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Hippocampal and entorhinal cortex size in subjects at-risk for Alzheimer's disease: A controlled MRI study

Jones, Ann Elizabeth, Ph.D.
The Ohio State University, 1993
HIPPOCAMPAL AND ENTOHRINAL CORTEX SIZE IN SUBJECTS
AT-RISK FOR ALZHEIMER'S DISEASE: A CONTROLLED MRI STUDY

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
Ann Elizabeth Jones, B.S.N., M.S.

************

The Ohio State University
1993

Dissertation Committee: Approved by
E.M. Burns
J.T. Nickel
H.A. Nasrallah

Elizabeth Burns R.N. Ph.D.
Adviser
College of Nursing
VITA

1972.................................B.S.N., The Ohio State University, Columbus, Ohio

1977.................................M.S., Arizona State University, Tempe, Arizona

1977-1979............................Clinical Nurse Specialist Eastway MHC, Dayton, Ohio

1980-1988............................Instructor, College of Nursing, The Ohio State University, Columbus, Ohio

1988-Present............................Staff Nurse, The Ohio State University Hospitals, Columbus, Ohio

1988-Present............................Clinical Nurse Specialist Veterans Clinic, Columbus Ohio

1991-1993............................Research Assistant, Department of Psychiatry, The Ohio State University, Columbus, Ohio

FIELDS OF STUDY

Major Field: Nursing
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CHAPTER I
INTRODUCTION

Background of the problem

The increased longevity of populations in industrialized nations offers a variety of challenges to the health care delivery system. Possibly the greatest of these challenges is dementia. As more people live into the seventh, eighth, and even ninth decade of life, the number experiencing loss of cognitive function increases. Dementia is a broad category encompassing conditions that result in the impairment of long-and-short memory including impairment in abstract thinking, judgment, and higher cortical function (DSM-III-R, 1987). Disorders that manifest dementia include primary degenerative dementia of the Alzheimer type, multi-infarct dementia, dementia associated with chronic alcoholism, and Parkinson's disease (DSM-III-R).

Primary degenerative dementia of the Alzheimer type or Alzheimer's disease (AD) accounts for two-thirds of the dementia cases in the United States (Katzman & Saitoh, 1991). This neurodegenerative disorder is characterized by progressive impairment of cognitive function resulting in physical disability and death usually within 5-10 years after diagnosis. Mental and physical disability of this
magnitude often results in the need for institutional care. In the United States it is estimated that the total annual cost of lost productivity, medical and nursing home care, social services, and early death due to AD is $90 billion (Progress Report on Alzheimer's Disease, 1992).

Alzheimer's disease also results in hidden costs to society. Many families care for AD patients at home at an estimated cost of $12,000 per year (Progress Report on Alzheimer's Disease, 1992). Undertaking home care reduces the earning power of the caregiver and increases the stress on the family leading to depression, isolation, and increased health problems. Caring for an AD patient in the home can result in unreimbursed medical costs to the family including respite care, special foods, medications, and supplies (Progress Report on Alzheimer's Disease, 1992). Early onset AD (before age 60) may prematurely end the career of the patient and can jeopardize retirement and health care benefits for the family.

Much has been learned about AD since it was first described by Alois Alzheimer M.D. in 1907, but much still remains unexplained. Brain autopsy data from patients with clinical symptoms of AD reveal a pattern of neurofibrillary tangles, neuritic plaques, and cell loss in the frontal, temporal, and parietal lobes of the brain. The diagnosis of AD can only be confirmed by neuropathological examination. Clinical diagnosis of "probable AD" is based on the
exclusion of other disorders via physical evaluation including blood and urine tests, as well as neuropsychological tests, computerized tomography (CT), magnetic resonance imaging (MRI), and electroencephalogram (EEG). By the time the patient's symptoms become apparent, significant brain changes have already occurred. To date, no method exists for early diagnosis of AD.

Two forms of AD sporadic and familial have been suggested. Sporadic AD is believed to represent isolated occurrence of the disease within a family. The familial form of AD occurs when many members of a family are affected by the disease (Progress Report on Alzheimer's Disease, 1992) and may be transmitted by an autosomal dominant gene (Cook et al., 1981). AD is believed to be a genetically heterogeneous disease with demonstrated linkage to chromosomes 14, 19, and 21 (Goate et al., 1989; Roses et al., 1990; Schellenberg et al., 1992; St. George-Hyslop et al., 1987; Weitkamp, 1983). Several research teams have found clear linkage to D14S43 located three-quarters of the way down on the long arm of chromosome 14 (Marx, 1992). The search to find the actual gene on chromosome 14, as well as on chromosomes 19 and 21 could answer several questions about the mechanism involved in AD including the elusive role of B-amyloid. It has also been suggested that further delineation of the genetics of familial AD will shed new
light on the role of inheritance in what is now seen as sporadic AD (Schellenberg et al., 1992).

First degree relatives of patients diagnosed with probable AD are believed to be at increased risk for the development of AD (Breitner et al., 1988; Huff et al., 1988). Findings vary as to the amount of increased risk among first degree relatives. Mohs et al. (1987) reported a morbid risk of AD-like illness approaching 50 per cent among first-degree relatives of clinical AD cases. Van Duijn et al. (1991) in a re-analysis of seven studies looking at family history of dementia found a relative risk of AD for those with at least one first degree relative with dementia to be 3.5 (95% CI 2.6-4.6).

Age has been studied as a risk factor for development of AD (Evans et al., 1989; Schoenber, et al., 1985; Sulkava et al., 1985; Amaduci & Lippi, 1990;). Evans et al., (1989) in a prevalence study of an East Boston community reported an overall prevalence rate for probable AD of 10.3% with a range from 3% for those 65-74, to 47.2% for those over 85. Other studies have also found an increase in AD with age ranging from 7% for individuals 80 years or older (Schoenberg et al., 1985), to 17.3% in those over 85 (Sulkava et al., 1985).

Research on alcohol use as an hypothesized risk factor for AD has yielded mixed results. Both AD and chronic alcoholism result in dementia with identical clinical
features. Graves et al., (1991) in a collaborative re-
analysis of case-control studies found no excess estimated
risk of AD in subjects categorized into three levels of
alcohol intake. Terri et al., (1989) found alcohol abuse
contributed most to cognitive decline in those already
diagnosed with AD. Ciesielski and Hontela (1985) suggest,
"The similarities of clinical and electrophysiological
deficits of Alcoholic Dementia and SDAT (Senile Dementia of
the Alzheimer's Type) suggest a possible compatible
similarity in the neuropathological pattern underlying these
two psychiatric entities" (p. 99). Freund and Ballinger
(1992) report a paucity of studies on the effects of alcohol
on the neurochemistry and neuropathology of AD concluding,
"It would be surprising if AD were not affected by exposure
to alcohol with its many effects on lipid membranes, ion
channels of the brain, and the immune system" (p. 239).

Current research into the effects of maternal alcohol
use on the developing fetal brain may shed some light on
brain changes in later life. Although fetal alcohol
syndrome with its characteristic neurological changes is now
widely recognized to be the result of maternal alcohol
ingestion during pregnancy, other less apparent brain
changes affecting brain plasticity throughout the life cycle
have yet to be definitively identified. It is possible that
maternal alcohol ingestion during pregnancy may promote
subtle interruption of synaptic connections that only become
apparent during the aging process. Alcohol ingestion in males has reported effects on sperm making alcohol ingestion by either or both parents an hypothesized risk factor in offspring for brain changes throughout the life cycle.

Brain imaging techniques such as magnetic resonance (MR) are increasingly recognized as a way to detect early brain changes in aging as well as a variety of disorders characterized by dementia (Jernigan & Butters, 1989; Jernigan et al., 1991a; Jernigan et al., 1991b; Jernigan et al., 1991c; Kesslak et al., 1991). MR is a noninvasive, high-resolution method for quantifying changes in the brain antemortem. MR uses an external magnet (resistive or superconducting) that emits electrical currents which act on hydrogen nuclei within brain cells in the patient, setting up resonance. A measurable signal is emitted when the pulse is turned off and the nucleus returns to normal alignment (Colon & Trimble, 1987).

MR produces an image that permits visualization of brain structures because of the tissue contrast between gray matter structures, white matter structures, and surrounding CSF. Computerized images of MR scans use signal values in the form of pixels to represent CSF, white matter, gray matter, or abnormal signal hyperintensities (Jernigan, et al., 1990). Structures can be measured after being outlined by a stylus or cursor. The computer then generates a number based on an integration of the number of pixels within the
outlined area (Kesslak, et al., 1991). It is also possible to produce images of the brain that replace the monochromatic tones of gray matter, white matter, and cerebral spinal fluid (CSF) with bright colors to highlight brain structures.

