeared only in the group statistics; individuals with CIND would not have had both language and memory deficits or they would have met criteria for dementia. Rather, some individuals had memory difficulties, while others had fluency or executive deficits without memory problems. The AD and VaD groups were significantly more impaired than the CIND group (by definition). However, there were no differences between the AD and VaD groups on measures of global cognitive impairment, functional status, memory, language, orientation or attention/working memory. Compared with the AD group, the VaD group had significantly greater impairment on tests of executive function including the attention sub-score of the DRS, the phonemic fluency (FAS) test and the perseveration to previous response measure from the WCST.

DISCUSSION

This chapter presents the core methods and the descriptive, neuroimaging and neuropsychological data collected from the participants of this thesis project. The data presented here will, in subsequent chapters, be used to explore brain-behavior relationships across the sample. However, before undertaking these analyses, a brief comparison of the data from the diagnostic groups is useful to highlight trends in the

sample. Analyses of the descriptive, volumetric and neuropsychological data each revealed important findings.

Descriptive data, including the age, sex, education and diagnostic group composition of this sample, corresponded well to those of other similar study populations (Kennedy *et al.*, 1989) (DeCarli *et al.*, 1992) (Kikinis *et al.*, 1992) (Jackson *et al.*, 1994). Also consistent with findings in other samples, the vascular dementia group was, on average, older than the control, CIND and AD groups. The risk of cerebrovascular disease increases with age, so it is not surprising that the VaD group was the oldest group. In subsequent statistical analyses, both across the sample and between groups, the effects of age must be co-varied.

Descriptive analysis of the location of cerebrovascular disease and the trajectory of decline highlight the importance of location of cerebrovascular disease. A small number of limbic brain regions were identified in which vascular disease had sudden cognitive consequences. Either the medial temporal lobe, the posterior cingulate, the anterior-medial thalamic region or isocortical association areas (most commonly in territories of the middle cerebral artery) were affected in twenty-one of the twenty-three individuals with step-wise progression in this sample. Since the same brain regions are affected early and severely by AD neuropathology (Braak, Braak, 1991) (Braak, Braak, 1995) (Brun, Englund, 1981), it is not surprising that injury to such areas would emerge as common causes of cognitive impairment in a cognitive neurology clinic referral sample. Infarct location is a key factor in determining both the severity and pattern of cognitive impairment.

Anterior-medial thalamic hyperintensities occurred more frequently than strategic cortical strokes, and were not always related to step-wise progression. Also unlike cortical strokes, thalamic infarcts were frequently not mentioned in radiologist reports. Anterior-medial thalamic hyperintensities may be an under-recognized cause of cognitive impairment due to CVD, even in AD and CIND. Previously, thalamic strokes have been cited as a cause of memory impairment in isolated case reports (Akiguchi *et al.*, 1987) (Goldenberg *et al.*, 1983) (Squire, Moore, 1979) (Mori *et al.*, 1986). However, only one previous study has investigated the potential relevance of small thalamic lesions in a series of individuals with probable Alzheimer's disease. In that study, computed tomography (CT) scan hypodensities in the dorsomedial thalamus correlated with attention and set shifting. A subgroup without these hypodensities had only visual recognition learning and memory impairments, but intact attention while those with both AD and thalamic lesions had learning, memory and attention impairments (Forstl, Sahakian, 1993). One other recent study (Fein *et al.*, 2000) has quantified total thalamic hyperintensities in normal, cognitively impaired and demented individuals with or without lacunar infarcts. However, anterior or medial thalamic infarcts were not considered separately from other locations within the thalamus (Fein *et al.*, 2000). The current study is the first to provide a quantitative assessment of anterior-medial thalamic hyperintensities and relate these findings to a sudden change in cognitive status. The results presented here suggest that anterior-medial thalamic lesions larger than 60 mm^3 may be especially important. This should be considered in future studies of vascular disease and cognition.

Group comparisons using the quantitative neuroimaging revealed two important findings. First, the medial temporal lobe was relatively spared in individuals with VaD, but was significantly affected in both AD and AD with co-occurring cerebrovascular disease. This measure of medial temporal thickness was interpreted to be an index of AD neuropathology, but neuropathological verification was not available in this study. Other studies have found similar differences between AD and normal aging (O'Brien *et al.*, 1997) (Killiany *et al.*, 1993) (Jack *et al.*, 1997), but few have directly examined AD compared to VaD. One previous study of cognitive impairment in AD and VaD found that hippocampal volumes were significantly greater in VaD compared to AD (Libon *et al.*, 1998). A visual rating of temporal atrophy has been shown to distinguish AD from a heterogeneous group of neurodegenerative diseases including VaD, Huntington's disease, schizophrenia, MCI and alcohol related cognitive decline (O'Brien *et al.*, 1997). However, AD and VaD groups were not directly compared. A different study used a linear medial temporal measure similar to the one used here, but on computed tomography (CT). In that study, individuals with depression and normal elderly were distinguished from those with dementia, but no differences were found between dementia types (AD, VaD and Lewy Body dementia) (O'Brien *et al.*, 2000). It may be that the improved resolution and reliability of our MRI protocol aided the discrimination of AD from VaD. It is also possible that the differences were due to different application of measurements. In individuals with strokes involving the medial temporal regions, our measure was taken on the unaffected side and thus did not reflect vascular disease.

The second key observation from the MR imaging analysis involved the quantification of white matter hyperintensities. Hyperintensities in the periventricular

region occur more frequently in dementia compared with normal controls (Pantoni *et al.*, 1999). Studies using visual rating scales have suggested that periventricular hyperintensities are more severe in dementia (Barber *et al.*, 1999) (Vermersch *et al.*, 1996). However, the volumes of these changes in a large sample of individuals with cognitive impairment have not been previously quantified. After excluding individuals with a clinical stroke or in whom the radiology report suggested a large degree of cerebrovascular disease (n=17/109), we expected the remaining selected AD sample would still show increased volume of white matter changes compared with the normal control sample. Indeed, the "pure" AD sub-group had significantly (2-3 times greater volumes) more hyperintensity than normal controls in the periventricular, deep white matter and basal ganglia regions. This was approximately one-third of the amount of white matter changes seen in the mixed or VaD groups. In the periventricular region, the AD group averaged eight cubic centimeters more hyperintensity compared to normal aging, which is close to the volume of white matter changes that have been found to correlate with cognitive impairment in studies of community-dwelling elderly (Boone *et al.*, 1992) (DeCarli *et al.*, 1995).

Neuropsychological differences between the diagnostic groups were largely driven by group definitions (normal controls performed better than those with CIND, who were significantly better than those with dementia). No difference in global cognitive or functional severity measures were found between the AD and VaD groups. Meta-analysis of 27 previous studies suggest that, when matched for age, education and severity of dementia, groups of VaD patients tend to show less verbal long-term memory impairment and greater frontal executive dysfunction compared with AD patients (Looi,

Sachdev, 1999). In the present sample, only measures of executive function, but not verbal memory, differed between the VaD and AD groups, even after excluding the subgroup of individuals with mixed AD and cerebrovascular disease. Eighteen of the studies included in the analysis by Looi and Sachdev investigated verbal memory; of these, six (33%) had similar findings to those reported here (no significant difference between the AD and VaD groups on verbal learning and memory).

Group comparison studies, such as those included in the above meta-analysis, often relate increased white matter hyperintensities to executive impairment in the VaD group, and medial temporal lobe atrophy to memory impairment in AD. Our results suggest that the situation is more complicated in two ways. First, participants with VaD have relatively little medial temporal lobe atrophy, yet still have significant memory impairments. Other factors, perhaps cerebrovascular disease, must be accounting for the effects on memory in these individuals. Second, the extensive white matter changes seen in VaD may not be related to the executive dysfunction we observed. Individuals with VaD also had more global brain atrophy, more cortical strokes and greater volumes of anterior-medial thalamic hyperintensities than those with AD, CIND or normal controls. The independent contributions of these brain changes must be explored to determine their relevance in the context of aging, co-occurring AD and multiple expressions of cerebrovascular disease.

In summary, analysis of this large, well characterized sample identified several "strategic" areas of gray matter regions in which strokes are related to a sudden change in cognitive status. Cerebrovascular disease affecting the anterior-medial thalamus was identified as a potentially under-recognized contributor to cognitive impairment,

especially when larger than $60mm^3$. The rapid medial temporal lobe thickness measure was most severely affected in AD, while subcortical and periventricular white matter hyperintensities were common in VaD, CIND and AD. Even the pure sub-group of AD showed more than twice the white matter hyperintensities of normal elderly. Since many imaging and neuropsychological variables differed between the groups, simply attributing executive impairments to hyperintensities and memory loss to medial temporal atrophy is overly simplistic. The emphasis of future studies should not be on group differentiation, but rather on exploring the relationship between cognitive outcomes and different types and locations of brain changes. It is likely that multiple pathologies can lead to similar cognitive or behavioral outcomes. More detailed analysis of the independent effects of these multiple brain imaging changes on cognitive function is warranted and will be explored in subsequent chapters.

CHAPTER IV
RISK FACTORS AND RELATIONSHIPS BETWEEN BRAIN CHANGES

INTRODUCTION

AD and cerebrovascular disease share several common risk factors. While age has long been appreciated as a risk factor for all types of cognitive impairments, recent evidence has mounted that many vascular disease risk factors increase the likelihood of cognitive impairment. Midlife and later-life hypertension (Kivipelto *et al.*, 2001) (Launer *et al.*, 2000) (Skoog *et al.*, 1996) (Tzourio *et al.*, 2001), atrial fibrillation (Ott *et al.*, 1997), diabetes (Ott *et al.*, 1999) (Stolk *et al.*, 1997), high fat intake (Kalmijn *et al.*, 1997), high serum cholesterol levels (Kivipelto *et al.*, 2001) (Kivipelto *et al.*, 2001) (Hebert *et al.*, 2000), postural hypo- or hypertension (Matsubayashi *et al.*, 1997), cardiovascular disease (Fahlander *et al.*, 2000), peripheral vascular disease (Skoog *et al.*, 1999) and past history of stroke or transient ischemic attacks (Yoshitake *et al.*, 1995) have all been related to increased risk of cognitive impairment or dementia in large, population studies. Further, anti-hypertensive therapy (Tzourio *et al.*, 2001) (Forette *et al.*, 1998) and statin therapy to lower cholesterol levels (Wolozin *et al.*, 2000) (Jick *et al.*, 2000) have both been shown to reduce the risk of cognitive impairment in elderly participants. It is not clear whether the effects of cerebrovascular disease risk factors on cognition are attributable to overt cerebrovascular disease or whether these risk factors accelerate neurodegenerative damage.

White matter hyperintensities on magnetic resonance images are strongly related to cerebrovascular disease risk factors including history of previous stroke (Sullivan *et al.*, 1990), mid-life hypertension (DeCarli *et al.*, 1999) (Swan *et al.*, 1998) (Skoog *et al.*, 1996), diabetes (Carmelli *et al.*, 1999), atherosclerosis (Yamauchi *et al.*, 1999)

(Wiszniewska *et al.*, 2000) (Longstreth *et al.*, 1998), high cholesterol (Carmelli *et al.*, 1999). However, the relationship between risk factors and brain atrophy measures is less clear. Some quantitative studies suggest that brain atrophy is correlated with cerebrovascular disease risk factors (Swan *et al.*, 1998) (DeCarli *et al.*, 1999) (Petrovitch *et al.*, 2000), while others have found that atrophy is not correlated with any vascular risk factors (Hirono *et al.*, 2000). The e4 allele of the apolipoprotein gene is associated with increased risk of AD and has been correlated with brain atrophy in some studies (DeCarli *et al.*, 1999) (Geroldi *et al.*, 1999) but not in others (Barber *et al.*, 1999) (Jack Jr. *et al.*, 1998) (Bartres-Faz *et al.*, 2001).

The current study was designed to assess the relationship between brain imaging measures and cerebrovascular disease risk factors. It is hypothesized that, if the increased risk of cognitive impairment with CVD risk factors is mediated by accelerated Alzheimer's degeneration, then CVD risk factors would be associated with increased global and medial temporal lobe atrophy in a cognitive neurology clinic sample. In contrast, if the increased risk from CVD risk factors is mediated though vascular damage to the brain, these risk factors would relate only to MR lesion measures.

METHODS

In multiple linear regression, there are three central assumptions that must be met for the analysis to be considered robust. First, variables are assumed to be normally distributed. Second, a minimum sample size is required. The most common convention states that there should be at least ten subjects for each predictor variable (Norman, Streiner, 1986). Third, both the cases and the predictor variables should be independent. In most brain-behavior analyses, each case is a separate individual and therefore

independent; however, the predictor variables (brain volumes) are not necessarily independent. An individual with larger volumes of periventricular hyperintensities might be more likely to have larger volumes of other cerebrovascular disease measures. Similarly, an individual with more focal atrophy of the medial temporal lobes might also have larger ventricular CSF volume. When measures are highly correlated (a problem called multicollinearity) these variables are unlikely to provide unique contributions to the dependent measure and therefore only one of the measures may enter a regression equation. Therefore, a careful analysis of the correlations between measures is required. If variables are highly correlated, it is possible to determine if some of these variables are actually reflections of a smaller number of underlying variables.

Correlations between the normalized and transformed brain measures were evaluated descriptively using bivariate Pearson correlation coefficients. To identify independent variables, a factor analysis was performed using the brain measures. Factor analysis is a statistical tool that can be used to identify simple patterns in the relationships amongst variables. In particular, this approach can be used to assess whether observed variables can be explained largely in terms of a smaller number of underlying variables, called factors. Factor analysis is commonly used as a data reduction tool, to identify a small number of factors that explain most of the variance observed in a larger number of variables. A second common use is to screen variables for subsequent analysis (for example, to identify collinearity prior to performing a linear regression analysis) (Norman, Streiner, 1986). Both these uses of factor analysis are applied in this thesis: in this chapter, factor analysis is used to explore collinearity amongst the brain measures

and in chapter five, factor analysis is used to reduce the number of cognitive variables for brain-behavior correlation analyses.