Several obstacles remain in measurement of cerebral structures using MR. The edges of structures are not always sharply defined allowing variability in determining the boundaries of structures. In some cases, boundaries between areas are not clearly defined even using gross morphology. Although every effort is made to align a subject's head and to secure it before imaging begins, movement can occur which has the potential to change significantly the appearance of brain structures (Jernigan, 1991a).

Albeit the above problems exist, MR offers much promise to researchers interested in early detection of dementia. If early diagnosis of AD could be made using MRI images that indicate beginning changes in the size of key brain structures, it might then be possible to intervene in this neurodegenerative process by using medication to replace depleting neurotransmitters and possibly to halt further brain deterioration.

A second advantage offered by using MR to obtain information about early brain changes lies in being able to study individuals before they become so demented that they are unable to provide much needed epidemiological
information. This information could be used to identify risk factors for the development of AD including pattern of alcohol use. It is hypothesized that environmental factors contribute to the development of AD, but to date it has not been possible to get valid information from people with AD about such exposures. Although a variety of risk factors for AD have been studied in the past two decades, results have been equivocal. People with early brain changes identified by MR imaging would be more intact cognitively and therefore better able to provide detailed information about exposures and risk factors possibly associated with AD. These individuals would also be younger than most diagnosed AD patients, and therefore the exposures would be more recent occurrences.

Problem statement

Alzheimer's disease is estimated to affect 4 million people in the U.S. at a total cost of greater than $90 billion dollars annually (Progress Report on Alzheimer's disease, 1992). The prevalence rate for this age-associated dementia approaches 47.2% in those over 85 (Evans et al., 1989). With more people living longer, the proportion of people surviving to the over 85 category will increase, therefore increasing the number of people diagnosed with AD.

Currently, AD is diagnosed only after behavioral symptoms occur, as a result of significant brain changes. Once behavioral symptoms occur, the AD patient's brain has
probably suffered extensive damage. These cognitively impaired patients become progressively incapable of caring for themselves, relying instead on family or extended care facilities at an estimated cost of billions of dollars. The emotional costs of this disease are inestimable.

AD is a slowly progressing dementia that may begin in the fifth or sixth decade of life by affecting structures in the limbic system that mediate memory. Autopsy data from AD patients indicate cell loss in the hippocampal formation and entorhinal cortex, limbic system structures key to memory formation. First degree relatives of patients diagnosed with probable AD are believed to be at risk for the development of AD (Breitner et al, 1988). It may be possible to identify early brain changes in this at-risk population using MR imaging before observable cognitive and behavioral impairments occur. MR provides non-invasive, high-resolution images of brain structures that can be quantitatively measured using specifically developed computer software. Currently this technology is in the developmental stage; accurate measures of brain structures using MR in aging are not yet available. Measurements of memory structures including the hippocampal formation and entorhinal cortex obtained from an at-risk population and then contrasted with images obtained from a comparison group with no family history of dementia, could provide a way to identify early brain changes possibly consistent with AD.
**Purpose of the Study**

This research is an extended analysis of a study entitled, "Predictors of AD in High-Risk Offspring" (Burns et al., 1987). The purpose of this extended analysis is to determine a reliable and valid rule set for measuring the hippocampal formation and entorhinal cortex from MR images. A second purpose is to determine if correlations exist between size of brain structures in two groups and known and hypothesized risk factors for AD.

**Objectives**

The study is guided by the following research objectives:

1. To establish reliable sectional measurements of the anatomic landmarks visible on MR images to be used as a guide for outlining and quantifying the hippocampal formation and entorhinal cortex.

2. To compare measurements of the hippocampal formation and entorhinal cortex in two populations: offspring of AD patients and offspring with no known family history of dementing illness.

3. To assess the relationships between the size of the hippocampal formation and entorhinal cortex and known risk factors for AD.

4. To assess the relationship between the size of the hippocampal formation and entorhinal cortex and hypothesized risk factors for AD.
DEFINITION OF TERMS

The following terms and definitions for data from a record review of information obtained in the Burns et al. (1987) study will be used in this study.

Alzheimer's disease—established based on DSM-III-R criteria.

At-Risk subject—offspring of AD patient diagnosed using DSM-III-R criteria based on chart review, family interview, and autopsy data when available.

Known risk factors—these will include:

- family history of dementia
- age

Hypothesized risk factors—

- alcohol use in subject (subjects ranged from no alcohol use to social use. No subjects met criteria for alcohol abuse or alcohol dependence)
- alcohol use in subject's parents

Hippocampal formation—medial-temporal lobe structure that includes the dentate gyrus, hippocampus proper, and subiculum.

Structure measurement—The Sun-III-160 Expandable Workstation and Pixar II Image Computer software (Department of Psychiatry, The Ohio State University, Columbus, Ohio) will be used to obtain measurements of brain structures using a combination of manual tracing and thresholding of structures. Individual sectional measurements will be obtained after the brain structure has been outlined and
will consist of cross-sectional surface area measurement x thickness of slice (5mm) = sectional volume measurement for each slice. Measurements from two slices will be summed and averaged and that figure will represent the sectional measurement.

Entorhinal cortex-gray matter structure inferior to the hippocampal formation.
CHAPTER II
THEORETICAL FRAMEWORK
and
REVIEW of LITERATURE

Theoretical Framework

Man has long sought to understand the intricacies of thought and behavior through study of the brain. Early attempts to map structures of the brain via autopsy were often carried out in secret under threat of imprisonment. Later study used animal models from which individual brain structures were systematically removed to determine which behaviors were affected. Modern neuroscience began in 1953 when John Eccles published the results of intracellular recordings from single nerve and muscle cells. These studies provided the basis for what is today the central philosophy of modern neuroscience: that all behavior is an expression of neural activity (Kandel & Schwartz, 1985).

Initial progress on the relationship among brain, mind, and behavior occurred as a result of the merging of the traditions of neuroanatomy, neurophysiology, biochemical pharmacology, and behavioral studies (Kandel & Schwartz, 1985). Further impetus for brain, mind, behavior
relationships came in the form of the neuron doctrine recognizing that the nervous system consists of numerous neurons or signaling units. It is these far-reaching neurons with their myriad of signal connections mediated by neurotransmitter systems throughout the brain that are the basis for brain function. Neural activity defines what man thinks, says, and does. When that neural activity is interrupted, man's functioning becomes impaired. Severe breakdown in neural activity can lead to complete loss of function. Synaptic transmission of neuronal signals enables interneuronal communication and relies on intact pathways and adequate amounts of neurotransmitters for appropriate information processing.

Mind (or mental) function occurs as a result of the integration of neural events. Stimuli are presented to the senses, the encoded information is processed at several levels within the brain, and a response is initiated. The concept of mind encompasses consciousness which Sperry (1990) defines as, "...a dynamic emergent of brain activity, neither identical with, nor reducible to, the neural events of which it is mainly composed. Consciousness is not perceived as an epiphenomenon, or an inner aspect, or other passive correlate of brain processing, but rather an active integral part of the cerebral process itself, exerting potent causal effects in the interplay of cerebral operations" (p. 382).
Behavior occurs as the final product of brain mind relationships. Rational, appropriate behavior reflects intact neuronal pathways integrated into complex responding units. A change in behavior often signals a breakdown in brain function. Neural activity defines what man thinks, says, and does. This study will use the concept of brain, mind, behavior relationships grounded in the modern neuroscience doctrine that all behavior is an expression of neural activity, as its theoretical framework.

The concept of brain, mind, behavior relationships can be applied to the brain changes in AD. Autopsy data on AD patients show marked atrophy, the result of cell loss in the association neocortex, hippocampus, parahippocampus, basal nucleus of Meynert, and olfactory cortex. Neuropathological hallmarks of AD accompany this cell loss and include neurofibrillary tangles (NFT) and neuritic plaques (NP). Neurofibrillary tangles are composed of paired helical filaments (Katzman & Saitoh, 1991) found in the temporal, frontal, and parietal lobes, and are species specific to humans (Kokmen, 1984). Neuritic plaques consist of a central core of extracellular amyloid surrounded by degenerating nerve ends (Katzman & Saitoh, 1991). The presence of these abnormal structures along with granulovacuolar degeneration and Hirano bodies result in significantly altered neuronal function in the brains of AD patients.
The hippocampal formation, a medial temporal lobe structure playing a key role in memory function, has been extensively studied in AD. Using modern staining techniques, researchers report a remarkable breakdown in brain function in AD due to cell loss resulting in the isolation of some structures, most notably the hippocampal formation (Hyman, et al., 1984). Hyman, et al., (1984) report autopsy data on AD patients that include cellular changes in major projecting systems of the hippocampal formation that act to isolate it from other areas including the association cortices, basal forebrain, thalamus, and hypothalamus.