Factor analysis expresses each variable as the sum of common and unique proportions. The common portions of all the variables are explained fully by the underlying factors, while the unique portions are uncorrelated with each other and with the factors. First a single factor is generated reflecting correlations with all variables. Each factor can be understood as a weighted combination of the original variables. If the unique, or unexplained, portions of the correlations are very high, then the single factor is insufficient and a second factor is explored. Additional factors are added to optimize the explanation of the relationships between the variables. Taken to the extreme, this would produce an overloaded solution in which there is one factor for each variable. This solution would explain 100% of the correlations, but each factor would essentially be a re-expression of each variable. Thus, ideally a factor solution will identify a minimum number of factors that explain most of the variance in the data. The amount of variance explained by each factor is expressed as the eigenvalue. Eigenvalues are standardized to the number of variables in the sample. Thus, in a factor analysis of 10 variables, the total sum of eigenvalues would be 10. In practice, it would take 10 factors to account for 100% of the variance in the population, but, three factors might, for example, account for 80% of the variance. In that case, the sum of eigenvalues from the three relevant factors would be 8. The variables with the largest eigenvalues account for largest amount of variance. The most common rule for selecting the number of factors to consider, is to use only those factors with eigenvalues greater than 1.0 or, in other words, only those factors that explain more than would be explained by a single variable.

A factor loading matrix shows the correlations between each variable and the factor. For the independent effects of the factors to be interpretable, a rotation of these correlations is performed. In an orthogonal rotation, each factor remains independent from the others. This is key if the goal is to reduce the number of independent variables for analysis or to generate independent measures for later brain-behavior analyses. Finally, of the many types of orthogonal rotations, the most widely used criterion is that the rotation should maximize the variance explained by each factor. This is called a varimax rotation.

Volumes of the hyperintensities in the periventricular, deep white matter, basal ganglia, posterior-lateral and anterior-medial thalamus, volume of strokes affecting the cerebral cortex, vCSF and sCSF volumes and medial temporal lobe thickness were entered as variables. A principal components factor analysis with varimax rotation was applied and only those factors with eigenvalues greater than one were retained.

To explore the relationship between each CVD risk factor and brain imaging measures, multiple analyses of covariance (ANCOVAs) were applied, one for each risk factor. Age at the time of imaging was entered as a covariate. ANCOVAs were performed, comparing those with/without six risk factors: ApoEe4, a history of hypertension, treatment for high serum cholesterol, a history of diabetes, a history of coronary artery disease or peripheral vascular disease and history of previous stroke or transient ischemic attack. Since there were six risk factors, the Bonferroni correction for multiple comparisons was used and total model significance levels were set at alpha=0.05/6 = 0.0083. In any significant total models, individual brain measures were compared at significance level alpha=0.05.

To determine whether age and number of cerebrovascular risk factors contribute independently to the brain imaging factors, three multivariate, step-wise linear regressions were performed. Significance levels were set at 0.05/3=0.016.

RESULTS

Correlations between brain imaging measures

Brain volumes were highly correlated (Table 4). Correlations between all cerebrovascular measures were statistically significant, but many were moderate in strength ($p<0.005$; Pearson correlation co-efficient between 0.2-0.8). Of all the cerebrovascular disease volumes, hyperintensities in the periventricular white matter and deep white matter were the most strongly correlated (Pearson correlation co-efficient = 0.76; Figure 6). Two atrophy measures, sCSF and medial temporal lobe thickness, were correlated with each other, but were not significantly correlated with cerebrovascular disease measures. In contrast, ventricular volume was significantly correlated with all measurements. Indeed, a post-hoc regression model showed that vCSF volume was independently related to age, medial temporal lobe thickness, periventricular white matter hyperintensities and volume of cortical stroke (model $R^2=0.44$; $p<0.0005$).

Table 4: Brain volume correlations Pearson correlation co-efficients for brain imaging measurements after appropriate transformations. All volumes were normalized to total intra-cranial capacity .

Measure	*sCSF*	*MTLT*	*DW*	*PV*	*PLT*	*AMT*	*BG*	*vCSF*	*Stroke*
SCSF									
MTLT	-0.38**								
DW	0.14	-0.16							
PV	0.17	-0.29**	0.76**						
PLT	0.15	-0.04	0.51**	0.56**					
AMT	0.07	0.08	0.31**	0.34**	0.55**				
BG	0.17	-0.08	0.55**	0.64**	0.63**	0.40**			
vCSF	0.33**	-0.47**	0.38**	0.53**	0.32**	0.20*	0.32**		
Stroke	0.14	0.13	0.20*	0.23*	0.31**	0.26**	0.23*	0.25**	

* Correlation is significant at two-tailed $p<0.005$

** Correlation is significant at two-tailed $p<0.0005$

sCSF = sulcal CSF volume. MTLT = width at the thinnest point of the medial temporal lobe. DW = transformed deep white matter hyperintensity volume. PV = transformed periventricular white matter hyperintensity volume. PLT = transformed posterior-lateral thalamic hyperintensity volume. AMT = transformed anterior-medial thalamic hyperintensity volume. BG = transformed basal ganglia hyperintensity volume. vCSF = transformed ventricular CSF volume. Stroke = transformed volume of all cortical strokes.

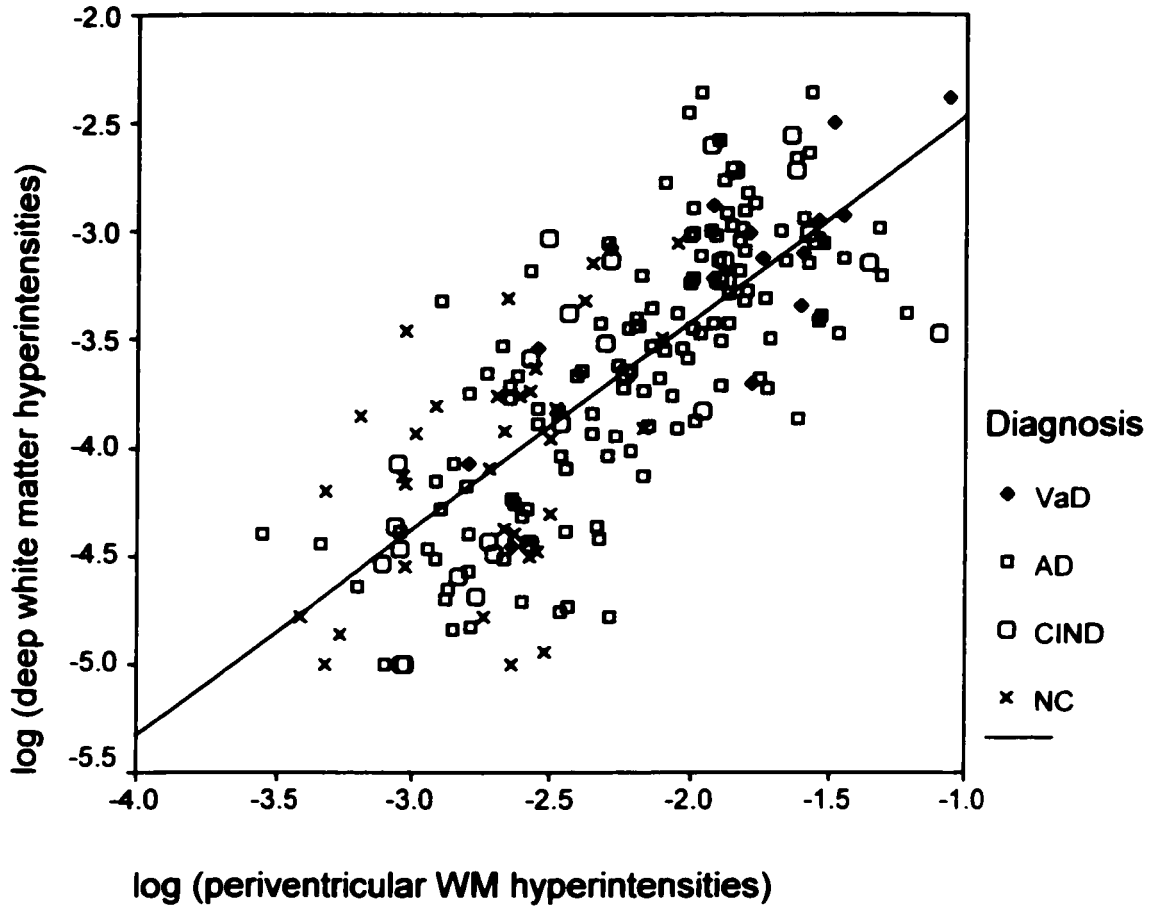

Figure 6: Periventricular and deep white matter hyperintensities when appropriately transformed were highly correlated (Pearson correlation co-efficient = 0.76), regardless of diagnosis.

In the factor analysis, three factors emerged with eigenvalues greater than 1 and accounted for 69% of the variance in the sample. Table 5 contains factor loadings for brain measures, reflecting the correlations of each variable with the final factor. The first factor (40.7% of total variance) reflected measures of small vessel disease (deep white matter, periventricular, basal ganglia and posterior-lateral and anterior-medial thalamic hyperintensities). The second factor (17.5% of total variance) was weighted by measures of atrophy (vCSF, sCSF and medial temporal lobe atrophy). The third factor (11.1% of

total variance) reflected strategic lesions, including large vessel strokes involving the cerebral cortex and small vessel disease affecting the anterior-medial thalamus.

Table 5: Factor analysis of volumetric brain measures. Strongest factor loadings are shown, except where one variable loaded equally (± 0.05) on multiple factors.

Factor Description	Brain Measure		Factor 2	Factor 3
Atrophy	VCSF		0.68	
	SCSF		0.76	
	Medial temporal lobe		-0.80	
Strategic lesions	Anterior-medial thalamic HI			0.51
	Large vessel stroke			0.84

Risk factors and brain volumes

In this sample, history of coronary artery disease or peripheral vascular disease, history of previous stroke or transient ischemic attack, and status of concurrent therapy for diabetes, high cholesterol and hypertension were available in all participants. Apolipoprotein genotyping was available for a sub-sample of participants. In total, 127 of the 205 individuals in the sample had ApoE genotyping, including 2/34 normal controls, 16/30 individuals with CIND, 97/126 with possible or probable AD and 12/15 with vascular dementia. History of hypertension was present in 47 individuals, 27 individuals had a history of high cholesterol, 35 individuals had a history of coronary artery or peripheral vascular disease, 34 individuals had a previous stroke or transient

ischemic attack and 14 individuals had diabetes. In total, one or more of the vascular risk factors were present in 8/34 normal controls, 20/30 CIND, 50/126 AD, 15/15 VaD.

All brain measurements were significantly correlated with age ($0.26<R<0.40$; all $p<0.0005$), except volume of cortical strokes ($R=0.05$; not significant). Thus, in all analyses of brain volumes with risk factors, the effects of age were co-varied. Since so few normal controls had ApoE genotyping data available and since the normal control group had very few cerebrovascular disease risk factors, comparisons were made using only those individuals with cognitive impairment. Thus, any differences between those with and without a risk factor will not reflect the predominance of healthy brains in the risk factor-negative groups.

Individuals with a history of high cholesterol had significantly larger s-CSF and v-CSF volumes and larger periventricular WMHI volumes compared to those without high cholesterol ($p=0.006$). Compared to those without cardiovascular disease, individuals with a history of coronary artery or peripheral vascular disease had significantly larger deep white matter, periventricular, posterior-lateral and anterior-medial thalamic hyperintensity volumes, larger vCSF volumes and larger volume of cortical strokes ($p=0.002$). Individuals with a history of previous stroke or transient ischemic attack had significantly more deep white matter, periventricular, posterior-lateral and anterior-medial thalamic and basal ganglia hyperintensities, larger volumes of cortical strokes and greater medial temporal lobe thickness ($p<0.0005$).

After accounting for the effects of age and for multiple comparisons, there were no significant differences between individuals with and without current (late-life) hypertension ($n_{positive}=47$) or diabetes ($n_{positive}=14$). There were also no significant

differences between carriers of the ApoEe4 allele ($n_{positive}$=65) and individuals without the ApoEe4 allele.

Multivariate linear regression models were used to predict individual brain imaging factor scores based on age and total number of cerebrovascular disease risk factors (Table 6). Age and number of risk factors were both independent predictors of small vessel disease ($p<0.0005$). In contrast, only age predicted the atrophy factor ($p<0.0005$) and only the number of CVD risk factors predicted the strategic infarct factor ($p<0.0005$). The age-by-risk factor interaction term did not contribute to any model.

Table 6: Relationship between brain imaging factors and risk factors.

Predictor variables	*Predicted Variables*		
	Factor 1 (small vessel disease)	**Factor 2 (atrophy)**	**Factor 3 (strategic infarcts)**
Age	0.142*	0.140*	–
# CVD risk factors	0.069*	–	0.151*
TOTAL MODEL	**0.211***	**0.140***	**0.151***

The predicted brain imaging factors are shown in each column. The amount of independent variance in each factor explained by age is in the first row and by number of CVD risk factors in the second row. The bottom row shows the total variance explained by each multivariate linear regression analysis.
* $p<0.0005$

DISCUSSION

This study evaluated the relationship between cerebrovascular disease risk factors and brain imaging measures in a cognitive neurology clinic sample. As expected, CVD risk factors were significantly related to the presence of multiple measures of cerebrovascular disease. Specifically, a history of coronary artery disease or peripheral vascular disease, stroke or transient ischemic attack were related to all small vessel and large vessel disease volumes. In contrast, a history of high cholesterol was correlated

with periventricular hyperintensities and ventricular size, but not with other small vessel or large vessel disease measures. This raises the possibility that more than one mechanism underlies periventricular white matter changes and ventricular dilation. Two other studies have suggested that periventricular and deep white matter hyperintensities may be partially independent. In one study, middle age aortic atherosclerosis was related to periventricular, but not deep white matter hyperintensities in later life (de Leeuw *et al.*, 2000). A second study of over 1000 elderly subjects showed that number of carotid artery plaques and increase in intima media thickness were related to the severity of periventricular, but not subcortical white matter, lesions (de Leeuw *et al.*, 2000). The data presented here suggests that periventricular hyperintensities and ventricular atrophy may be related to subtle vascular changes from elevated cholesterol levels and, along with other subcortical white matter lesions, may be related to symptomatic vascular disease (history of coronary artery disease, peripheral vascular disease, transient ischemic attacks and stroke).