Cell loss, plaques, and tangles have been found to occur in the hippocampal formation in predictable patterns within specific cell layers in AD. When compared to controls, Van Hosen and Hyman (1990) found thinning of the cortex in the CA1/subicular area in AD while the dentate gyrus, CA4, and CA3 regions were not markedly different. Van Hosen and Hyman (1990) further describe a distinctive pattern of NFT formation resembling the pattern of cell loss in AD patients. Neurofibrillary tangles were observed in the subicular/CA1 area, layers II and IV of the entorhinal cortex, and layers III and V of the posterior parahippocampal cortex while the dentate gyrus, CA4, and CA3 were generally unaffected. Neuritic plaques were found in a line along the middle and outer portions of the molecular
layer of the dentate gyrus, subiculum, and CA4, CA3, CA2, and CA1 (Van Hosen & Hyman, 1990).

Whitehouse et al., (1982) documented a profound and selective degeneration of the neurons of the nucleus basalis of Meynert in AD. Kalus et al., (1989) used silver impregnation techniques for identification of amyloid and neurofibrillary tangles and found pathology in the presubicular region in AD patients.

The above authors have gone on to link the patterns of pathology in AD to the synaptic connections i.e., the fine structural anatomy of the affected areas. Using mostly non-human primates for study, several pathways and their connections have been identified. The subicular/CA1 area is known to have direct connections to the entorhinal, cingulate, posterior parahippocampal, and prefrontal cortices as well as to subcortical structures including the mammillary bodies, nucleus accumbens, parts of the thalamus, and the amygdala (Van Hosen & Hyman, 1990). Hippocampal efferents to the cortex are also affected due to NFT in layer IV of the entorhinal cortex (Hyman et al., 1989).

Conversely, the perforant pathway (a neuronal projection originating in layers II and III of the entorhinal cortex and believed to be the major source of cortical input to the hippocampal formation) has its function interrupted by NFT in AD (Hyman et al., 1986). The loss of neurons in the nucleus basalis of Meynert, a major
source of cholinergic innervation of the cerebral cortex, suggests a pathological substrate of the cholinergic deficiency in the brains of AD patients (Whitehouse et al., 1982).

The combination of the above pathology seen within the context of afferent and efferent neuronal pathways within the limbic system and neocortex demonstrates a process that effectively disconnects the hippocampal formation from the rest of the brain. Within this perspective, the cognitive changes that consistently accompany the development of AD become explainable. Loss of neuronal input to and output from the hippocampus, a major morphophysiological substrate of memory, leaves the AD patient with an increasingly compromised memory system. Initially the patient is incapable of forming new memories and progressively loses the ability to retrieve old memories.

Memory loss also occurs from chronic alcohol abuse. As early as 1879, Maudsley described chronic alcoholism with its clinical picture that, "...resembles not a little in mental symptoms...the last stage of senile dementia." This similarity in clinical picture is currently reflected in the Diagnostic and Statistical Manual (DSM-III-R) of the American Psychiatric Association diagnostic criteria for dementia associated with alcoholism (291.20) which specifies the occurrence of a global dementia after prolonged, heavy alcohol intake, persisting for at least 3 weeks after
cessation of alcohol intake, and when other causes of
dementia (including AD) have been excluded. Conversely, AD
is diagnosed after other forms of dementia including multi-
infarction and alcohol-associated dementias (DSM-III-R,
1987) have been ruled out.

Evidence from several areas suggests the dysfunction
seen in chronic alcoholism to be the result of hippocampal
dysfunction. EEG studies of P-3 with its hippocampal origin
and link to memory acquisition and consolidation shows
reduced amplitude in patients with alcohol induced brain
deficits (Ciesielski 1985). Goodin et al. (1978) report
differences in wave form, amplitude, and latency of P3 in
dementia.

Cerebral atrophy is a hallmark at necropsy of brains of
chronic alcohol users. In a neuropathological study of
brain damage caused by alcohol, Harper (1982) found low
brain weight, cerebral atrophy, and ventricular dilation.
Beverage alcohol is water soluble allowing it to diffuse
easily and quickly across bodily membranes and into the
brain where its neurotoxic effects are widespread (Tabakoff
et al., 1990). A number of neurotransmitters including
serotonin, norepinephrine, and dopamine (Tabakoff et al.,
1990) are affected by alcohol. Rats fed alcohol when
compared to a control group showed an 83% reduction in AChE
positive neurons in the basal nucleus of Meynert complex
along with significant reductions in ACh content and AChE
activity in the cortex, hippocampus, and amygdala (Arendt et al., 1988). Studies in humans have reported decreased CAT activity in chronic alcoholic patients (Antuono et al., 1980; Nordberg et al., 1980). Freund and Ballinger (1988) autopsied the brains of 79 chronic alcoholics and found a 30% reduction in muscarinic cholinergic synaptic receptor density in the hippocampus and a 30% reduction in benzodiazepine receptor density when compared with 25 matched nonalcoholic controls.

With the advent of modern neuroimaging techniques, most notably MR, researchers and clinicians can see these brain regions in living subjects. MR is based on the "interaction between radio waves and hydrogen nuclei in the body in the presence of a strong magnetic field " (Bradley, 1985, p. 1). Magnetization of hydrogen nuclei allows determination of physical characteristics of tissue which can be translated into picture elements (Bradley, 1985). Differences in relaxation times between different tissue elements allow the creation of images that, in the brain, delineate gray matter, white matter, and CSF. Using recently developed computer software programs, it is possible to determine the size of selected brain structures based on tissue properties and to re-image subjects over time to determine where tissue loss is occurring and to quantify that tissue loss. This technology suggests the possibility of early diagnosis of the dementing process with greater chance for
pharmacological intervention before widespread cell loss has occurred.

Based on the above patterns of pathology in AD patients, this study will use MR imaging linked to a software program to measure the hippocampal formation and entorhinal cortex in first degree relatives of AD patients and controls to determine if early changes in those structures can be identified before cognitive and behavioral changes are manifested. It is expected that early pathological changes will occur in the areas later identified to have suffered significant cell loss as well as NFT's and NP's due to the progressive nature of the dementing process in AD. This approach to the study of AD with its focus on neuronal pathways as key structures in normal memory function is consistent with the above described neuron doctrine that posits brain mind behavior function to be the result of neural activity.

In the future it may be possible to use MR imaging as a diagnostic tool to identify early AD before clinical symptoms appear. The utility of MR as an early diagnostic tool would depend upon its validity measured in terms of sensitivity and specificity. Sensitivity is a measure of the tool's ability to identify those who truly have the characteristic being tested for. Conversely, specificity is the tool's ability to identify those who do not have the characteristic being tested for (Kelsey, 1986, p. 286). A
score of 100% reflects no measurement error. Most tools contain measurement error in terms of false positives and false negatives. If the tool is designed to identify a high percent of those with the characteristic (sensitivity) then it will err by also identifying some false positives—those who do not have the characteristic but test positive. Conversely, if the tool has a high specificity it will identify most without the condition but will also include some false negatives—those with the condition who test negative. Tolerance of false positives and false negatives depends upon the characteristic being tested for, the consequences of the characteristic, and the cost of testing (Kelsey et al., 1986; Polit & Hungler, 1987).

Review of Literature

Alzheimer's disease (AD) is a chronic organic brain syndrome characterized by insidious onset with gradual deterioration of cognition, memory, psychomotor, and sensory-perceptual functioning. AD is believed to account for up to 70% of the dementia cases (those 65 and older) in the United States (Cote, 1985; Katzman & Saitoh, 1991). Evans et al. (1989), in a study of noninstitutionalized people over age 65, found an overall prevalence rate for probable AD of 10.3%. The prevalence rate was age associated increasing from 3% in the 65-74 age range to 47.2% in those over age 85 (Evans et al. 1989).
Findings vary as to the percent of AD that is sporadic versus familial. Heston et al., (1981) report 30% of AD to be familial while Katzman and Saitoh (1991) place that figure at 40%. First degree relatives of patients with sporadic AD have four times the incidence of AD when compared with the general population (Terry & Davies, 1983). Several researchers have hypothesized that susceptibiity to AD is transmitted by an autosomal dominant gene (Cook et al., 1981; Huff et al., 1988) with chromosome 21 implicated as a possible site for the genetic defect (Goldgaber et al., 1987). Age and family history are currently accepted as risk factors for the development of AD. Studies of other etiological factors have been equivocal.

Alzheimer's disease was recognized in 1905 by Dr. Alois Alzheimer in his report on the death of a 55 year old woman with progressive dementia whose autopsy revealed brain abnormalities in the form of abnormal nerve cell tangles and clusters of degenerating nerve endings (Katzman, 1986). This cluster of behavioral and neuropathological changes has become the hallmark of Alzheimer's disease. The neuropathological changes documented by Alzheimer, using silver staining techniques, have been further delineated through the use of advanced staining methods. The advent of electron microscopy in conjunction with new staining methods, has led to elaboration and expansion of the knowledge of the neuropathological changes in AD. Pertinent
findings now include neurofibrillary tangles, characterized by paired helical filaments species-specific to humans, neuritic plaques occurring as broken-off nerve fibers surrounding a dense amyloid core and correlating with the severity of dementia, and cell loss of larger versus smaller neurons (Kokmen, 1984).

Diagnosis of definite AD can only be made based on neuropathological analysis at autopsy. Findings include widespread atrophy of the association neocortex and the hippocampus as well as atrophy in parahippocampal structures, the cholinergic forebrain basal nucleus of Meynert, the dorsal tegmental nuclei, and the locus ceruleus (Katzman & Saitoh, 1991). DSM-III-R (1987) criteria for diagnosis of probable AD include exclusion by history, physical examination, and laboratory tests of all other specific causes of dementia with the diagnosis of Primary Degenerative Dementia of the Alzheimer Type being "limited to cases in which there is clear evidence of progressive and significant deterioration of intellectual and social or occupational functioning" (p. 121).

This review will begin by addressing salient literature on brain changes in AD and in alcohol use based on the results of neuropathological studies. The role of MR in brain imaging in the detection of early brain changes in dementia including AD and alcoholic dementia will then be discussed with a focus on developing reliable measurement
protocols for targeted brain structures. The last section will review validity issues surrounding self-reports of alcohol use.

Neuropathology of AD

Probable AD is diagnosed clinically based on behavioral changes including decline in thinking, judgment, and intellectual function followed by deterioration in language and motor deficits. Definitive diagnosis of AD can only be made after histopathologic evaluation of the brain either on biopsy or at autopsy (Jellinger, 1990). Characteristic morphologic changes in AD include reduction of fresh brain weight, decreased hemispheric volume with atrophy of medial temporal lobes and hippocampus, increased ventricular volume, and weight loss in the cerebral cortex, with loss of large neurons in the hippocampus and midfrontal cortex (Jellinger, 1990).

Atrophy of the AD brain is widespread but not uniform with involvement of the association neocortex, "hippocampus and parahippocampal structures including the entorhinal cortex, olfactory cortex and olfactory bulb, the cholinergic forebrain basal nucleus (of Meynert), the dorsal tegmental serotonergic nuclei, and the locus ceruleus noradrenergic nuclei" (Katzman & Saitoh, 1991, p. 278) while sparing the primary somatosensory, visual, and auditory cortices, the motor cortex, cerebellum, basal ganglia, brain stem, and much of the thalamus (Katzman & Saitoh, 1991).
The neuritic plaques (NP) and neurofibrillary tangles (NFT) within the neocortex and hippocampus identified by Alzheimer in 1906 have become hallmarks of AD. The neuritic plaque forms around a central core of amyloid protein which is in turn surrounded by degenerating nerve endings of dendrites and axons. The neurites also contain paired helical filaments (PHF) and stain for antibodies for a number of different neurotransmitters, suggesting that several neurotransmitter systems contribute to the formation of NP (Katzman & Saitoh, 1991).

Neurofibrillary tangles occur within the neuronal cell body and are composed of paired helical filaments occurring as two twisted filaments coiled counter clockwise around each other. Tau and ubiquitin are the major proteins in NFTs (Katzman & Saitoh, 1991).

Alzheimer's disease is manifested by memory impairment beginning with loss of recent memory and proceeding to almost total amnesia. Studies of human amnesia and of nonhuman primate brain function reveal memory related structures to include the hippocampus and parahippocampal gyrus, the amygdala, the cholinergic cells of the nucleus basalis of Meynert and septal nuclei, midline thalamic nuclei, and the higher order association cortices that mediate different modalities (Hyman et al., 1989, p. 121; Damasio et al., 1985).
These studies suggest that information from different association cortices converges in the limbic system in a step-wise fashion. Input to the hippocampus is directed through relay neurons in the entorhinal cortex of the parahippocampal gyrus (area 28) with higher order sensory-specific association areas projecting to layer II of the entorhinal cortex. Layer II and superficial layer III cells of the entorhinal cortex give rise to connections to the dentate gyrus of the hippocampus, known as the perforant pathway as its axons perforate the subiculum, pass through the hippocampal fissure, and terminate on the outer portion of the dentate gyrus granule cells and on the hippocampal CA1/subicular pyramidal cells (Hyman et al., 1989).

The subiculum is the primary output structure for the hippocampal formation sending hippocampal efferents to cortical areas in the temporal lobe, other limbic structures, the amygdala, thalamus, hypothalamus, as well as basal forebrain structures including the septum, nucleus accumbens, and diagonal band nuclei. Many of these cortical and subcortical areas then project to association cortices including a strong projection from the subiculum to layer IV of entorhinal cortex which projects to multiple association areas and is a parallel of the perforant pathway in the opposite direction (Hyman et al., 1989).

Neuropathological studies of AD brains compared to brains of controls as well as to brains identified to have
Huntington's Disease (HD), pseudodementia, or mixed dementia, suggest that AD pathology disrupts connections in the memory related structures of the limbic system and association cortices. Specifically, the neurons of origin of interconnecting projections of the entorhinal cortex, the hippocampal formation, and the amygdala are affected by neurofibrillary tangles (Hyman et al., 1986; Hyman et al., 1990). Hyman et al. (1989) remark "the distribution of NFTs respects almost entirely cytoarchitectural and laminar principles" (p. 123). They report layer II, the superficial part of layer III, and layer IV of the entorhinal cortex are severely affected by NFTs with layers V, VI, and the remainder of layer III showing few NFTs. As noted above the perforant pathway, the major projection from entorhinal cortex to the hippocampal formation, arises from layer II. The major projection from entorhinal cortex to the amygdala is from layer IV. Layer IV projects to periamygdaloid cortex, accessory basal, medial basal, and lateral basal nuclei (Hamos et al., 1989; Hyman et al., 1990).

The neurons of the CA1/subicular area are also markedly affected by NFTs. The prosubiculum, the area encompassing the overlap between the CA1 and subiculum, gives rise to hippocampal formation projections to the entorhinal cortex and the amygdala as well as direct projections to limbic and association areas (Hyman et al., 1990, p. 172). The accessory basal and lateral nuclei are the nuclei of origin
for amygdala efferents to the entorhinal cortex with amygdala projections to the hippocampal formation arising from the accessory basal, medial basal, and cortical nuclei, and cortical transition zone. Neuropathological study of AD brains reveals NFTs in the accessory basal and cortical nuclei and the cortical transition area (Hyman et al., 1990). The combination of the above neuropathological changes in AD results in isolation of the hippocampal formation from the cerebral cortex through destruction of the input and output connections of the hippocampal formation (Hyman et al., 1984; Hyman et al., 1989).

Neuritic plaque formation has been identified in several memory related structures in the brains of AD patients. Neuritic plaques disrupt the termination zone of the perforant pathway which exists in a band in the outer portion of the molecular layer of the dentate gyrus (Hyman et al., 1986). Several authors (Hyman et al., 1986; Purohit et al., 1989) have suggested that the distribution of plaques in the terminal zone of the perforant pathway supports the idea that plaque formation is related to cortical neuronal disease. Another band of NP has been identified in the molecular layer of the subiculum and prosubiculum, an area that receives input from temporal lobe association areas and from the brainstem (Van Hoesen & Hyman, 1990). Neuritic plaques have also been identified in
the medial basal nucleus and accessory basal nucleus of the amygdala (Hyman et al., 1990).

Patterns of neural pathology in AD are not limited to the above changes. Other cortical and subcortical areas important in memory as well as other cognitive functions are also impaired. Rudelli et al., (1984) examined the thalamic nuclei, basal forebrain, and hypothalamus and found neuritic plaques in the thalamus and mammillary bodies as well as in the septum and substantia innominata, site of the nucleus basalis of Meynert. The substantia innominata contains large cholinergic neurons termed the basal nucleus of Meynert. The basal nucleus of Meynert has widespread projections to the cortex and amygdala. These neurons are the major source of cholinergic innervation for the neocortex (Carpenter, 1991). Davies et al., (1976) found decreased levels of cholinergic neurotransmitters in AD. This neurochemical alteration has been explained by the loss of cholinergic cells in the nucleus basalis (Whitehouse et al., 1981). Allen et al., (1988) found that large neurons in the nucleus basalis in AD were not lost but had decreased in size, becoming small cells that were excluded from previous studies counting large cells.