Regardless of the route of damage, however, periventricular and deep white matter hyperintensities were highly correlated. Studies attempting to disambiguate their contributions to cognitive impairment should be undertaken with caution. A recent analysis of periventricular and subcortical white matter lesions was undertaken in a large, population sample of healthy elderly. In this sample, both periventricular and subcortical white matter hyperintensities, when analyzed separately, correlated with neuropsychological measures. When analyzed conditionally on each other, only periventricular white matter hyperintensities were correlated with global cognitive function whereas the relationship with subcortical white matter hyperintensities was no

longer significant (de Groot *et al.*, 2000). However, since the two measures are highly correlated, it is unlikely that both variables would independently relate to variance in any outcome measures. Whichever measure happened to have a stronger relationship with the outcome variable might enter the model first and the other measure would not account for more variance. Thus, despite the fact that subcortical white matter lesions did not contribute any more than periventricular white matter lesions to cognitive status, it cannot be concluded that the cognitive measures are unrelated to subcortical white matter lesions. A more fruitful approach would be to explore the relationship between statistically independent measures, such as those identified by the principal components factor analysis in the present analysis, and cognitive outcomes. These relationships will be analyzed in the following chapter.

Other risk factors did not show significant relationships with the brain measures. While diabetes was not significantly related to brain measures, only fourteen individuals were diabetic in this sample. Thus, the lack of relationship between diabetes and brain imaging findings should be interpreted with care. In contrast, over half the sample were ApoEe4 carriers (65/127) and ApoE status was not related to any brain imaging measures. Much of the evidence surrounding the effects of ApoE suggest that it is a risk factor that predisposes to development of AD at an earlier age, but that it is not related to the progression of symptoms or signs (Swartz *et al.*, 1999). Finally, a history of hypertension was also not related to brain imaging measures. There is considerable evidence linking hypertension to cognitive decline (Launer *et al.*, 2000) (Kivipelto *et al.*, 2001) (Forette *et al.*, 1998) (Swan *et al.*, 1998) (Tzourio *et al.*, 2001) and imaging changes (DeCarli *et al.*, 1999) (Swan *et al.*, 1998); however, most of these studies relate

untreated, mid-life hypertension to late-life outcomes. In this analysis, mid-life blood pressures were not available and hypertension was largely controlled. It is likely, based on the above studies, that mid-life hypertension would be a much stronger correlate of brain imaging findings than treated hypertension in later life.

In contrast to the expected relationships between risk factors and hyperintensity volumes, the relationships between the risk factors and atrophy measures were more complex. Individuals with history of stroke or TIA had, unexpectedly, larger medial temporal lobes. This suggests that the cognitive impairment in these individuals was likely attributable to brain injury from the strokes, and occurred relatively independent of AD-related atrophy.

An additional complication was identified in the correlations between ventricular volume, commonly used as an indirect measure of brain atrophy, and all hyperintensity and large vessel stroke volumes. In our regression model, vCSF volume was independently related to age, focal atrophy, small vessel disease and large vessel disease. vCSF may therefore be a useful measure that reflects multiple brain pathologies. This finding may account for some of the discrepancy in the literature concerning white matter hyperintensities. Several studies have found correlations between white matter hyperintensities and brain atrophy (Giubilei *et al.*, 1997) (DeCarli *et al.*, 1995) (Salonen *et al.*, 1997), while other studies have concluded that white matter hyperintensities and brain atrophy are independent (DeCarli *et al.*, 1996) (Hirono *et al.*, 2000). The studies that identified correlations between hyperintensities and atrophy used ventricular size as a measure of atrophy. In contrast, medial temporal lobe atrophy, and sulcal CSF volumes were used as measures of atrophy in the studies that found no relationship between

atrophy and hyperintensities. These findings suggest that sCSF and medial temporal lobe measures may be relatively independent from the effects of cerebrovascular disease.

The relationships between brain hyperintensity volumes, and between ventricle size and multiple pathologies, present substantial difficulties for brain-behavior analyses. In this study, factor analysis was used to identify statistically independent variables. The atrophy factor was independent of volumes reflecting small vessel disease or strategic infarcts. One variable, anterior-medial thalamic hyperintensities, contributed equally to both the first and third factors. The vascular supply to this region is mediated by small, perforating vessels that can be affected by the same pathological processes that predispose to other small vessel disease measures, such as periventricular hyperintensities. The anterior-medial and posterior-lateral thalamic regions are supplied by different perforating vessels. The anterior and dorsomedial regions of the thalamus receive blood from the anterior and posterior thalamosubthalamic paramedian arteries. The posterior thalamus is supplied by the geniculothalamic artery and the posterior choroidal artery. Finally, the anterior choroidal artery supplies the lateral thalamus and posterior internal capsule (where the white matter fibers of the mamillothalamic tract enter/exit the thalamus). Since the anterior and dorsomedial thalamus have multiple important interconnections with memory processing regions including the hippocampus, mamillary bodies and posterior cingulate, strokes in the thalamus are strategically located to cause memory problems. Individuals with lesions in this area, like those with strokes affecting the medial temporal region, can present to a cognitive neurology clinic with cognitive impairment in the absence of any other cerebrovascular disease or AD pathology (Squire, Moore, 1979).

To test the hypothesis that the increased risk of cognitive impairment with CVD risk factors is mediated by both neurodegeneration and symptomatic vascular disease, multiple linear regression analyses were performed. Age and number of vascular risk factors were both significant independent predictors of small vessel disease. Many qualitative studies have related age to small vessel disease changes (Longstreth *et al.*, 1998) (Longstreth *et al.*, 1996) (Sullivan *et al.*, 1990) (Sander *et al.*, 2000). In contrast, CVD risk factors were not related to the brain atrophy factor in this study. CVD risk factors were related to small vessel disease and strategic infarcts, but not to brain atrophy, suggesting that the increased risk of cognitive impairment with CVD risk factors was not simply mediated by atrophic brain changes. Rather, damage to the brain detectable as MR hyperintensities, from either infarction or sub-acute ischemic changes, correlated with ischemic risk factors and may have exerted direct effects on cognitive status. As discussed above, vascular disease can result in cognitive impairment with less co-occurring AD pathology, compared to those with AD pathology alone (Snowdon *et al.*, 1997) (Lee *et al.*, 2000). Studies that have demonstrated reduced risk of dementia with prospective treatment of hypertension (Tzourio *et al.*, 2001) (Forette *et al.*, 1998) and cholesterol (Wolozin *et al.*, 2000) (Jick *et al.*, 2000) did not relate treatment to imaging findings. The results presented here suggest that the increased risk of cognitive impairment with cerebrovascular disease risk factors is mediated via cerebrovascular injuries to the brain, and not via accelerated AD-related brain atrophy. Studies that directly address the relationship between independent brain imaging measures and cognitive status are warranted.

CHAPTER V
BRAIN-BEHAVIOR RELATIONSHIPS

INTRODUCTION

We have previously demonstrated that cerebrovascular disease risk factors are related to increased volumes of white matter hyperintensities, deep gray matter hyperintensities and cortical strokes (see previous chapter). However, the relevance of these brain changes to cognitive impairment is controversial. While some strokes, for example those affecting the medial temporal lobe (Weiskrantz, 1977) (Takahashi *et al.*, 1997), clearly can cause cognitive impairment in isolation, the impact of diffuse white matter or deep gray matter hyperintensities is poorly understood.

Most studies exploring the cognitive consequences of cerebrovascular disease use a group comparison approach. The most common design compares individuals with clinically diagnosed VaD and AD. This approach assumes that differences between the groups will reflect the contributions of vascular disease. A meta-analysis of 27 studies comparing AD and VaD on neuropsychological test scores concluded that, when matched for age, education and severity of dementia, VaD patients have relatively less impairment in verbal long-term memory and relatively greater impairment on frontal executive functions compared with AD patients (Looi, Sachdev, 1999). A recent study showed that two cognitive tasks, testing verbal recognition memory and word association (executive) functions, may be useful for differentiating individuals with AD from those with subcortical ischemic VaD (Tierney *et al.*, 2001). Our own results (Chapter 3) also support the finding that executive functions are relatively more severely affected in VaD compared with AD, for equivalent levels of global impairment.

A second comparison approach involves contrasting those with CVD (either normal controls or AD+CVD) with those without CVD on tests of cognitive function. The largest study of this sort published to date found that, among those with lacunes, atrophy correlated with global severity of cognitive impairment but measures of cerebrovascular disease did not (Fein *et al.*, 2000). However, this study only examined global measures of cognitive impairment (MMSE and CDR). The independent contributions of atrophy and cerebrovascular disease to a range of neuropsychological tests, including tests of frontal lobe function, were not explored. A second study of the same sample showed that, after accounting for the effects of cortical gray matter atrophy or hippocampal atrophy, subcortical lacunar infarcts were not related to cognitive impairment on any cognitive domain (Mungas *et al.*, 2001). However, this study was heavily weighted with normal control individuals (57% of the sample had no global cognitive impairment). Further, the correlations between measures of atrophy and cerebrovascular disease were not addressed. Cerebrovascular disease was correlated to cortical gray matter atrophy in that sample, so that both variables would be unlikely to contribute independently to regression models (Mungas *et al.*, 2001).

Frontal lobe functions may be impaired in individuals with white matter hyperintensities. In otherwise healthy elderly, those with global atrophy and white matter hyperintensities (WMHI) have slower speed of cognitive processing and impaired executive function compared to those without atrophy and WMHI (Schmidt *et al.*, 1993) (Swan *et al.*, 2000) (La Rue *et al.*, 1995) (DeCarli *et al.*, 1995) (Ylikoski *et al.*, 1993). Similarly, those with AD+WMHI have greater visuospatial dysfunction (Amar *et al.*, 1996), attention/concentration impairment (Tsiskaridze *et al.*, 1998), slower speed of

cognitive processing and worse executive functions (Tsiskaridze *et al.*, 1998) (Amar *et al.*, 1996) (Libon *et al.*, 1998) compared to those with only AD. However, these relationships are inconsistent: other studies have found no differences between those with AD only and those with AD+WMHI in neuropsychological performance (DeCarli *et al.*, 1996) (Doddy *et al.*, 1998) (Bartres-Faz *et al.*, 2001). While neuropsychological test scores did not differ, functional imaging studies suggest that those with AD alone and those with AD+WMHI might be reaching the same level of impairment by different mechanisms (DeCarli *et al.*, 1996) (Reed *et al.*, 2000).

In contrast to the subtle and controversial cognitive correlates of CVD-related MRI hyperintensities, MRI measures of atrophy have shown stronger and more consistent relationships with cognitive impairment. In individuals with cognitive impairment, medial temporal lobe atrophy is correlated with impairments in verbal memory (Kohler, 1994) (Laakso *et al.*, 1995) (Libon *et al.*, 1998) (Stout *et al.*, 1999) (Kopelman *et al.*, 2001) and with greater general impairment (Laakso *et al.*, 1995) (Launer *et al.*, 1995) (Pantel *et al.*, 1997). Global brain atrophy is also correlated with overall severity of cognitive impairment (Bracco *et al.*, 1993) (Brunetti *et al.*, 2000) (Mungas *et al.*, 2001).

Few studies have examined the independent effects of atrophy and cerebrovascular disease. One study of 52 individuals with AD showed that both gray matter volume and white matter hyperintensities were independently associated with global cognitive severity assessed using the MMSE and DRS (Stout *et al.*, 1996). In contrast, another study of 76 individuals with AD+WMHI found that, after controlling for atrophy, duration and demographic factors, white matter hyperintensities were not associated with global cognitive impairment or dementia severity (Hirono *et al.*, 2000).

In that study, however, all individuals with lacunar infarcts (n=84) were excluded from the analysis. Further, whole brain atrophy was normalized to total intra-cranial capacity, while white matter hyperintensities were not. Both the Hirono et al. and Stout et al. studies use only global cognitive severity measures, without separately examining memory or executive functions.

In summary, several trends in relating brain changes to behavior in aging and dementia have emerged: medial temporal lobe atrophy correlates with memory and global cognitive impairments, brain atrophy correlates with overall severity of impairment and white matter hyperintensities seem to be related to frontal executive functions.

However, the contribution of cerebrovascular disease (white matter changes, cortical strokes and deep gray matter hyperintensities) independent of the effects of atrophy are not clear. Four methodological problems have limited previous approaches to the role of CVD in cognitive impairment. First, the design of most studies have involved group comparisons. When these groups are based on diagnostic categorizations (e.g. AD versus VaD), the results may be affected by pathologic overlap (see chapter 1). Second, in those studies which contrast those with or without hyperintensities within a single diagnostic group (AD or normal controls), there is considerable variability in the methods employed (quantitative analysis versus rating scales), in defining groups (which lesions or hyperintensities are used to divide the groups) and in the cognitive outcome measures used (global severity, memory, executive function). Third, these types of analyses, while demonstrating cognitive differences between groups, do not assess the independent contributions of different brain measures. In most cases, groups with

lacunes or hyperintensities also have greater global atrophy (Swan *et al.*, 2000) (La Rue *et al.*, 1995) (Mungas *et al.*, 2001), so it is difficult to draw any conclusions regarding the independent contributions of atrophy and hyperintensities. Finally, many previous studies lack sufficient sample sizes for multivariate analyses.

Thus, the purpose of this study was to explore the relationships between quantitative brain measures and the pattern of cognitive impairment in a large cognitive neurology clinic sample. Based on the findings from group comparison studies, we hypothesize that volumes of CVD and atrophy will independently correlate with measures of cognitive impairment. Specifically, MRI hyperintensities reflecting small vessel disease will contribute to frontal-lobe mediated tasks; in contrast, MRI volumes reflecting atrophy will be the strongest predictors of memory and global cognitive function.

METHODS

Participants underwent MR imaging with post-processing quantification and neuropsychological testing as described above (chapter 3).

Correlations between cognitive test scores were evaluated descriptively using bivariate Pearson correlation coefficients. To identify a smaller number of independent variables, a factor analysis was performed. DRS total score and the functional rating scale were not entered into the factor analysis, so that they could be used as measures of general severity in separate analyses. All other neuropsychological sub-test scores were entered into a principle components factor analysis with varimax rotation. Since individuals with incomplete neuropsychological data ($N_{\geq 1 \text{ incomplete tests}} = 15$; see chapter 2.2) were equally or more severely impaired compared with those in the same diagnostic

categories with complete data, missing values were replaced with the average for the diagnostic group (AD, VaD, NC) (Streiner, 2002) (Sinharay *et al.*, 2001). Only those factors with eigenvalues greater than one were retained.

Brain-behavior relationships were explored using multiple linear regression models. Analyses were performed for DRS total score, FRS, and for each significant factor from the behavior analysis. For all models, the effects of age and education were accounted for by entering these variables in the regression. Then, the independent brain factors identified previously (see chapter 3) were used as predictor variables in step-wise regression modeling. The cutoff for significance of F change was 0.05/3, or <0.016, since three variables were entered into the step-wise analysis. The Bonferroni correction for multiple analyses was used to determine the criterion for significance of the total models (0.05 divided by the number of regression models performed).

RESULTS

Descriptive data summarizing the neuropsychological test performance across the sample can be found in chapter 3.

As expected, the cognitive test scores were highly correlated (table 7).

Table 7: Pearson correlation co-efficients for neuropsychological tests and sub-scores. Bolded correlations are significant ($p<0.0005$).

Measure	FRS	MMSE	DRS total	DRS atten	DRS init	DRS const	DRS conc	DRS mem	CVLT acq	CVLT sdfr	CVLT sdcr	WCST cat	WCST corr	WCST nper	WCST ppc	WCST ppr	Boston naming	FAS	Benton	Forward DS
FRS																				
MMSE	**-.73**																			
DRStotal	**-.81**	**.86**																		
DRS attention	**-.45**	**.58**	**.68**																	
DRS initiation	**-.69**	**.68**	**.85**	**.48**																
DRS const	**-.38**	**.46**	**.48**	**.47**	**.33**															
DRS concept	**-.58**	**.63**	**.80**	**.55**	**.56**	**.36**														
DRS memory	**-.78**	**.80**	**.83**	**.39**	**.62**	**.29**	**.51**													
CVLT acquisition	**-.73**	**.69**	**.77**	**.39**	**.64**	**.31**	**.53**	**.79**												
CVLT sdfr	**-.68**	**.61**	**.69**	**.31**	**.55**	**.24**	**.44**	**.78**	**.87**											
CVLT sdcr	**-.69**	**.63**	**.72**	**.35**	**.60**	**.29**	**.48**	**.75**	**.89**	**.91**										
WCST # cat.	**-.60**	**.51**	**.55**	**.30**	**.50**	**.27**	**.39**	**.51**	**.61**	**.54**	**.54**									
WCST # correct	**-.50**	**.39**	**.43**	.22	**.37**	**.26**	**.31**	**.42**	**.48**	**.40**	**.38**	**.74**								
WCST npersev	**-.35**	**-.32**	**-.29**	-.10	**-.26**	-.19	-.19	**-.33**	**-.36**	**-.31**	**-.29**	**-.66**	**-.83**							
WCST ppc	.02	.08	-.01	-.08	-.00	.03	-.01	.05	.03	.05	.03	.21	.17	**-.67**						
WCST ppr	**.46**	**-.33**	**-.40**	-.19	**-.35**	**-.26**	**-.30**	**-.37**	**-.43**	**-.36**	**-.35**	**-.62**	**-.84**	**.71**	-.11					
Boston naming	**-.57**	**.63**	**.69**	**.38**	**.56**	**.32**	**.57**	**.61**	**.61**	**.56**	**.63**	**.39**	**.37**	**.27**	.01	**-.37**				
FAS	**-.59**	**.59**	**.68**	**.40**	**.59**	**.26**	**.59**	**.53**	**.63**	**.53**	**.57**	**.47**	**.40**	**-.34**	.09	**-.39**	**.53**			
Benton	**-.57**	**.58**	**.62**	**.51**	**.46**	**.48**	**.53**	**.47**	**.45**	**.41**	**.41**	**.47**	**.41**	**-.35**	.13	**-.35**	**.43**	**.48**		
Forward DS	**-.35**	**.41**	**.42**	**.36**	**.38**	**.28**	**.35**	**.27**	**.31**	.18	.20	.24	.20	-.13	-.02	-.18	**.29**	**.43**	**.27**	
Backward DS	**-.45**	**.51**	**.55**	**.49**	**.47**	**.34**	**.43**	**.38**	**.45**	**.33**	**.37**	**.41**	**.26**	-.17	-.03	**-.25**	**.28**	**.55**	**.42**	**.57**

Table 7 Key

FRS = Functional Rating Scale
MMSE = Folstein Mini-Mental Status Examination
DRS = Dementia rating scale
- att = attention sub-scale;
- init = initiation sub-scale;
- const = construction sub-scale
- conc = conceptualization sub-scale
- mem = memory sub-scale

CVLT = California Verbal Learning Test
- A1-5 = acquisition score
- sdfr = short delay, free recall
- sdcr = short delay, cued recall

WCST = Wisconsin Card Sort Test
- cat = # categories correctly identified
- corr = # correct categorizations
- nper = # non-perseverative errors
- ppc = perseverative errors to previous category
- ppr = perseverative error to previous response

FAS = Phonemic fluency (for words beginning with F, A, and S)
Benton = Benton line orientation test
DS = Digit span

The factor analysis identified four salient variables with eigenvalues greater than one, which accounted for 71.3% of the variance in the sample. Table 8 shows the factor loadings for all variables entered into the analysis. One variable (FAS fluency) contributed equally (loadings <0.05 apart) to factors one and two (Norman, Streiner, 1986). Factor one (45.4% of total variance) reflected short-term memory and language skills (the acquisition, short-delay free recall and short-delay cued recall sub-scores of the CVLT, DRS memory, and, more weakly, the DRS initiation, Boston naming, FAS fluency and MMSE scores). Factor two (12.4% of total variance) reflected tests of working memory and attention (forward and backward digit span, DRS attention, construction and conceptualization, Benton line orientation and FAS fluency). Factor three (8.7% of total variance) encompassed four of the five WCST measures, a problem solving task requiring abstraction and set shifting. This has been summarized with the term "mental flexibility". WCST perseveration to previous category was poorly correlated with most other tests (table 7) and emerged as a separate factor (factor four in table 8, 5.3% of total variance).

Table 8: Factor analysis of neuropsychological test scores. Relevant factor loadings (>0.50) are shown, except where one variable loaded equally (± 0.05) on multiple factors.

Factor Description	Brain Measure		Factor 2	Factor 3	Factor 4
			0.51		
Working memory & Attention	DRS attention		0.74		
	Digit span – forwards		0.72		
	Digit span – backwards		0.70		
	DRS conceptualization		0.61		
	DRS construction		0.58		
	Benton line orientation		0.56		
	WCST # correct			0.93	
	WCST # errors			0.70	
	WCST non-perseverative errors			-0.78	
	WCST previous response perseveration			-0.90	
Previous category perseveration	WCST previous category perseveration				0.98

Since four factors were identified, there were six brain-behavior regression models performed (DRS, FRS and each behavior factor). Age and education were forced into all models in the first block and the brain factors were evaluated step-wise in the second block. The first five models were significant ($p<0.0005$), while the model for neuropsychological factor 4 (WCSTpc) was not significant. Regression models demonstrated that both global cognitive (DRS total) and functional (FRS) impairments are independently related to brain atrophy and small vessel disease. Table 9 shows the results of these two regression models.

Regression models for the three significant brain factors are presented in Table 10. All three brain imaging factors were independently related to short-term memory and language (factor 1). However, the atrophy factor was the strongest predictor, accounting for ten times the variance in factor 1 compared with the small vessel and strategic infarct factors. The atrophy and small vessel disease factors were negatively correlated with poorer short-term memory/language performance, meaning that increased atrophy or small vessel disease predicted decreased memory/language performance. In contrast, larger strategic infarcts were positively correlated with short-term memory/language performance, suggesting that, after accounting for the effects of age, atrophy and small vessel disease, greater strategic infarct volumes related to less impairment in memory and language function. Factor 2 (working memory/attention) was associated with atrophy and strategic infarcts. In contrast, factor 3 (mental flexibility) was only significantly related to small vessel disease. Except for the association between strategic infarcts and memory mentioned above, all other relationships were in the expected directions.

Table 9: Whole sample regression model of DRS total score and FRS.

Variable	Predictor Variables	R^2 change	F change significance	Standardized Beta	Significance (Beta)
Global severity (DRS TOTAL) Model R^2=0.33 p<0.0005	Age	0.04	0.025	0.148	0.032
	Education			0.146	0.013
	Atrophy factor	0.25	<0.001	- 0.581	<0.001
	Small vessel factor	0.04	0.001	- 0.208	0.001
	Strategic infarct factor	–	–	–	–
Functional impairment (FRS) Model R^2=0.35 p<0.0005	Age	0.05	0.008	- 0.130	n.s.
	Education			- 0.143	0.026
	Atrophy factor	0.24	<0.001	0.578	<0.001
	Small vessel factor	0.06	<0.001	0.266	<0.001
	Strategic infarct factor	–	–	–	–

The amount of independent variance explained by each predictor variable in the final model is shown, along with the significance of the change in the model, the standardized beta for each variable in the final model and the significance of the variable weighting. The bottom row shows the total variance explained by each model.

Table 10: Whole sample regression models for the neuropsychological factors.

Variable	Predictor Variables	R^2 change	F change significance	Standardized Beta	Beta significance
Factor 1	Age	0.13	<0.001	- 0.097	n.s.
Short-term memory	Education			0.086	n.s.
& language	Atrophy factor	0.22	<0.001	- 0.537	<0.001
model R^2=0.40	Small vessel factor	0.02	0.015	- 0.148	0.015
p<0.0005	Strategic infarct factor	0.02	0.010	0.145	0.009
	Age	0.06	0.001	0.319	<0.001
Factor 2	Education		0.200	0.003	
Working memory	Atrophy factor	0.07	<0.001	- 0.288	<0.001
model R^2=0.16	Small vessel factor	–	–	–	–
p<0.0005	Strategic infarct factor	0.03	0.015	- 0.159	0.015
	Age	0.06	0.003	- 0.001	n.s.
Factor 3	Education			0.169	0.013
Mental flexibility	Atrophy factor	–	–	–	–
model R^2=0.10	Small vessel factor	0.04	0.003	- 0.241	0.001
p<0.0005	Strategic infarct factor	–	–	–	–
Factor 4 **Previous category perseveration**	no significant model				

The amount of independent variance explained by each predictor variable in the final model is shown, along with the significance of the change in the model, the standardized beta for each variable in the final model and the significance of the variable weighting. The bottom row shows the total variance explained by each model.

Outliers for all models were investigated. In all models, outliers were individuals with younger onset of cognitive impairment. Post-hoc sub-group analyses were therefore performed for two age groups (see Table 11), those over 65 (N=155) and under 65 (N=50) at time of MR imaging. There were no significant differences between those over and under 65 in years of education, number of cerebrovascular disease risk factors or sex distribution. The two groups also did not differ on the degree of global cognitive or functional impairment (Table 11). However, individuals in the younger group had milder short-term memory and language impairment, with more severe dysfunction in working memory. In older individuals (table 12, top), the models were largely consistent with the whole group models. Atrophy was the strongest correlate of global impairment and memory and language dysfunction. Atrophy and small vessel disease were both independently correlated with mental flexibility. Small vessel disease was also related to general severity. Strategic lesions were independent contributors to short-term memory/language impairments and were the chief correlate of working memory. In contrast, the group of individuals under age 65 (table 12, bottom) showed that atrophy was the only significant correlate of general severity, short-term memory/language function and working memory. Models for mental flexibility and WCSTpc were not significant. The effects of age and education were minimal in the younger group.

Table 11: Age differences Demographic and cognitive factor z-scores for participants under 65 versus 65 and over.

Variables	**Under age 65** *Mean ± Standard deviation*	**Over age 65** *Mean ± Standard deviation*	**p*** (univariate)
N	50	155	
Age (years)	58 ± 6.3	76 ± 5.5	(selected)
Education (years)	15 ± 3.4	14 ± 3.5	n.s. (0.07)
Sex (% females)	60	54	n.s. (0.10)
# CVD risk factors	0.56 ± 1.0	0.83 ± 1.0	n.s. (0.10)
Functional impairment (FRS)	15 ± 6.8	17 ± 5.7	n.s. (0.14)
Global severity (DRS)	123 ± 21	121 ± 15	n.s. (0.41)
Short-term memory and language (z-score)	0.49 ± 1.0	-0.16 ± 0.94	**<0.0005**
Working memory (z-score)	-0.40 ± 1.2	0.13 ± 0.91	**<0.0005**
Mental flexibility (z-score)	0.12 ± 0.88	-0.069 ± 1.0	n.s. (0.15)
Previous category perseveration (z-score)	-0.11 ± 1.0	0.034 ± 1.0	n.s. (0.43)

* In the multivariate model, the two groups differed on cognitive variables, after accounting for education ($p<0.0005$).
n.s. = not significant (univariate $p>0.05$)

Table 12: Regression analyses for older and younger participants.

Age ≥ 65:	Global severity (DRS)	Functional impairment (FRS)	Short-term memory & language	Working memory	Mental flexibility
Age and education **Atrophy** **Small vessel disease** **Strategic lesions**	partial $R^2 = 0.07$ partial $R^2 = 0.20$ partial $R^2 = 0.05$	partial $R^2 = 0.08$ partial $R^2 = 0.23$ partial $R^2 = 0.08$	partial $R^2 = 0.08$ partial $R^2 = 0.23$ partial $R^2 = 0.02$ partial $R^2 = 0.03$	partial $R^2 = 0.07$ partial $R^2 = 0.06$	partial $R^2 = 0.06$ partial $R^2 = 0.04$ partial $R^2 = 0.04$
TOTAL MODEL	R^2=0.32; p<0.0005	R^2=0.39; p<0.0005	R^2=0.36; p<0.0005	R^2=0.13; p<0.0005	R^2=0.14; p<0.0005

Age < 65:	Global severity (DRS)	Functional impairment (FRS)	Short-term memory & language	Working memory	Mental flexibility
Age and education **Atrophy** **Small vessel disease** **Strategic lesions**	partial $R^2 = 0.41$	partial $R^2 = 0.32$	partial $R^2 = 0.06$ partial $R^2 = 0.26$	partial $R^2 = 0.37$	
TOTAL MODEL	R^2=0.41; p<0.0005	R^2=0.32; p<0.0005	R^2=0.32; p<0.0005	R^2=0.13; p<0.0005	n.s.