In a study of cell loss from the nucleus basalis of Meynert, Doucette et al., (1986) examined 4 severely demented brains and 4 less demented brains and found the degree of neuronal loss increased in accordance with the
severity of the dementia in the intermediate and posterior subdivisions but not in the anterior portion of the nbM. Wilcock et al., (1983) reported an approximate 50% reduction in cell density in the nbM as well as a significant reduction in level of CAT activity in the frontal and temporal lobe cortex in AD patients versus controls. Arendt et al., (1983) compared loss of neurons in the nbM in AD, Paralysis Agitans, Korsakoff's disease, and several other neurological disorders and found the greatest loss in AD (70%) and Paralysis Agitans (77%) with a 47% loss in Korsakoff's disease. No marked reduction in nbM neurons was found in postencephalitic parkinsonism, HD, chronic alcoholism without dementia, schizophrenia, and infantile brain damage. The adjacent external segment of the globus pallidus in these patients was unaffected suggesting these changes to be specific rather than due to diffuse cortical atrophy (Arendt et al., 1983).

Studies of aging in mammalian species including mice, rats, monkeys, and humans have shown cognitive decline in later phases of life (Bartus et al., 1980; Lippa, 1980; Strong, 1980). CNS changes have been targeted to play a major role in this decline although Bartus et al., (1982) also recognized the role of sociocultural, economic, and psychological factors.

The cholinergic hypothesis has been advanced as one explanation for cognitive deficits in aging. This
hypothesis targets changes in the cholinergic neurotransmitter system as responsible, in part, for cognitive deficits in aging. The clinical findings of progressive memory loss coupled with neuropathological findings of decreased choline acetyltransferase (CAT) activity in the brains of AD patients has provided a model for the cholinergic hypothesis. As stated above, the nucleus basalis of Meynert is the source of cholinergic neurons projecting to the neocortex and amygdala and neuropathological study has found a loss of cells in the nbM in AD (Whitehouse et al., 1981).

Pharmacological agents have been used to mimic memory deficits. Young monkeys given the central cholinergic receptor blocker scopolamine showed similar memory deficits to those of aged monkeys on the same memory tasks (Bartus & Johnson, 1976). These results appear to provide further evidence for the cholinergic hypothesis as an explanation for memory deficits. The question of deficits in other neurotransmitter systems in AD has been studied by several groups. Perry et al., (1981) used cases of AD, unipolar depression, and normal controls to determine histologically the relationship between neuron numbers in the locus ceruleus and the levels of the noradrenergic enzyme dopamine B-hydroxylase (DBH) in the cortex of demented and non-demented cases as well as examining the relationship between DBH levels and severity of dementia. The locus ceruleus is
a major source for noradrenergic neurons that have extensive axonal connections with the entire forebrain (Kandel & Schwartz, 1985). Measures of AD type abnormalities including mean plaque counts and mental test scores were not significantly related to cortical activity of DBH while the activity of acetyltransferase did correlate significantly with AD type abnormalities, leading these authors to conclude that changes in the cholinergic system are more involved with AD abnormalities then are changes in the noradrenergic system (Perry et al., 1981).

AD can only be diagnosed based on neuropathologic study of cortical and subcortical areas including the presence of a requisite number of NPs and NFTs. The etiology of these neuronal abnormalities in AD is still unknown. No consensus has been reached on the question of which came first, the NP or the NFT. Further questions revolve around how or whether plaques and tangles contribute to the memory deficits characteristic of AD. Neuropathological studies at autopsy of patients diagnosed with probable AD show significant loss of cells in the medial-temporal lobes specifically the hippocampal formation and entorhinal cortex. These structures are believed to play a key role in the storage and retrieval of memory. Various cell layers within the HF and EC act as information pathways receiving input from other limbic system structures and sending this information on to the association cortex. Input from the association
cortex is also shunted through the HF and EC on its way to other limbic system structures.

Clinical diagnosis of AD is made based on observable patient behaviors usually beginning with memory dysfunction. By the time enough memory loss has occurred to meet criteria for the diagnosis of possible or probable AD, significant cell loss in the HF and EC, key memory structures, has already occurred. Neuroimaging technology including MR may provide a way to image and measure memory structures to determine a pattern of cell loss before that loss becomes widespread and possibly before behavioral symptoms have occurred.

Neuropathology of alcohol abuse

AD is only one of many dementias characterized by memory loss. A clinical challenge in diagnosis of AD is to differentiate AD from other dementias including Pick's disease, Wernicke-Korsakoff syndrome, alcohol dementia, multi-infarct dementia, and senile dementia. These disorders, among others, present with symptoms of confusion and memory loss in the early stages. The similarity in symptoms between AD and alcohol related dementias has led to several hypotheses concerning a possible relationship between AD and alcohol use (Freund, 1982; Lishman, 1986). These hypotheses have been tested by research focused on the specific neurotransmitter systems involved in alcoholic dementias (Antuono et al., 1980; Nordberg et al., 1980).
Arendt et al. (1988) suggest, "The psycho-pathological similarities between alcohol related impairment of cognitive function and AD might reflect similarities in the neurobiological substrate of these disorders, suggesting a common crucial step in the pathogenesis of the mental impairment under both conditions" (p. 563).

Two alcohol related syndromes have been suggested in the literature. Wernicke encephalopathy, a neurological disorder occurring in alcoholic or nutritionally deficient patients, is characterized by nystagmus, abducens, and conjugate gaze palsies, unsteadiness of stance and gait, and a confusional-apathetic state (Victor et al., 1989). Victor et al., (1989) reported that most of the patients they studied who survived the acute stage of Wernicke encephalopathy went on to display the Korsakoff amnestic-state with its memory and learning deficits in otherwise cognitively intact patients. Neuropathologic studies of alcoholics with Wernicke-Korsakoff syndrome report lesions of the mammillary bodies of the hypothalamus, the dorsomedial thalamic nucleus, and the nerve fibers connecting these two structures along with widening of sulci, enlarged lateral ventricles, lesions of the wall of the third ventricle, and atrophy of the superior cerebellar vermis (Oscar-Berman, 1990; Victor et al., 1989).

Alcoholic dementia has been suggested as a distinct disorder but remains difficult to classify (DSM-III-R, 1987;
Lishman, 1986). Victor et al., (1989) state, "...the status of alcoholic dementia as a clinical-pathologic entity remains in limbo" (p. 139). On autopsy, patients who have the clinical diagnosis of primary alcoholic dementia have shown lesions as in the Wernicke-Korsakoff syndrome (Victor et al., 1989). Freund (1985) acknowledges there may not be ethanol-specific morphologic changes in a given brain suggesting instead that chronic alcohol abuse may have a role in modifying other ongoing processes such as aging or other causes of dementia. He posits the existence of two types of anterograde amnesia in alcoholics. One includes the Korsakoff syndrome with its diencephalic lesions, while the second is characterized by lesions in the medial bitemporal cerebral cortex, hippocampus, and amygdala (Squire, 1981).

Several animal studies have looked at brain structures after ingestion of alcohol. Arendt et al., (1988) fed rats alcohol for twelve weeks and found the alcohol fed rats when compared to controls to have an 83% reduction of AChE positive neurons in the basal nucleus of Meynert complex. A 74% reduction in activity of ChAT and an 81% reduction in AChE in the basal forebrain of the alcohol fed rats compared to controls were also found. Significant reductions in ACh content and AChE activity in the cortex, hippocampus, and amygdala were also present in the alcohol fed rats but no difference in ChAT activity between the alcohol fed rats and
the controls was found in those brain structures. A significant reduction in the number of AChE positive neurons in the three subdivisions of the basal nucleus complex in the alcohol fed rats was also present. These authors suggest that ethanol causes the death of cholinergic neurons in the basal nucleus complex.

The brains of human alcohol abusers have been studied in an attempt to determine possible alcohol related neurotransmitter changes. Nordberg et al., (1980) looked at the activity of CAT and MAO along with the number of muscarinic binding sites in different brain regions in AD, chronic alcoholics, and controls and found decreased CAT activity and reduced number of muscarinic binding sites in chronic alcoholic patients. Antuono et al., (1980) used discrete sampling of brain tissue in AD (n=2), alcoholic (n=3), and control subjects (n=5), and reported decreased CAT activity in the frontal, temporal, and insular cortex, as well as in the hippocampus area and a slight, nonsignificant decrease in muscarinic receptor binding in AD and alcoholic patients in this limited numbers of subjects.

Nordberg et al., (1982) studied 20 chronic alcoholics and 14 controls on postmortem to determine cholinergic activity in the hippocampus. Findings included nonsignificantly lower ChAT in the hippocampus of the chronic alcoholics as compared to the controls along with lower numbers of muscarine-like binding sites which reached
significance in the oldest group of alcoholics compared to controls. Arendt et al., (1983), as reported earlier, found a 47 per cent decrease in neurons in the nucleus basalis of Meynert in the brains of subject's with Korsakoff's disease when compared to controls on autopsy.