DISCUSSION

In this study, independent measures of brain atrophy and cerebrovascular disease, previously identified using principal components factor analysis, were related to separate domains of cognitive impairment. This approach has the advantage of avoiding assumptions regarding diagnostic categorizations and instead focuses on the relationship between brain changes and cognitive or functional status. Diagnostic group comparisons can be problematic due to pathological overlap: roughly one-third of individuals with AD have some cerebrovascular disease (Kalaria, Ballard, 1999) and at least half of those with clinical diagnoses of VaD have some degree of AD pathology (Victoroff *et al.*, 1995) (Gold *et al.*, 1997) (Kosunen *et al.*, 1996) (Swan *et al.*, 1999). Dividing groups based on presence or absence of CVD, which can be readily detected on T2-weighted MR images, is a more fruitful approach. However, this approach is limited by correlations between CVD and atrophy measures. The principle components factor analysis used in this analysis has the advantage of identifying independent variables, in this case, reflecting atrophy, small vessel disease and strategic infarcts. Additionally, the completion of a test battery, encompassing several key neuropsychological domains, by a large sample, provided sufficient data for factor analysis of cognitive functions. It must be noted that the findings from these analyses reflect trends across this cognitive neurology clinic sample. Therefore, applying these results to individual cases is precarious. Despite this limitation, the approach taken here has the advantage of approaching the role of cerebrovascular disease from a functional, real-world perspective. Rather than selecting a small number of individuals with isolated lesions, this study was designed to assess the

role of these lesions in individuals with effects from aging, other cerebrovascular changes and, possibly, AD taken into account.

The variables that emerged from the analysis of neuropsychological tests reflected underlying cognitive skills affected by AD and cerebrovascular disease. Specifically, short-term memory/language function, working memory and mental flexibility. The factors were named with descriptive nomenclature, rather than using specific neuropsychological terminology, since each factor represents contributions from multiple tests or sub-tests. The first factor reflected short-term memory and language abilities, two domains most commonly impaired in AD, and in other dementias. A study by Stuss et al., showed that these two domains are highly correlated in individuals with frontal lobe injury (Stuss *et al.*, 1994). In addition, the tests of short-term memory used in this analysis (e.g. CVLT, DRS memory) were largely verbal tests, thus partially accounting for the correlation with language tests. The second factor reflected scores on the DRS attention, the digit span task, the DRS conceptualization and construction subscores and the Benton line orientation task. These tests all require the short-term storage, manipulation and utilization of mental representations; for example, comparing one figure to a set of figures and identifying the matching figure, or recalling and listing a set of numbers in reverse order. The two visuospatial tasks (DRS construction and Benton line orientation) similarly require storage, manipulation and utilization abilities, but using visuospatial representations rather than language or numbers. These tests were the weakest contributors to the factor score, suggesting that the factor is more closely related to the attention and working memory components of the tasks than to visuospatial abilities. Thus, this factor was labeled "working memory and attention" as a way of

describing the cognitive requirements that underlie these tasks. Finally, the third factor was related to most WCST sub-scores. This test assesses the ability to identify features for categorization of cards (by the number, color or shape of figures on the cards), the ability to recognize when the categorization rules have changed and the ability to identify the new rule of sorting the cards. Perseveration, in this context, refers to continued use of one rule set despite evidence that the rule is no longer appropriate. Since participants are not told before the decision rule is altered, all participants will show perseveration to the previous category each time the rule is switched. Thus, even normal controls have several of these "errors". It was not surprising, therefore, that the fourth factor (perseveration to previous category) was an independent measure, unrelated to other measures of cognitive impairment, or to brain volumes. In contrast, the total number of non-perseverative errors, the number of perseveration to previous (erroneous) responses, and the total number of correct categorizations, all reflect the ability (or inability) to shift categorization rules, or "mental flexibility".

Since factor analyses require large samples and multiple tests, they are not routinely used in clinical neuropsychological studies. However, two recent studies have employed factor analyses to explore cognitive domains in Alzheimer's disease. The first study identified three factors reflecting working memory, memory-language abilities and visuospatial functions in a group of mild AD (Kanne *et al.*, 1998). The second identified four factors in AD, reflecting the cognitive domains of attention/registration, verbal fluency/reasoning, graphomotor/praxis and recent memory (Pappas *et al.*, 2000). While data on graphomotor and praxis did not emerge as a separate factor in the present sample,

the remaining factors in both studies are similar to those reported here. Neither study explored the relationship of these cognitive impairments with cerebrovascular disease.

Since younger individuals were consistently outliers in the regression models generated for the entire group, separate analyses were performed for those under 65 and those 65 and over. Age at the time of MR imaging was used instead of the conventional age at disease onset to divide subjects. A study by Sullivan et al. (Sullivan *et al.*, 1993) showed that onset date adds little to the prediction of brain or behavior variables after accounting for age at scanning. Age at onset is difficult to assess accurately, especially in patients with very slow progression. Additionally, it is difficult to define "onset" for individuals with cognitive impairment who do not meet criteria for dementia and, by definition, there can be no age of onset for healthy elderly controls. Age at time of scanning, in contrast, could be used to divide both the normal control and the cognitive impairment samples equivalently. Thus, the age at time of scan was used in this study.

Cerebrovascular disease measures were important predictors of cognitive status in older, but not younger individuals in this cognitive neurology clinic sample. The different brain-behavior relationships in older and younger participants likely reflects different patterns of both brain pathology and neuropsychological impairments. First, the prevalence of cerebrovascular disease is significantly greater in older individuals. Population studies have shown that increasing age is the strongest correlate of lacunar infarctions (Longstreth *et al.*, 1998) (Longstreth, Jr., 1998) and silent strokes (Lee *et al.*, 2000). Additionally, greater age is associated with increased ratings of white matter hyperintensities in a population sample (Schmidt *et al.*, 1993). The different brain-behavior relationships may also reflect different patterns of neuropsychological

impairments in younger individuals. Our results suggest that, with equivalent global severity, early-onset individuals tend to have greater deficits in working memory and later-onset individuals had more severe short-term memory and language dysfunction. These findings confirm earlier reports in which tests of attention and working memory were more impaired in younger onset and tests of memory and naming were impaired in later-onset individuals (Jacobs *et al.*, 1994) (Reid *et al.*, 1996) (Sevush *et al.*, 1993).

An additional difference between the older and younger individuals relates to the role of age and education. In the younger group, these co-variates did not contribute significantly to most models. For those over age 65, age and education were more relevant and may be due to correlations with cerebrovascular disease. In an autopsy-verified study, patients with less education had significantly more cerebrovascular disease (Del Ser *et al.*, 1999). Thus, the co-variates of age and education may be accounting for some of the variance related to vascular disease in older individuals. This relationship would not be expected, and was not identified in this analysis, in individuals under 65.

In both age groups, atrophy was the strongest predictor of functional status, global cognitive impairment, short-term memory and language functions. In the older sample, atrophy was also related to mental flexibility. Independent of the effects of age, education and atrophy, small vessel disease was a predictor of impaired mental flexibility. Working memory and mental flexibility are both types of "executive" functions. These impairments are key components of the cognitive decline in AD and vascular dementia. Indeed, a prospective longitudinal community-based study found that those who develop AD within two years show the largest declines in memory and

executive functions (Chen *et al.*, 2001). The Wisconsin Card Sort test, the sub-scores of which loaded highly onto the mental flexibility factor, activates large areas of the frontal cortex in functional imaging studies of normal volunteers (Berman *et al.*, 1995). WCST impairments are related to frontal lobe atrophy (Kopelman *et al.*, 2001) and the test is sensitive to the effects of frontal lobe damage in individuals with focal lesions (Stuss *et al.*, 2000). Thus, small vessel disease-related hyperintensities in the periventricular and subcortical white matter may be interrupting the frontal-subcortical network and impairing frontal lobe function. In contrast, large vessel cortical strokes and anterior-medial thalamic hyperintensities were related to working memory and short-term memory-language functions. This is consistent with findings in individuals with isolated thalamic lesions that result in amnestic syndromes (Van der Werf *et al.*, 1999).

Atrophy measures explained most of the variance in cognitive function, compared to the other MRI measures. However, while small, the effects of cerebrovascular disease are measurable, and independently contribute to impairment in multiple areas of cognition and in functional abilities, especially in those at greatest risk for cognitive impairment (those over age 65). The results presented here account for some of the clinical heterogeneity of AD, VaD and mixed disease. Patterns of impairment are different in younger and older individuals, and the relationship between brain imaging abnormalities and cognitive functions differ as well. The regional location of pathology, whether atrophy or cerebrovascular disease, relates to variations in the clinical presentation. Variations in the distribution of the pathology of AD account for some of the observed clinical heterogeneity (Cummings, 2000). Similarly, variations in the distributions of cerebrovascular disease are related to type and severity of impairment.

Rather than focusing on differences between diagnostic categorizations, more attention should be paid to the behavioral consequences of regional atrophy and cerebrovascular disease.

The results presented here provide *in vivo* evidence that cerebrovascular disease may worsen AD-related cognitive impairments by affecting short-term memory, working memory and mental flexibility. As mentioned above, previous autopsy-based studies have shown that vascular brain lesions increase the expression of dementia in those with co-occurring AD pathology (Snowdon *et al.*, 1997) (Lee *et al.*, 2000) (Esiri *et al.*, 1999).

The results of this study suggest that in younger individuals brain atrophy, but not small vessel disease or strategic infarcts, correlates with most cognitive impairments. In older individuals, brain atrophy and cerebrovascular disease are independently related to mental flexibility, short-term and working memory difficulties. These results provide a biological rationale for recent empirical findings in which prospective treatment of cerebrovascular disease risk factors (e.g. therapy for high cholesterol (Wolozin *et al.*, 2000) (Jick *et al.*, 2000) and high blood pressure (Tzourio *et al.*, 2001) (Forette *et al.*, 1998)) reduce the incidence of dementia. Prevention of small and large vessel cerebrovascular disease may be beneficial for preventing cognitive and functional decline, especially in those over age 65.

CHAPTER VI
STRATEGIC WHITE MATTER HYPERINTENSITIES?
Vascular disease affecting acetylcholinergic white matter pathways

INTRODUCTION

One of the most consistent neurochemical deficits in Alzheimer's disease (AD) is a loss of cortical cholinergic markers (Perry *et al.*, 1980) (Perry, 1980) (DeKosky *et al.*, 1992) (Bierer *et al.*, 1995). A major focus of therapeutic interventions has thus been the acetlycholine (ACh) neurotransmitter system. Acetylcholinesterase inhibitors show modest benefit in short-term symptomatic treatment of AD and are considered standard treatment for mild to moderate AD (Doody *et al.*, 2001). The "cholinergic hypothesis" of AD ascribes much of the cognitive and neuropsychiatric aspects of the disease to the cholinergic dysfunction (Bartus *et al.*, 1982) (Collerton, 1986) (Cummings, Kaufer, 1996).

The principal cholinergic innervation of the cerebral cortex is supplied by cholinergic neurons of the nucleus basalis of Meynert (nbM) in the basal forebrain (Mesulam, 1996) (Mesulam, Geula, 1988) (Mesulam *et al.*, 1983). The trajectories of the cholinergic white matter pathways (one medial and two lateral axon bundles) linking the nbM with the cerebral cortex have been traced immunohistochemically in the human brain (Selden *et al.*, 1998). The cholinergic neurons of the nbM show severe loss in AD (Whitehouse *et al.*, 1981) (Saper *et al.*, 1985) (Lehericy *et al.*, 1993) (Baloyannis *et al.*, 1994). Degradation of this system correlates with intellectual impairment, predominantly memory and attention deficits (Lehericy *et al.*, 1993) (Lawrence, Sahakian, 1995).

Increasingly, epidemiological and experimental evidence support the role of vascular risk factors in the development of AD (Kivipelto *et al.*, 2001) (Launer *et al.*, 2000) (Forette *et al.*, 1998) (Wolozin *et al.*, 2000). While CVD in isolation may be a relatively

rare cause of dementia (Hulette *et al.*, 1997) (Snowdon *et al.*, 1997). small strokes to the deep white matter and deep gray nuclei have been shown to increase the likelihood of expressing dementia twenty-fold in those with co-occurring AD neuropathology (Snowdon *et al.*, 1997). Studies of selective nbM lesions in rodents and monkeys have shown that damage to the ACh system is related to impaired visuospatial attention and increased perseverative behavior, with relative sparing of learning and memory performance (Wenk, 1997) (Muir *et al.*, 1993) (Voytko *et al.*, 1994) (Pang *et al.*, 1993).

The AD-related degenerative destruction of cells within the nbM and subsequent loss of cholinergic input to the cortex have been well studied, to our knowledge the frequency and consequences of vascular lesions in acetylcholinergic white matter pathways have not been previously investigated. While specific subcortical gray matter nuclei have long been recognized as strategic for cognition, the concept of strategic location of cerebrovascular disease has not been applied to subcortical white matter. This study was undertaken to determine the frequency of these vascular lesions and to test two hypotheses concerning their consequences. First, based on animal models, we hypothesized that white matter changes affecting the cholinergic system would correlate with visuospatial attention and executive function impairments, with equivalent global and memory impairments. Second, we reasoned that, for the same degree of general cognitive impairment, medial temporal lobe atrophy would be less severe in individuals with cholinergic white matter changes. A visual MRI rating scale of hyperintensities within ACh white matter pathways was developed and applied to the cognitive neurology clinic sample (see chapter 3) to describe the frequency, severity and cognitive correlates of ACh white matter hyperintensities.

METHODS

The patient sample, normal volunteers, neuropsychological test battery, MR imaging protocol and methodology for the medial temporal lobe thickness measure are described in Chapter 3. Individual neuropsychological test scores were chosen for analysis in this section in order to address the specific hypotheses raised above. Specifically, the working memory and attention factor score from Chapter 5 included visuospatial tasks, but was more closely related to mental storage and manipulation rather than visuospatial abilities. The hypothesis based on animal models suggested more specific effects on visuospatial attention and mental flexibility, tasks that are not directly reflected in the factor scores. Therefore individual neuropsychological tests were chosen for analysis.

Rating hyperintensities in acetylcholinergic white matter pathways

Volumetric T2- and PD-weighted MR images from the 205 participants were compared with published immunohistochemical tracings of acetylcholinergic pathways (figure 7). Axial slices from both T2 and PD images were displayed simultaneously on a computer screen, with the ability for the rater to scroll between slices from the top of the head to the bottom of the cerebellum. The following areas were inspected with reference to the neuroanatomical localization described by Selden et al. (Selden *et al.*, 1998): 1) the estimated location of the lateral pathway through the centrum semiovale and the external capsule, (figure 7 a-d; light gray lines) 2) the estimated route of the medial white matter pathway, including the white matter deep to the cingulate gyrus (anterior and posterior), (figure 7 a-d , dark lines) and 3) the estimated location of the nucleus basalis of Meynert in the basal forebrain (figure 7 c,d, small arrows).

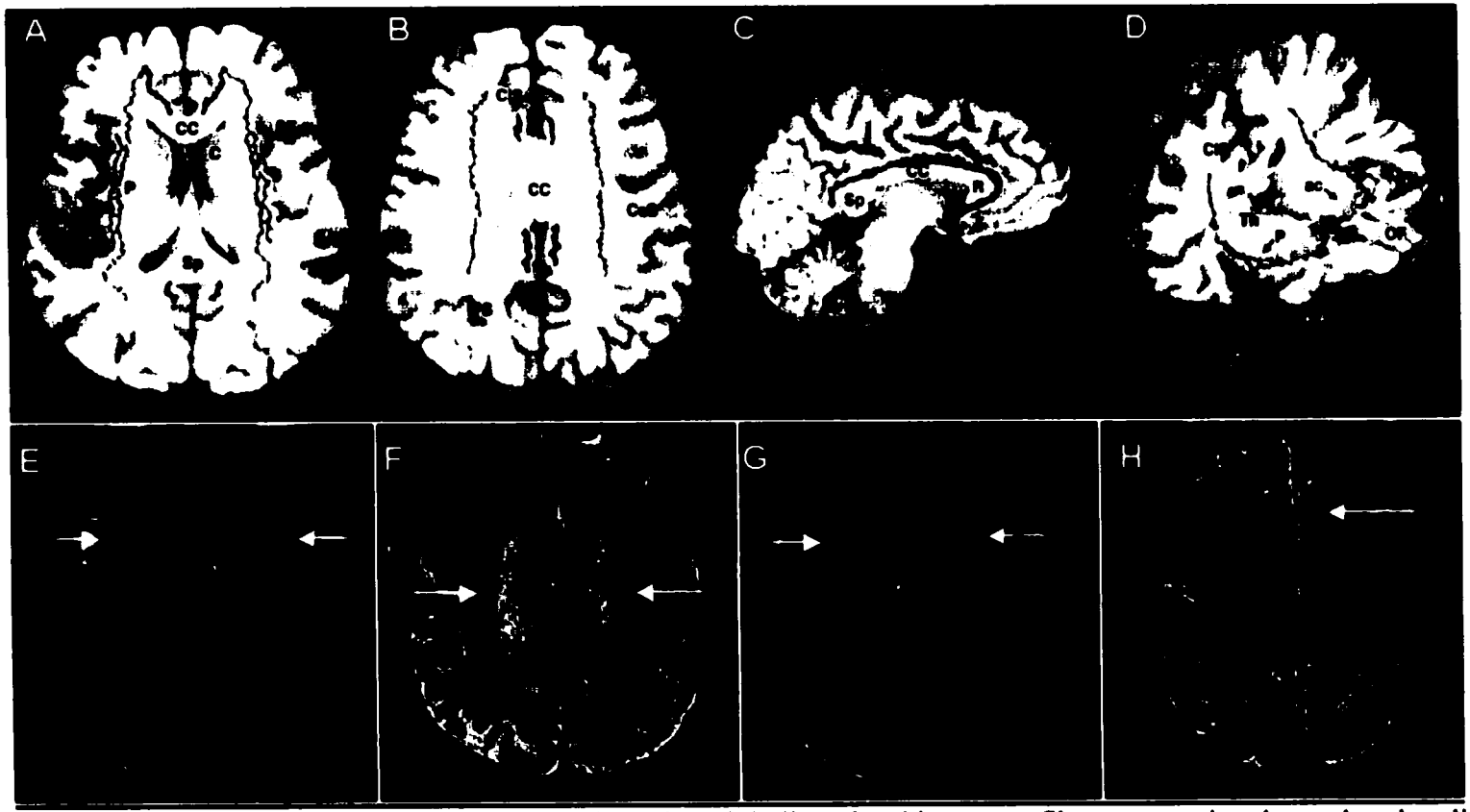

Figure 7: Hyperintensities in ACh pathways The trajectories of cholinergic white matter fibers connecting the nucleus basalis of Meynert (small arrow) with the cerebral cortex are shown on (a) a lower axial section, (b) a higher axial section, (c) a sagittal projection and (d) a coronal and axial cut-out through the thalamic nuclei. Dark lines represent the medial pathway and light gray lines represent the two divisions of the lateral pathways. The lateral cholinergic pathway was commonly affected by one or more of: (e) external capsule hyperintensities, (f) multiple deep white matter infarcts, or (g) extensive periventricular white matter changes. Rarely, (h) small lesions occurred in the medial cholinergic pathway. (a-d adapted from Selden et al., with permission) (Selden *et al.*, 1998) ac=anterior commissure, C=caudate, CC=corpus callosum, CeS=central sulcus, CiS=cingulate sulcus, IPS=intraparietal sulcus, OF=orbitofrontal cortex, P = Putamen, R = rostrum of corpus callosum, SF = sylvian fissure, Sp = splenium of corpus callosum, Th = thalamus

Hyperintensities affecting cholinergic pathways were categorized as minimal (none to single small lesions), moderate (single large or multiple small lesions) or severe according to the decision rules in Table 13. Cutoff values for the three groups were selected to facilitate reliable ratings within and between raters. Both size and location of hyperintensities were considered (e.g. either small hyperintensities in multiple parts of the pathways or large hyperintensities in a single part of the ACh pathway were designated moderate). MR scans from three individuals exemplifying the decision rules for each category are shown in Figure 8. All ratings were performed over a period of three days, in random order, blind to all clinical and demographic information, by a single observer. A second rater was trained using a sample of ten scans. Inter- and intra-rater reliability was assessed using 40 scans randomly selected from the total set (excluding the training scans). MR images were loaded and displayed in random order and rated independently by two observers, blind to all clinical and demographic data.

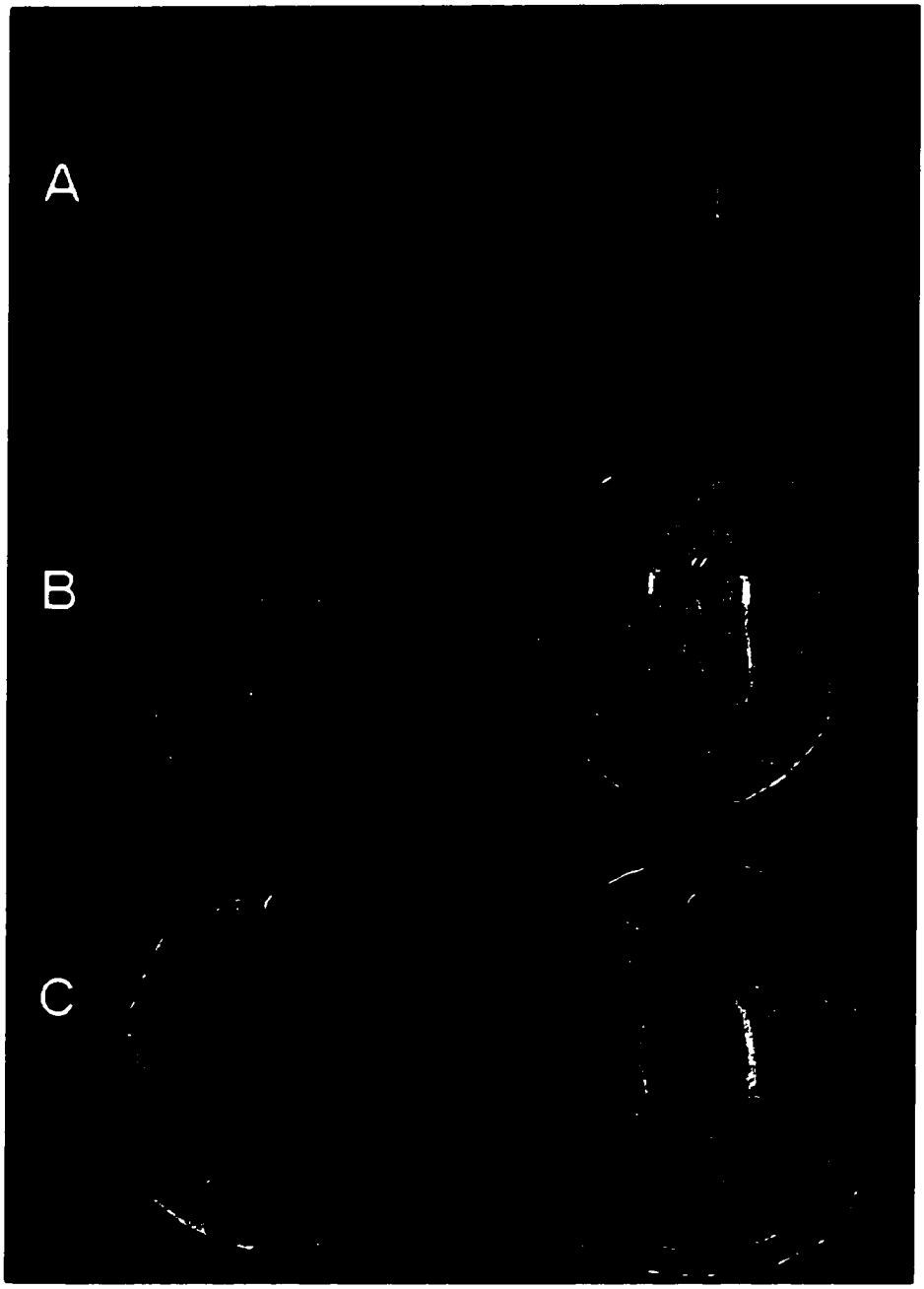

Figure 8: ACh ratings of minimal, moderate and severe T2- and proton density-weighted MRI scans of individuals with a) minimal; b) moderate and c) severe vascular disease affecting acetylcholinergic white matter tracts. Note that, while only a single slice is displayed, the white matter lesions in figures b and c extended vertically through several slices.

Table 13: Decision rules for rating hyperintensities in acetylcholinergic white matter pathways as either minimal, moderate or severe.

RATING	DECISION RULE
Minimal	a) No lesions to the nbM, no medial pathway or external capsule hyperintensities, AND, b) No more than 1 small (<1 cm^2) focal lesion or 3 tiny (<0.5 cm^2) hyperintensities in the lateral pathway.
Moderate	a) Single large focal (>1 cm^2), or more than one small focal (<1 cm^2) or >3 tiny (<0.5 cm^2) hyperintensities in either the lateral or medial pathway, with no external capsule hyperintensities OR, b) External capsule hyperintensities plus no more than 1 small (<1 cm^2) focal lesion or 3 tiny (<0.5 cm^2) hyperintensities elsewhere in the lateral pathway.
Severe	a) Infarction of nbM, OR, b) Multiple large focal or confluent hyperintensities in the lateral pathway, OR, c) Hyperintensities to both lateral and medial pathway OR, d) External capsule hyperintensities plus a single large focal (>1 cm^2), or more than one small focal (<1 cm^2) or >3 tiny (<0.5 cm^2) hyperintensities in either the lateral or medial pathway.

Statistics

The weighted kappa statistic was used to assess reliability, since the categorizations were ordinal (Fleiss, 1986). All comparisons between the groups with mild, moderate and severe ACh hyperintensities were performed using data only from those individuals with cognitive impairment so that differences would not simply reflect the presence of a disease state. To explore whether the categories of vascular damage to ACh tracts differed significantly on demographic measures, an analysis of variance (ANOVA) was performed, including age at scan, duration of illness, years of education, sex and number of CVD risk factors (minimum = 0; maximum =5). An analysis of co-variance (ANCOVA) was performed to compare the ACh rating groups on the

neuropsychological tests scores, after co-varying the effects of age, education and medial temporal atrophy. A linear regression model was used to determine whether ACh group was a significant predictor of medial temporal lobe atrophy after accounting for the effects of age.

RESULTS

The three point visual rating scale of hyperintensities in ACh white matter pathways had good reliability (weighted kappa statistics: intra-rater agreement = 0.79, inter-rater agreement = 0.74) (Fleiss, 1986).

All 34 normal elderly controls had no to minimal cholinergic pathway involvement. Moderate or severe cholinergic pathway involvement was identified in 35% (59/171) of those with cognitive impairment. Of this total, moderate or severe hyperintensities were found in: 30% (38/126) individuals with possible or probable AD, 60% (9/15) individuals with probable VaD and 40% (12/30) individuals with cognitive impairment who did not meet criteria for dementia. The diagnostic frequencies in each ACh rating category is provided in Table 14 and demographic characteristics of each category are given in Table 15. Individuals with minimal ACh hyperintensities were significantly younger than those with moderate or severe ACh hyperintensities (Table 15). Therefore, subsequent statistical analyses were performed co-varying for the effects of age. Individuals in the severe group had, on average, twice the number of cerebrovascular risk factors compared with those with minimal or moderate hyperintensities in ACh white matter pathways.

Table 14: Clinical diagnoses in ACh groups. Clinical diagnoses of participants with minimal, moderate and severe acetylcholinergic white matter disruption.

	ACh pathway hyperintensities		
Diagnosis	***Minimal***	***Moderate***	***Severe***
Normal elderly controls	34	0	0
Cognitive impairment, no dementia	18	5	7
Possible or probable AD	88	17	21
Probable VaD	6	1	8
TOTAL	**146**	**23**	**36**

Table 15: ACh group demographic characteristics. Demographic characteristics of participants with cognitive impairment who have minimal, moderate or severe vascular compromise of acetylcholinergic white matter tracts.

	ACh pathway hyperintensities		
Variable	***Minimal*** (n = 112) mean (s.d.)	***Moderate*** (n = 23) mean (s.d.)	***Severe*** (n = 36) mean (s.d.)
Age at scan	70.7 (9.9) [a]	76.6 (6.1)	75.3 (8.7)
Years of education	13.6 (3.7)	14.4 (3.4)	13.4 (3.7)
Duration	3.7 (2.5)	4.2 (3.6)	5.0 (4.1)
Sex (% female)	44	48	61
# CVD risk factors (0-5)	0.7 (1.0)	0.7 (0.7)	1.4 (1.2) [b]
MMSE	23.0 (5.0)	23.7 (4.7)	22.4 (4.2)

MMSE = Folstein Mini-Mental Status Examination (maximum = 30)
Groups were significantly different (ANOVA total model $p<0.0001$), driven by:
[a] The minimal ACh group was significantly younger than the other two groups (post-hoc analysis $p=0.003$).
[b] those with severe ACh hyperintensities had significantly more CVD risk factors (post-hoc analysis $p=0.001$).