More recently, Freund and Ballinger (1988) autopsied the brains of 79 chronic alcoholics in a study that controlled for or excluded other brain diseases, clinical (including liver) diseases, medications, age, and postmortem conditions, and reported a 40 per cent decrease in cholinergic muscarinic receptor binding but not affinity, in the frontal cortex when compared with matched controls of the same age. The results of this study are important because other conditions known to cause dementia in alcoholics were excluded, so the reported brain changes are more likely the result of the chronic neurotoxicity of alcohol itself.

Neuropathologic studies are fraught with methodological issues and the above reported studies are no exception. Some of these issues include small sample size, variation in age and cause of death, amount of time from death to harvesting of the brain, different methods used in obtaining tissue samples, as well as the use of a variety of methods to analyze the tissue. The fact that fairly consistent changes in cholinergic neurotransmitter function in alcohol abuse were found, given all of the aforementioned
methodological issues, suggests that these changes are probably real and that the condition of the cholinergic system may be fairly stable for a number of hours after death.

Arendt et al., (1988) have suggested that the neurotoxic effects of ethanol on the cholinergic basal forebrain system may indicate a "pathoplastic" role of ethanol in the development of dementing disorders characterized by degeneration in NBM such as AD. Freund (1982) has questioned whether alcohol abuse protects against or predisposes drinkers to AD. Lishman (1986) hypothesized that the similarity between AD and alcoholic dementia is attributable to the fact that both disorders have "analogous neurochemical deficits" (p. 1185) i.e. cortical cholinergic deficiency resulting from involvement of basal forebrain nuclei in a Wernicke-type pathology. These theoretical issues, coupled with the results of neuropathological changes in the cholinergic system in the nucleus basalis of Meynert, a basal forebrain structure, in both AD and alcohol abuse, seem to suggest a need for further study of the effects of alcohol use in the development of AD.

It can be hypothesized that alcohol's effect on the cholinergic transmitter system may increase the rate of cognitive decline in AD, a condition known to affect the cholinergic transmitter system, by impacting an already compromised system. Terri et al., (1989) investigated
factors that might influence rate of decline in 106 patients with AD. Cognitive function as measured by Mini-Mental Status Exam in alcohol abusers declined 5 points per year faster than in nonabusers. Alcohol abuse as a risk factor contributed the most to cognitive decline in AD although only 5 subjects in the study were designated as alcohol abusers.

The above studies reflect the controversy as to the role of alcohol in the neuropathological changes found in chronic alcoholics. The Wernicke-Korsakoff syndrome with its diencephalic cell loss and anterograde memory loss is well documented. The existence of an alcoholic dementia with medial-temporal lobe changes and AD like memory deficits has been suggested. Animal and human studies have documented the effects of alcohol on the cholinergic system. Some suggested alcohol-related changes parallel brain changes in normal elderly. Alcohol may have a role in the development of AD or in the rate of progression of AD. The possible role of alcohol in AD needs further study.

Role of MR imaging in AD and alcohol

With the evolution of sophisticated imaging techniques such as magnetic resonance, observations of neuropathologic changes are no longer limited to autopsy studies. Imaging analysis of the living human brain reduces the number of confounding variables involved in fixed brain studies including time between death and harvest of the brain,
effects of fixing brain tissue, and fragility of some brain structures. MR imaging allows visualization and measurement of cerebral gray matter, white matter, and CSF, moving quantification of brain changes beyond the techniques of weighing and visual inspection that characterize autopsy studies. MR imaging allows quantification of structure(s) in terms of volume or surface area as well as percent of total area. MR imaging possesses specific advantages over computerized tomography (CT). MR imaging is capable of imaging brain regions in all planes while CT is limited to a transverse plane. Andreasen (1988) lists four applications for MR imaging: "morphometric studies of brain structure, assessment of tissue function and possible tissue pathology through measurement of T1 and T2 relaxation times, measurement of metabolic function through magnetic resonance spectroscopy, and assessment of blood flow and possibly metabolism through paramagnetic tracers" (p. 1385). Many of these applications have no parallel in CT. Spatial resolution can be improved in CT through the use of increased radiation. MR imaging does not require exposure to radiation (Bradley, 1985).

MR imaging is based on the ability of the body to become magnetized when placed in a magnetic field. Hydrogen, the most abundant element in the body, contains a proton in its nucleus that possesses a property when magnetized called "spin" (Bradley, 1985). Magnetic moments
are created when spin occurs causing hydrogen nuclei to behave like bar magnets that align with a strong magnetic field. Application of a small, low-energy pulse of the proper frequency will cause the sample to tilt out of alignment with the magnetic field. Once tilted, the magnetic moment begins to precess, a motion in which it rotates slowly in a cone-like path. This rotation causes the emission of a radio frequency termed the Larmor frequency. Each type of nucleus has a unique Larmor frequency (Morgan & Hendee, 1984).

The speed and pattern with which a substance returns to realignment with the field after being pulsed out of alignment accounts for the various types of images available with MR. Spin-lattice relaxation time or T1 is one type of realignment. T1 times vary for different tissues. A second type of realignment is spin-spin or T2 relaxation which also varies with the tissue type being studied. T1 and T2 values in milliseconds for normal and pathological specimens have been computed for various tissues. By employing different scanning methods, various aspects of a tissue or structure can be enhanced. For instance the relative signal amplitudes for white matter, gray matter, and CSF obtained during a brain scan can be altered to increase certain signal intensities thereby enhancing gray matter structures over white matter ones (Morgan & Hendee, 1984).
Validation studies have been employed to determine the accuracy of MR imaging for obtaining measurements of structures in vivo. Once MR images are obtained, computer generated software packages allow the investigator to measure surface area or volume of specified structures using a drawing cursor. Jack et al., (1990) applied three different techniques for MR imaging of volume in brain structures to four cylinders of known volume: tracing, thresholding, and random marking. Results suggest that the use of a combination of tracing and thresholding to obtain volume measurements can be made with high precision and reproducibility (Jack et al., 1990). Nadich et al., (1987) correlated MR images with cryomicrotome sections and formalin-fixed sections of human brain and found that spin-echo MR imaging with short repetition time/short echo time pulse sequences performed well when displaying gyri and sulci of the inferomedial temporal lobe and the gross anatomic features of the major gray and white components of the hippocampal formation. Areas including the subspenial, supercallosal, and paraterminal components of the limbic lobe were poorly imaged using this MR protocol (Nadich et al., 1987).

Attempts have been made to determine volumetric measurements of brain structures in normal persons. Two studies by Jack et al., (1988; 1989) compared volumes of several structures on the right versus left side of the
brain. The first study (Jack et al., 1988) reported the nondominant (right) temporal lobe volume to be significantly greater than the dominant (left) in a sample of 25 mostly right handed normal adults. The second study (Jack et al., 1989) measured the volume of the right and left anterior temporal lobes and hippocampal formation of 52 healthy volunteers age 20-40. Results indicated right-left asymmetry in the volumes of both areas to be a normal finding. The anterior temporal lobe of the non-dominant (right) hemisphere was larger than the left by a statistically significant amount. A statistically significant difference was also obtained in volume measurements of the hippocampal formation with the right hippocampal formation having a larger volume than the left in all subjects.

Jernigan et al., (1991a) used MR volume measurements of selected brain structures to determine age related changes. Significant decreases occurred in the volume of the caudate nucleus, anterior diencephalic structures, and in the gray matter of most cortical regions in subjects between 30 and 79 years of age with the thalamus and the anterior cingulate cortex remaining unchanged. Age related changes in white matter were reflected in lengthened T2 values (Jernigan et al., 1991a).

Dementia in general and AD specifically have been studied using a variety of MR protocols. Kesslak et al.,
(1991) compared 8 patients diagnosed with AD to 7 age-matched controls and found greater than a 40% reduction in the volume of the hippocampus and entorhinal cortex in the AD patients. Areas like the striatum not involved in the degenerative process in AD showed no volumetric changes. Volumes of the hippocampus and entorhinal cortex correlated with scores on the Mini-Mental State Examination (r=0.89).

Jernigan et al., (1991b) also used MR imaging volume studies to provide an MR analog to existing neuropsychological and neuropathologic comparisons of AD and Huntington's disease (HD). These studies showed the greatest volume reductions in HD patients in striatal structures with reductions also detected in the thalamus and inferior cortical areas, especially in mesial temporal lobe structures. The AD group experienced widespread cortical volume reductions, reaching highest losses in mesial cortices and including subcortical losses particularly in the thalamus. The HD group had significantly more severe white matter abnormalities with the AD group showing more white matter abnormalities than the control but less than the HD group.

Using dual sequence MR imaging that compensates for partial volume averaging (mixing of signal from gray matter and white matter or gray matter and CSF), Rusinek et al., (1991) measured the distribution of cerebral gray matter, white matter, and CSF in 14 AD patients and 14 healthy controls. Overall percentage of gray matter was
significantly decreased in AD patients when compared to controls with the most significant reduction occurring in the temporal lobes and a central region. Other areas of significant loss included the frontal lobe and the occipital lobe. CSF volume increases were identified in the temporal, occipital, and frontal regions. No significant changes in white matter content were identified in any region. The authors report the above findings to be consistent with pathologic observations of cortical cell loss (Rusinek et al., 1991).