Most commonly, the lateral cholinergic pathway was affected by various combinations of external capsule hyperintensities (figure 7.e), multiple deep white matter hyperintensities (figure 7.f), or severe periventricular hyperintensities that extended into the region of ACh white matter tracts (figure 7.g). In one case, a large anterior cerebral artery infarct disconnected the medial pathway from the nucleus basalis of Meynert to the

cerebral cortex. Smaller lesions affecting the medial cholinergic pathway were also rare (n=3; figure 7.h).

Complete neuropsychological test battery scores were available for 163 of the 171 individuals with cognitive impairment, with eight individuals refusing or being unable to complete one or more of the Boston naming (3), Benton line orientation (5), FAS fluency (2) and digit span (3) tests. Of the eight individuals with incomplete neuropsychological battery, six had probable AD (four with minimal ACh, and two with moderate ACh involvement), and two had probable VaD (one with minimal and one with severe ACh hyperintensities).

In the 163 individuals with complete neuropsychological assessments, age (total model $p<0.0005$), years of education (total model $p=0.027$) and medial temporal atrophy (total model $p=0.001$) were significant co-variate predictors of neuropsychological measures. Thus, the groups of mild, moderate and severe hyperintensities in ACh white matter tracts were compared after first accounting for the effects of these variables. The groups differed significantly on neuropsychological functions (Table 16; multivariate model $p=0.002$). There were no significant differences between the groups in general cognitive function (DRS), short-term memory (CVLT) and working memory (digit span) (Table 16). Significant differences were identified in visuospatial function (Benton line orientation) and executive function tests, including FAS fluency and WCST sub-scores (total number correct, number of non-perseverative errors and perseveration to previous response). These differences were not driven by individuals with VaD and remained significant even after restricting the analysis only to those with possible or probable AD. In post-hoc comparisons, impairment on the visuospatial and executive function

measures was greatest in the group with severe hyperintensities in ACh white matter tracts, intermediate with moderate ACh tract hyperintensities and mildest in the minimal ACh tract hyperintensities (total model p=0.002; between group comparisons p<0.05).

Table 16: Neuropsychological scores in ACh groups Average neuropsychological test scores in those with minimal, moderate and severe vascular compromise of acetylcholinergic white matter tracts (multivariate model p=0.002).

Neuropsychological measure	*ACh pathway hyperintensities*			
	Minimal mean (SEM)	***Moderate*** mean (SEM)	***Severe*** mean (SEM)	**P value***
DRS total	119 (1.38)	120 (3.15)	116 (2.14)	n.s. (0.55)
CVLT – acquisition	23.0 (1.0)	20.3 (2.4)	21.4 (1.8)	n.s. (0.51)
CVLT – short delay free recall	2.0 (0.25)	2.8 (0.57)	2.3 (0.44)	n.s. (0.49)
CVLT – short delay cued recall	3.5 (0.27)	4.6 (0.63)	4.5 (0.48)	n.s. (0.10)
WCST – # categories	1.5 (0.13)	0.91 (0.30)	1.0 (0.23)	n.s. (0.10)
WCST – # correct	37 (1.0)	32 (2.4)	31 (1.8)	**0.011**
WCST – non-perseverative errors	16 (1.5)	22 (3.3)	24 (2.5)	**0.034**
WCST – perseveration to previous category	9.3 (0.85)	8.9 (1.9)	7.9 (1.5)	n.s. (0.73)
WCST – perseveration to previous response	6.1 (0.51)	8.4 (1.2)	9.5 (0.89)	**0.004**
Boston naming	20 (0.59)	22 (1.3)	20 (1.0)	n.s. (0.40)
Phonemic fluency (FAS)	30 (1.3)	27 (3.0)	22 (2.3)	**0.012**
Benton line orientation	19 (0.91)	15 (2.1)	13 (1.6)	**0.014**
Forward digit span	8.3 (0.22)	7.9 (0.51)	7.8 (0.39)	n.s. (0.56)
Backward digit span	5.3 (0.21)	5.3 (0.49)	4.6 (0.37)	n.s. (0.20)

* Univariate comparisons after adjusting for the effects of age, education and medial temporal lobe atrophy (covariates entered in the model: age = 72.72, education = 13.60, medial temporal lobe thickness = 7.96 mm). n.s.= not significant

A linear regression model was performed to test the hypothesis that medial temporal lobe width would be smaller in those with less cholinergic pathway disruption. After accounting for age, medial temporal lobe atrophy was most severe in those with minimal ACh hyperintensities (average = 7.54 mm), intermediate in those with moderate ACh hyperintensities (average = 8.18 mm) and mildest in those with severe ACh hyperintensities (average = 9.20 mm) ($p_{regression}$=0.043).

DISCUSSION

In this study, we evaluated hyperintensities in acetylcholinergic white matter pathways of individuals with cognitive impairment from a large, cognitive neurology clinic sample. Hyperintensities in acetylcholinergic white matter pathways were common in those with cognitive impairment. Approximately one-third of all individuals with cognitive impairment had moderate or severe hyperintensities in the ACh pathways, in contrast to no healthy elderly controls. The healthy elderly group was separated from those with cognitive impairment using neuropsychological testing, but without reference to the MR images. Many of the MR features identified in this study would previously have been described as "non-specific white matter changes". This common description may be more dismissive than is prudent. Our results suggest that at least some of these "non-specific" changes occur in strategic locations with measurable clinical effects. White matter changes occur commonly in elderly samples (Longstreth *et al.*, 1996) (de Groot *et al.*, 2000) (Lee *et al.*, 2000), and neuropathological correlates include demyelination and axonal loss, gliosis, small lacunar infarcts, and increased periependymal extracellular fluid (Pantoni *et al.*, 1999) (van Gijn, 1998). While some of these changes may truly be silent, our initial observations suggest that those hyperintensities which affect ACh tracts have quantifiable consequences. There is a need to prospectively evaluate the sensitivity and specificity of hyperintensities in ACh white matter tracts for cognitive impairment. The view that all white matter changes are equivalent and non-specific may need to be re-considered.

Not only were hyperintensities in cholinergic white matter tracts common, but also, as predicted, they were correlated with executive and visuospatial dysfunctions.

Impairments in these domains have been demonstrated in animal studies in which the cholinergic system has been selectively damaged. Initial experimental models considered performance decrements after excitotoxic nbM lesions to be the result of learning and memory impairments. However, recent studies in which more selective and discrete nbM lesions were achieved suggest that selected aspects of attention, and not learning or memory, are mediated by the nbM-cortical ACh system (Wenk, 1997). Specifically, both rodents (Muir *et al.*, 1993) and monkeys (Voytko *et al.*, 1994) with excitotoxic nbM lesions have shown great difficulty in shifting visuospatial attention, but not in learning or memory. Further, Pang et al. demonstrated in rats that both the ability to discriminate between two perceptual stimuli and the tendency to make perseverative responses to a single stimulus, are tasks sensitive to damage of nbM cholinergic neurons (Pang *et al.*, 1993). While it is hazardous to directly compare cognitive domains across species, these behavioral deficits are intriguingly consistent with our findings. In the WCST, the patient has to infer rules for sorting cards by category (color, shape, number) and recognize when the category for sorting is arbitrarily changed by the tester. The deficits in set shifting and choosing categorization rules in the Wisconsin Card Sort Test parallel the perseverative responses seen in the animal models. Further, the line orientation task, which requires the subject to match the angle between two lines to a semi-circular array of lines, parallels the perceptual attention deficits demonstrated in the animal models.

In the previous chapter, small vessel disease did not correlate with the working memory and attention factor, while here ACh hyperintensities were related to visuospatial abilities. These findings are not contradictory since, the factor score was most strongly related to mental storage and manipulation functions rather than to visuospatial abilities.

In addition, the small vessel disease factor reflected not only ACh hyperintensities, but also other deep white matter hyperintensities, periventricular hyperintensities, and subcortical gray matter hyperintensities.

As expected based on the above animal models of ACh tract damage, we found no significant differences between the groups on tests of learning/short term memory (CVLT), working memory (digit span) or overall impairment (DRS) after accounting for age and medial temporal lobe atrophy. This suggests that individuals with and without ACh tract damage may be reaching equivalent levels of cognitive impairment by different routes. Indeed, the significant inverse relationship between medial temporal lobe atrophy and vascular ACh tract damage suggests that those without ACh damage may have more severe progression of AD, and thus greater medial temporal lobe atrophy, for the same degree of cognitive impairment. This finding is consistent with the autopsy studies discussed in previous chapters, in which in individuals with co-occurring CVD have less AD pathology, with equivalent pre-mortem cognitive impairment, compared to those with AD only (Snowdon *et al.*, 1997) (Lee *et al.*, 2000). While ACh white matter tracts were not specifically examined in these studies, the infarcts may have aggravated existing cholinergic deficits. ACh tract damage may be partially responsible for increasing the expression of dementia symptoms in those with co-occurring AD. The study by Snowdon et al. also demonstrated that in those with little AD neuropathology, brain infarcts were only weakly associated with poor cognitive function (Snowdon *et al.*, 1997). One possibility raised by our results may be that those without AD neuropathology may be better able to withstand small vascular injuries to cholinergic white matter tracts than those with AD-related cholinergic degeneration.

The pattern of deficits found in our sample is also consistent with *in vivo* studies of white matter hyperintensities in cognitive impairment. As in our study, Hirono et al. showed that, after controlling for atrophy, white matter changes were not correlated with global cognition or dementia severity in AD (Hirono *et al.*, 2000). However, our study also identified deficits in executive and visuospatial function. Executive dysfunction has been correlated with extensive white matter changes both in AD (Tsiskaridze *et al.*, 1998) and in community dwelling elderly (DeCarli *et al.*, 1995) (Boone *et al.*, 1992) (Ylikoski *et al.*, 1993). Visuospatial dysfunction has also been correlated with widespread white matter changes in individuals with AD (Amar *et al.*, 1996). It has been suggested that some threshold volume of white matter changes must be achieved before cognitive deficits are seen (DeCarli *et al.*, 1995) (Boone *et al.*, 1992) (Amar *et al.*, 1996). Periventricular hyperintensities above the threshold volumes usually extend well out into the centrum semiovale and are more likely to encroach on the lateral acetylcholinergic white matter pathway (see figure 7.g). Thus, cognitive deficits observed with extensive periventricular white matter changes may reflect damage to cholinergic tracts.

Given the specific effects of these hyperintensities, the efficacy of cholinergic therapeutic agents may be influenced by the degree of damage to ACh white matter tracts. Recent preliminary studies have suggested efficacy of acetylcholinesterase inhibition in VaD and mixed disease (Bullock, Lilienfeld, 2001). This finding may be partially explained by the high prevalence of co-occurring AD even in those with probable VaD (Gold *et al.*, 1997) (Roses, Saunders, 1997). Positive responses, according to this argument, stem from treatment of AD-related cholinergic deficit in those with VaD. In addition to this effect, our results suggest that cholinergic deficits may also arise

as a specific consequence of white matter lesions. In our sample, approximately half of the individuals with mixed or vascular dementia, and a quarter of the individuals with probable AD had significant cholinergic tract damage which may benefit from cholinergic therapies. Prospective evaluation of these hypotheses in large treatment studies, comparing responses to therapy with varying severity of ACh white matter tract damage, will need to be performed to address this possibility more conclusively.

Several important limitations of this analysis should be emphasized. First, the findings reported here are correlative and do not directly measure cortical cholinergic markers. Second, medial temporal lobe atrophy was used as a convenient correlate of AD pathology; this was not a neuropathological study and the observed medial temporal lobe atrophy measure is not necessarily specific to AD. Studies using functional brain imaging or neuropathological examination of cholinergic markers and medial temporal AD pathology in those with varying vascular ACh damage are warranted. Third, many pathological processes may manifest as hyperintense on MRI (reviewed in Pantoni et al. (Pantoni *et al.*, 1999) and Ogata (Ogata, 1999)). However, the heterogeneous histological causes of deep white matter and periventricular hyperintensities have been widely studied as macroscopic phenomena with complex, and often subtle, cognitive and clinical correlates. They have been correlated with impairments in cognitive processes (such as attention and speed of processing), incontinence and motor slowing, in both dementia (Ylikoski *et al.*, 1993) (Hirono *et al.*, 2000) (Amar *et al.*, 1996) and healthy elderly cohorts (Longstreth *et al.*, 1996) (DeCarli *et al.*, 1995) (de Groot *et al.*, 2000). Our results provide a novel framework with which to interpret these results and suggest that cholinergic dysfunction may be contributing to these deficits. Finally, this study was

performed using a convenience sample drawn from a university cognitive neurology clinic. The frequency of ACh tract hyperintensities needs to be validated in other samples.

The approach presented here is simple, rapid and reliable to perform prospectively or retrospectively. Over a quarter of individuals with probable AD had moderate or severe vascular involvement of cholinergic white matter tracts, which may exacerbate degenerative cholinergic deficits. This data raise a cholinergic hypothesis for vascular cognitive impairment in which deficits previously correlated with extensive white matter changes may be attributable to the specific effects of vascular cholinergic tract damage. This may be especially relevant in the subgroup recently referred to as subcortical ischemic vascular dementia (Erkinjuntti *et al.*, 2000), but also applies to many individuals with clinical diagnoses of AD. The degree of vascular compromise of cholinergic white matter pathways should be considered in studies of cholinergic therapies in AD and VaD, and in assessments of individual responses to treatments under study. The cholinergic hypothesis of cognitive impairments in AD should be extended to include both degenerative damage to basal forebrain cholinergic neurons and vascular damage to cholinergic axons.

CHAPTER VII
THESIS DISCUSSION

The cluster of impairments that defines dementia can result in devastating changes in personality, cognition and daily function for affected individuals. These changes place tremendous physical, psychological and economic burdens on affected individuals and families. The social costs of dementia are great, especially as a large segment of our society approaches the ages at highest risk. There is a need to improve understanding of the causes of cognitive impairment in the elderly so that preventative and therapeutic strategies can be developed and tested.