Bondareff et al., (1988) used the T2 component of the MR signal to measure 11 brain loci in patients diagnosed with probable AD and found T2 values to be significantly correlated to dementia severity as measured by the Blessed-Roth Dementia Scale. T2 values for the right and left hemispheres and in gray and white matter did not differ significantly, although the mean T2 value for left hemisphere structures was more closely correlated with the dementia score. As T1 and T2 relaxation times are affected by tissue water content, these authors suggest that more severe dementia in AD is associated with more water in the brain. Harrell et al., (1987) obtained MR imaging on seven patients who had been diagnosed with primary degenerative dementia (Alzheimer's, Pick's) after clinical, laboratory, and CT evaluation. T1 image findings supported the CT findings but T2-weighted images showed high-density lesions
0.5-2 cm in size in white matter areas. The authors report these high-density lesions to be consistent with, but not diagnostic of, cerebral infarction. While not excluding the original diagnosis, these findings may suggest a role for MR imaging in differentiating among various forms of dementing illnesses (Harrell et al., 1987).

Press, Amaral, and Squire (1989) developed a high resolution MR protocol for imaging the human hippocampus that provides cytoarchitectonic detail. The authors adjusted the head position of the subject so that the hippocampus could be imaged perpendicular to its long axis. T1-weighted sequences of six interleaved 5 mm-thick slices with no gap were obtained with details including the pyramidal cell fields of the subiculum and hippocampus, the fimbria, perforant path, and molecular layer of the dentate gyrus. This protocol was applied to the study of three male amnestics and four male controls who showed nearly identical temporal lobe area measurements. The area of the hippocampal formation in the amnestics was 49 per cent of that in the controls. The authors conclude, "Because damage to the hippocampal formation occurs prominently in Alzheimer's disease, and memory impairment is one of its earliest symptoms, this protocol might be useful in achieving an early diagnosis of the disease" (p. 57).

Squire, Amaral, and Press (1990) then applied their high-resolution protocol for imaging the hippocampus and one that
imaged the mammillary nuclei to the study of 4 amnesic patients with alcoholic Korsakoff's syndrome and 4 non-Korsakoff amnestic patients. MR images of the alcoholic Korsakoff patients showed abnormally small mammillary nuclei with normal sized temporal lobes, hippocampal formation, and parahippocampal gyrus. The non-Korsakoff amnestic patient's images revealed a markedly reduced hippocampal formation with no detectable change in the size of the temporal lobe. The mammillary nuclei showed some reduction in volume but were reported to be considerably larger than in the Korsakoff's patients. Overall anatomical findings for individual patients corresponded to the severity of their memory impairment. The authors suggest that neuroimaging can be used to distinguish between medial temporal lobe amnesia and diencephalic amnesia (Squire et al., 1990).

A number of MR studies of chronic alcoholics have been reported in the literature. Jernigan et al., (1991c) used magnetic resonance imaging to compare 8 patients with alcoholic Korsakoff's syndrome with age-matched normal controls and nonamnesic chronic alcoholic patients. Though both nonamnesic chronic alcoholics and alcoholic Korsakoff patients showed CSF increases, the KS patients could be differentiated from the chronic alcoholics by widespread reductions in grey matter which reached greatest proportions in diencephalic structures. Korsakoff's syndrome patients demonstrated greater volume losses in the anterior portions
of the diencephalon, mesial temporal lobe structures, and the orbitofrontal cortices, when compared to the non-Korsakoff alcoholics suggesting that damage to the hypothalamus and hippocampus may contribute to the amnesic symptoms in Korsakoff's syndrome. Both groups showed similar losses in the posterior diencephalon (Jernigan et al., 1991c).

Jernigan et al., (1991d) extended the above study to a larger and younger population (28 non-amnesic chronic alcoholics and 36 age and sex-matched non-alcoholic controls) who underwent a series of neuropsychological tests as well as MR imaging. Alcoholics 4-5 weeks into detoxification showed increases in sulcal and ventricular CSF, significant volume reduction in subcortical gray matter structures and cortical regions including the diencephalon, caudate nucleus, dorsolateral frontal, and parietal cortex, and mesial temporal lobe structures. Increments in cortical and ventricular CSF and decrements in the volume of cortical and subcortical gray matter were significantly correlated. Although CSF volumes and some neuropsychological performances were correlated, Jernigan et al. conclude that the data provides little evidence of significant relationships between cognition and gray matter volumes.

Chick et al., (1989) compared T1 relaxation time in the whole brain and four regions of interest (frontal white, parietal white, frontal gray, parietal gray matter) in 69
detoxified alcoholics compared to age matched controls. The authors hypothesized that the brains of detoxified alcoholics would be overhydrated two weeks into abstinence when compared to age and sex matched non-alcoholics. T1 relaxation time is related to the state of water in the tissue and increases as the proportion of free-to-bound water within the tissue increases. Subjects also underwent cognitive testing (Category Sorting Test) and were assessed for cumulative lifetime alcohol intake. Results showed increased T1 relaxation time in whole brain, gray matter, and parietal white matter in the detoxified alcoholics when compared to controls. A significant relationship was also found between T1 measurements, cognitive impairment, and total lifetime consumption of alcohol in the detoxified alcoholics. These results, according to the authors, suggest MR T1 to be of clinical value as a marker of alcohol-related brain damage (Chick et al., 1989).

Magnetic resonance imaging allows non-invasive quantification of brain structures in vivo based on the unique molecular properties of tissues. Gray matter, white matter, CSF, as well as signal hyperintensities can be visualized and measured using a variety of software programs. Several validation studies have proven the accuracy of MR for measurement of brain structures. Brain MR imaging of normal subjects as well as those diagnosed with AD, HD, Korsakoff's syndrome, and to chronic alcoholics have the potential to
determine the unique neuropathology of disease states before extensive damage has occurred. MR offers the opportunity for early diagnosis as well as a better understanding of the development and progression of brain disorders. It may also provide some answers to questions about the possible interactions between AD and alcohol use. MR has the potential to quantify brain structures therefore allowing for early diagnosis and treatment of chronic, progressive brain disorders.

Validity of alcohol use data

Obtaining valid alcohol use data continues to challenge researchers. To date, no "gold standard" exists against which other measures of alcohol use can be compared (Midanik, 1988). CDTect is the trademark for a laboratory test that provides for quantitative determination of carbohydrate-deficient transferrin (CDT) in human serum. CDT concentration levels become elevated with daily alcohol intake of more than 60 grams of ethanol for at least one week. A recently modified CDT assay has a reported sensitivity of 93 per cent and specificity of 99 per cent (Stibler, 1991). With further validation studies CDTect may become a gold standard against which to measure results from self-reports, collateral reports, diary, official records, interviewing methods, and other laboratory tests. Until that time efforts to determine current and life-time alcohol
intake will continue to be obtained from a variety or combination of sources.

Much of the controversy surrounding validity of alcohol use reports has centered on self-reports. Commonly used self-report tools include the CAGE (Ewing, 1984), the MAST (Selzer, 1971), and the SMAST (Selzer et al., 1975) along with longer standardized diagnostic interviews including the Schedules for Clinical Assessment in Neuropsychiatry (SCAN), (Wing, 1989), the Structured Clinical Interview for DSM-III-R (SCID), (Spitzer et al., 1988), and the Alcohol use Disorders and Associated Disabilities Interview Schedule (AUDADIS), (National Institute on Alcohol Abuse and Alcoholism, 1990).

Analysis of these methods varies. Watson et al., (1984) compared self-reports to collateral reports and stated, "The results support a moratorium on the use of patients' self-reports in follow-up studies on alcohol consumption" (p. 344). Polich (1982) looked at alcoholic's reports of their drinking and behavioral problems and concluded that most types of self-reports are valid. A Special Medical Report from the NIAAA (1992) stated, "Self-report questionnaires and interviews have greater sensitivity and specificity than routine blood tests for biochemical markers" (p. 1664).

After an extensive review of the literature on the validity of self-reported alcohol use, Midanik (1988)
concluded that it is inappropriate to seek a definitive answer to the question of the validity of self-reports of alcohol use. She instead focused on concurrent criterion-oriented validity in her analysis and suggested that research in this area should focus on specific processes involved in providing accurate responses. Specifically, Midanik (1988) stresses the importance of the interactions of the respondent, the interviewer, the information being obtained, and the context of the interview to maximize valid responses.