The study of dementia is both old and new and views concerning the role of vascular disease have fluctuated considerably. The occurrence of cognitive impairment with advanced age was noted by early Greek law makers, including Plato and Solon. The terms delirium and dementia were first coded in Roman times. The word "morosis" (dementia) was included in Galen's list of medical diseases, and old age was specified as one of the situations in which it occurred. There are relatively few written references to dementia, however, between the Roman era and the 1800's. That century witnessed the rise of pathology and localization of function. In 1838, one of the landmark neurologists, Esquirol, defined "senile" dementia. In 1864, brain atrophy was first associated with dementia, and in 1868, Otto Binswanger first used the term "presenile" dementia to differentiate it from that occurring with old age. It was not until the first decade of the 1900's, with two publications by Alois Alzheimer, that specific neuropathological correlations were made with the clinical syndrome of early-onset, or presenile, dementia. In 1910, Emil Krapelin named this entity "Alzheimer's disease". In contrast, senile

dementia was considered a more normal part of aging, associated with atherosclerotic disease. Since Alzheimer's original case descriptions almost one hundred years ago (Alzheimer, 1907; translated by L.Jarvik and H.Greenson, 1987), views of the importance of vascular disease have waxed and waned.

For the first half of the 20th century, senile dementia, or senility, was commonly referred to as "hardening of the arteries", which reflected the presumption of vascular disease associated with aging. AD was thought to represent as different disease, including only those cases with young onset (called "presenile dementia"). In the 1960's and 70's, however, the application of electron microscopy to brains of individuals with presenile and senile dementia revealed that both diseases have the same microscopic brain changes. Therefore, through the 1970's the distinctions blurred between presenile and senile dementia, and the pendulum swung towards AD as the primary cause of cognitive impairment with aging. At the same time, the concept of multi-infarct dementia was crystallized as a contrasting pathological entity from AD (Hachinski *et al.*, 1975). A second concurrent trend involved revolutionary advances in the ability to detect parenchymal brain pathology, including cerebrovascular disease, *in vivo*. The advent of CT began in the mid 1970's and was followed by rapid advances in MRI technology in the 1980's. These tools enabled, for the first time, direct visualization of atrophy and cerebrovascular lesions *in vivo*. The greater contrast and sensitivity to pathology of MRI, along with advances in computerized analysis such as tissue segmentation, set the stage for this study.

Recently, the role of vascular disease has received renewed interest. The risks of AD and vascular disease increase with age and the two diseases occur together more

frequently than would be expected based on their independent risks. Brain imaging tools have demonstrated that multi-infarct dementia, rare isolated strokes, lacunes and periventricular white matter hyperintensities are potential causes of cognitive impairment. On one hand, it is rare to find individuals with typical dementia who show absolutely no neuropathological findings of AD (Hulette *et al.*, 1997). On the other hand, vascular disease commonly co-occurs with AD, and may lower the threshold of AD pathology at which symptoms become apparent. Much of what is currently understood about the role of brain-behavior relationships in dementia has come from studies of selected individuals or groups of patients. Brain structures involved in both memory and executive functions were initially identified by observing behavioral changes in individuals with sudden brain injuries (for example due to strokes, penetrating trauma or surgical ablation) and by relating these changes to the neuroanatomical changes. Analyses of individuals with Alzheimer's disease in the absence of vascular disease have further reinforced the close association between medial temporal lobe atrophy and memory function, and between global brain atrophy and overall severity of cognitive decline. However, the cognitive correlates of vascular disease, in the common and complex setting of aging, with or without AD, are much less clear.

Since the pathogenesis, potential prevention and treatments of AD and cerebrovascular disease differ in many respects, it is important to understand the relationship between these pathologies and cognitive impairment. Thus, this project set out to address four major objectives: 1) to develop a method to evaluate cerebrovascular disease and atrophy quantitatively, 2) to explore the role of risk factors, 3) to determine the contribution of independent brain variables (including cerebrovascular disease and

atrophy measures) to cognitive impairment and 4) to determine the relevance of location of cerebrovascular disease.

First, a method for quantifying brain atrophy and vascular disease was developed, tested for reliability and shown to be useful in investigating brain-behavior relationships in a preliminary sample. The value of considering subcortical gray matter lesions, atrophy and subcortical hyperintensities separately was shown in these preliminary brain-behavior analyses. These analyses also demonstrated that thalamic lesions might be important, not just as a rare cause cognitive impairment, but as a more common contributing factor to multiple cognitive domains.

The MRI processing method was then applied to scans from a large cognitive neurology clinic sample in which detailed neuropsychological testing and clinical histories were available. Examination of individuals with step-wise decline reinforced the concept of strategic infarcts involving a limited number of gray matter structures. This was the first study to suggest that anterior-medial thalamic infarctions larger than 60 mm^3 are especially relevant, while smaller lesions to this area do not tend to result in sudden step-wise declines in cognitive status. While not common in those with step-wise decline, small anterior-medial thalamic hyperintensities were more common than previously thought in the AD and CIND subgroups. Greater attention should be focused on these lesions in future studies, especially because our results suggest that these lesions are commonly under-recognized in radiologist reports.

The role of demographic and medical risk factors was explored and correlations between brain compartment volumes were assessed. This analysis showed that brain volumes are correlated in a complex manner. Deep and periventricular white matter

hyperintensities were highly correlated. In addition, ventricular size was the most important single brain measure for a reflection of multiple brain pathologies. Factor analysis was used to identify three statistically independent underlying variables or factors (reflecting atrophy, small vessel disease and strategic infarcts). Again the unique nature of anterior-medial thalamic hyperintensities was underscored in the contribution to the strategic infarct factor. Apolipoprotein E genotype, a risk factor for the development of AD, was not related to any brain imaging findings in this sample. Despite the association of vascular risk factors such as hypertension and high cholesterol with AD, these risk factors did not relate to the factor underlying atrophy measures. CVD risk factors were associated with sub-cortical brain hyperintensities (the factor labeled small vessel disease), and only CVD risk factors related to strategic infarcts.

It was hypothesized, therefore, that the contribution of CVD risk factors to cognitive impairment may be mediated via small vessel disease and strategic infarcts. However, there has been no clear evidence that these measures contribute independently to cognitive impairment, after accounting for confounds such as age and atrophy. Thus, the third goal of the study was to evaluate the independent contributions of atrophy and vascular disease to the pattern and severity of cognitive impairment. In this analysis, four independent factors underlying the cognitive impairments in this sample were identified and the relationships with the brain factors were explored. The results confirmed the importance of brain atrophy for cognitive impairment: atrophy was the strongest correlate of most domains and was especially relevant for younger individuals. The analysis also revealed that small vessel disease and strategic infarcts independently contribute to global cognitive and functional impairments and to difficulties in mental flexibility, short-term

memory/language and working memory in older individuals. Thus, treatments aimed at preventing cerebrovascular disease may play a significant role in preventing the onset, or ameliorating the severity, of cognitive decline in older age.

Finally, the concept of "strategic" cerebrovascular disease was expanded in this project with the hypothesis that hyperintensities in the acetylcholinergic neurochemical pathway would correlate with specific neuropsychological impairments. This analysis showed that small vessel disease-related hyperintensities in subcortical white matter, previously considered diffuse, commonly affect strategic brain pathways and result in specific cognitive deficits. These results give new rationale for investigating acetylcholinergic therapies in individuals with vascular dementia or mixed disease.

Much of the current controversy concerning the role of cerebrovascular disease in cognitive impairment stems from methodological difficulties. Five major methodological issues were addressed by the design of this study. First, a reliable method was developed to quantify brain atrophy, white matter lesions, and cortical and subcortical gray matter lesions simultaneously. This avoided the complex issue of which rating scale to use, and which types of brain changes to rate, while providing quantitative data for statistical analysis. Second, the independent effects of different brain volumes on behavioral scores were explored across the entire sample instead of comparing average functional or cognitive scores between groups of individuals. Third, this analysis was not restricted to examining global cognitive function (e.g. only acquiring MMSE or DRS scores). Rather, data reflecting multiple cognitive domains were collected and analyzed. Fourth, detailed analysis of correlations between measures was undertaken. Many of the controversies in current literature may stem from relationships between variables. For example, many

studies have concluded that white matter hyperintensities are not relevant for cognitive impairment after accounting for the effects of atrophy. These studies use ventricular size or cortical gray matter volumes, which are correlated with cerebrovascular disease, as measures of atrophy. The correlation between CVD and brain atrophy may begin quite early in life and thus has the potential for significant long-term impact (Seshadri *et al.*, 2001). In addition, the role of deep white matter versus periventricular white matter hyperintensities is widely debated, yet the two variables, when appropriately transformed, are highly correlated and should not be considered independently. Fifth, this study had sufficient sample sizes to enable multivariate statistical analyses.

These methodological features are key strengths of the analyses, but there are also several important limitations to these methods. Statistical transformations and factor analysis are powerful tools, yet are difficult to apply to individuals, either within a study or from an independent population. For example, setting a cutoff or threshold of periventricular hyperintensity volume, an approach used in analyses of healthy elderly individuals (Boone *et al.*, 1992) (DeCarli *et al.*, 1995), presents many difficulties. First, the statistics used in this analysis were head-size normalized and log transformed. Previously published thresholds from either raw volumes (such as 10 cm^3), or size-corrected volumes (e.g. 0.5% of intra-cranial capacity), are thus not easily applicable to these analyses. Further, periventricular hyperintensities were highly correlated with multiple other subcortical hyperintensities. Thus, two individuals might have the same factor score (e.g. small vessel disease) for different reasons (e.g. a high deep white matter hyperintensity volume versus high basal ganglia lesion volume). A given factor score

cannot be assumed to represent a specific periventricular white matter (or any other) hyperintensity volume.

A second limitation of these methods lies in the group analysis. This approach revealed useful trends across the cognitive neurology clinic sample, but cannot necessarily be applied to individuals. For example, while the small vessel disease factor accounted for a statistically significant percentage of variance across the sample, it may be more or less relevant in specific individuals. There were several participants, most commonly those under age 65, with substantial cognitive impairments yet no appreciable small vessel disease or strategic infarcts.

This study was also limited to specific T1-, T2- and PD-weighted MR pulse sequences. In the time since data acquisition began, improved sequences have been developed that provide better contrast between gray matter and white matter or between lesion and normal brain tissues. Additional sequences can be used to measure diffusion along white matter tracts, or to examine the chemical content of brain tissue. These approaches are powerful new tools for studying CVD in cognitive impairment; however, they were not applied to the current question for several reasons. First, the sequences were retained from the start of the study to facilitate comparison between participants, for example those who enrolled in 1993 and those who enrolled in 1998. This consistency has enabled the collection of the large sample analyzed in this study. Additionally, while not part of the present project, participants were followed longitudinally. Any change in scanning methodology could compromise individual comparisons over time. Finally, adding sequences to the protocol would require greater imaging time. Participants were

not sedated or restrained for scan acquisitions, and longer imaging times likely would have been intolerable for many of those with cognitive impairment.

Finally, statistical significance does not equate to practical relevance. The regression models relating brain factors to behavioral measures demonstrate that relatively little variation in behavior factors is independently accounted for by small vessel disease or strategic infarcts (ranging from the weakest relationship – small vessel disease contributed a partial R^2 of 0.02, or 2% of the sample variance to the model of short-term memory/language in older individuals – to the strongest contribution 8% of the variance in functional impairment of older individuals). Thus, cerebrovascular disease accounts for less than twenty percent of the explained variability. If, however, treatments for controlling or preventing vascular disease are widely applied, it might be possible to reduce the frequency or severity of short-term memory/language dysfunction in a small, but significant proportion of individuals. Recent results from treatment of late-life hypertension show that if 1000 individuals undergo effective blood pressure control for five years, 19 new cases of dementia (2% of the sample) might be prevented (Forette *et al.*, 1998). While a small change, a 2% risk reduction in a large at-risk population, such as those over age 65, would result in tremendous benefits.

Several areas of future investigation are initiated by these results. First, a more detailed analysis of the relationship between small vessel disease and acetylcholinergic pathway damage should be undertaken. The specificity of the relationship with cognitive impairment should be addressed by examining cognitive correlates of ACh-pathway hyperintensities compared to the correlates of other periventricular and subcortical white matter hyperintensities. In addition, a sample that includes individuals from outside the

cognitive neurology clinic (e.g. a post-stroke cohort, a multiple sclerosis sample) may facilitate comparison between the cognitive effects of ACh-pathway hyperintensities and hyperintensities which affect other neurochemical pathways. Also, the effects of disruption of other association pathways that course through the periventricular region, such as the superior longitudinal fasciculus, ought to be evaluated. Finally, the relevance of acetylcholinergic ratings for predicting therapeutic response could be investigated as part of a multi-center longitudinal study of acetylcholinesterase inhibitors for vascular dementia and mixed disease.

Beyond lesions which affect acetylcholinergic pathways, the role of localization should be investigated further. Our descriptive findings from individuals with sudden onset or step-wise decline suggest that a small number of strategic locations in the brain might be identifiable. Strokes affecting primary sites of damage in AD, including the amygdala, hippocampus, entorhinal cortex, and pre-subiculum areas of the medial temporal lobe, as well as the posterior cingulate gyrus, mamillary bodies and anterior-medial thalamus may be key locations in which a small volume of damage can be sufficient to cause dementia. Confirmation of the frequency and importance of anterior-medial thalamic hyperintensities in an independent sample is important. In addition, small infarcts which destroy white matter tracts connecting key limbic regions (such as the fornix or the mamillo-thalamic tract) may also be sufficient to cause dementia. In contrast, cerebrovascular disease affecting isocortical association areas, in the absence of AD neuropathology, may only result in clinical symptoms if extensive. When these strokes occur together with small amounts of AD neuropathology, however, they might increase the severity or expression of cognitive dysfunction. Similarly, white matter

hyperintensities affecting either frontal-subcortical circuits or cholinergic pathway projections may also be more relevant in the context of unmasking or worsening existing AD neuropathology, rather than as a sole cause of dementia. We propose to build on the preliminary descriptive findings from this study towards a prospective model for predicting post-stroke dementia in a large cohort. The combination of lesion localization and regional atrophy measures may be very powerful in identifying those at greatest risk for future cognitive decline.

Finally, the results presented here provide further impetus to study interventions which might slow or halt the development of cerebrovascular disease. Preventing cerebrovascular damage to the brain may be a complementary approach to therapies aiming to slow or halt AD neuropathology. Based on our data, CVD prevention would not eliminate dementia, but would be expected to reduce the incidence and severity of dementia and reduce the expression of AD pathology. Aggressive treatment of midlife cardiovascular and cerebrovascular risk factors is warranted, especially for individuals at risk (e.g. those with a positive family history). The results of this thesis, however, also suggest that preventing cerebrovascular disease alone is not enough. The strongest correlate of cognitive function is atrophy. Clearly, interventions that retard, prevent or reverse the brain-damaging effects of AD pathology are also crucial goals to pursue.

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