Until a "gold standard" is developed for the measurement of alcohol use, the above controversies will continue to exist. Self-reports of alcohol use have been both criticized and supported. It has been suggested that more than just the tool itself needs to be evaluated. The process of obtaining alcohol use information needs to be further studied and understood. Clearly, several issues remain to be resolved in this controversy.
CHAPTER III
PROCEDURES

This research is an extended analysis of existing data from a larger study (Burns, et al., 1987) conducted through the Department of Psychiatry at The Ohio State University. The larger study was funded by the State of Ohio Department of Aging. Criteria for subject selection and methods used for data collection for the Burns et al. (1987) study will be described in the first section of this chapter. The second section will describe the procedures used in this extended analysis to address the research objectives listed in Chapter I.

SECTION 1
STUDY DESIGN:

Subject selection

Burns et al. (1987) conducted a cross-sectional study of subjects, age 40-60, having a parent with AD and controls with no family history of AD to determine whether or not first-degree relatives of AD patients show a pattern of brain structural and functional abnormalities predictive of AD. Group 1 consisted of first-degree relatives of AD patients selected from the families of AD patients in
Columbus and central Ohio including the OSU Hospitals Cognitive Disorders Clinic, Alzheimer disease support groups, and from persons who responded to advertisements in the Alzheimer's disease and Related Disorders Association (ADRSA) Newsletter (a major source of subjects) and local community and university newspapers.

Group 2 subjects were selected from persons with no family history of AD who responded to advertisements in local community and university newspapers. A number of Group 2 subjects were the spouses of Group 1 subjects who themselves had no family history of AD. All subjects selected and tested in the Burns' study were included in this extended analysis.

Subjects were screened for inclusion in the study based on the following criteria:

Diagnostic criteria for AD:

Diagnostic records were obtained (after gaining the consent of the patient or legal guardian) on the parent of Group 1 subjects to assure that the diagnosis met established diagnostic criteria for AD as stated in the Report of the NINCDS-ADRSA Work Group (McKhann et al., 1984) and in the DSM-III-R (1987). Currently, AD is diagnosed definitively by autopsy and presumptively by combined medical history, neurologic, psychiatric, and neuropsychological studies.
I. Criteria for the clinical diagnosis of PROBABLE AD include:

Dementia established by clinical examination, documented by the Mini-Mental Status Test or other mental examination, and confirmed by neuropsychological testing:

Deficits in two or more areas of cognition (sufficiently severe to interfere with social/occupational functioning);

Progressive worsening of memory and other cognitive functions;

No disturbance of consciousness (e.g., the absence of delirium);

Onset between ages 40-90, most often after 65, duration > 6 months; and

Absence of systemic disorders or other brain diseases that could account for the progressive deficits in memory and cognition.

II. The diagnosis of PROBABLE AD was supported by:

Progressive deterioration of specific cognitive functions such as language (aphasia), motor skills (apraxia), and perception (agnosia);

Impaired activities of daily living and altered patterns of behavior;

Family history of similar disorders, neuropathologically confirmed; and
Laboratory results of:

a. Normal lumbar puncture as evaluated by standard techniques;
b. Normal pattern or nonspecific changes in EEG, such as increased slow wave activity; and
c. Evidence of cerebral atrophy on CT (or MRI) with progression documented by serial observation.

III. Other clinical features consistent with the diagnosis of PROBABLE AD, after the exclusion of dementia other than AD, include:

Plateaus in the course of progression of the illness;
Associated symptoms of depression, insomnia, incontinence, delusions, illusions, hallucinations, catastrophic verbal, emotional, or physical outbursts, sexual disorders, and weight loss;
Other neurological abnormalities, especially in patients with more advanced disease, including increased muscle tone, myoclonus, or gait disorder;
Seizures in advanced stages of the disease; and CT normal for age.

IV. Features that make the diagnosis of PROBABLE AD uncertain or unlikely include:

Sudden, apoplectic onset;
Focal neurological findings (hemiparesis, sensory loss, visual field deficits, and incoordination) early in the course of the illness; and
Seizures or gait disturbances at the onset or very early in the course of the illness.

V. **Clinical diagnosis of POSSIBLE AD:**

May be made on the basis of dementia in the absence of other neurological, psychiatric, or systemic disorders sufficient to cause dementia, and in the presence of variations in onset, presentation, or clinical course of the illness;

May be made in the presence of a second systemic or brain disorder sufficient to produce dementia, which is not considered to be the cause of the dementia; and

Should be used in research studies when a single, gradually progressive severe cognitive deficit is identified in the absence of other identifiable cause.

VI. **Criteria for diagnosis of DEFINITE AD are:**

The clinical criteria for PROBABLE AD; and

Histopathological evidence from a biopsy or autopsy;

VII. **Classification of AD for research purposes should specify features that may differentiate subtypes of the disorder, such as:**

Familial occurrence; Onset before age 65; Presence of trisomy-21; and Coexistence of other relevant conditions, e.g. Parkinsonism.
VIII. Exclusion criteria used in the diagnosis of AD:

Subjects were excluded for evidence of:

A. Parkinsonism or other neurologic disorder (epilepsy or seizure disorders);
B. Past or present major depressive episode;
C. Circulatory disturbances including: Multi-infarct dementia (MID); aneurysm; cerebrovascular or hypertensive disease requiring treatment;
D. Neoplasia;
E. Trauma such as subdural hematomas or cerebral hemorrhage;
F. History of toxicity due to: alcoholism or other drug abuse; heavy metals; organicides;
G. Nutritional deficits;
H. Active infections:
I. Endocrine abnormalities (e.g., hypothyroidism);
J. Uncorrected significant hearing or visual problems;

IX. Criteria for exclusion of a potential subject from the study:

Subjects were excluded for:

A. Any of the following health problems:
   Neurological disease of any form (e.g., Parkinsonism or epilepsy or other seizure disorder); Neoplastic lesions; Significant head
trauma (subdural hematomas; cerebral hemorrhage); History of endocrine abnormalities (e.g., hypothyroidism); History of a major depressive episode; Toxicity (substance abuse, diagnosis of alcohol abuse or dependence, infections, heavy metals, organics); Circulatory disturbances (MID, aneurysm, arteriovascular or hypertensive disease; Nutritional deficiency; and, Uncorrected hearing or visual problems.

B. Inability to be drug free from any medications affecting the CNS for two weeks prior to assessments, since such medications affect the parameters under study.

Complete physical and neurological examination:

After a thorough medical history was obtained, a complete physical and neurological examination was performed by a physician investigator.

Complete psychiatric examination and family history:

The structured clinical interview for diagnosis (SCID, Spitzer et al., 1987) was administered to each subject by a trained research assistant; the results were reviewed by a psychiatrist. If any psychiatric illness was detected in a potential subject, the person was excluded from the study. The complete medical, psychiatric, and family history was
based on the Family History Research Diagnostic Criteria (FHRDC, Endicott et al., 1978).

METHODS

MRI studies:

The SUN-III-160 Expandable Workstation with Pixar II Image Computer software (Department of Psychiatry, The Ohio State University, Columbus, Ohio) was available for use in the analysis of MRI scans. The SUN-III utilizes MRI processing, data base, and statistical software developed by The Ohio State University computer graphics research group. Magnetic tapes containing MRI scan data for each subject were processed using the SUN-III allowing specific measures of area and volume to be made. The SUN-III facilitates evaluation of regional neuropathology, e.g., the presence of hyperintense MRI signals and enables measurement of the hippocampal formation and entorhinal cortex.

1. Scanning parameters:

Each study included a set of transverse images (5 mm thickness, skip interval 1 mm) obtained using a T2 weighted spin echo sequence (TE1=200; TE2=75; TR=2500) for detection of unsuspected lesions and morphological abnormalities in this plane. A set of coronal images (5 mm thickness, skip interval 5 mm) obtained using a T1 weighted inversion recovery pulse sequence (T1=800; TR=1500) and a sagittal series, encompassing a 3-4 cm band about the mid-sagittal plane (3 mm thickness, skip interval 0.5 mm) using
the parietal saturation pulse sequence (TE=20; TR=600) for morphological assessment were also obtained.

2. **Clinical radiographic assessment:**
Clinical radiographic assessment was made by a qualified radiologist with experience in MRI.

3. **Other measurement issues:**
Parameters measured, measurement method, and MRI data analysis for the expanded analysis will be discussed below in Section 2. Burns et al., (1987) also performed event related potential (ERP) studies and cognitive assessment using an extensive neuropsychological test battery. Data from those tests were not included in this extended analysis study.

**SECTION 2**

**Power analysis**

Data on the variables of interest were available for 62 subjects, of whom 39 were cases and 23 were controls. Power analysis for this sample size was calculated as >.90, based on an estimated model of 5 predictor variables, $R^2 = 25$, and alpha = .05 (Cohen & Cohen, 1983).

**Measurement criteria**

The first research objective (to establish reliable sectional measurements of the anatomic landmarks visible on MR images to be used as a guide for outlining and quantifying the hippocampal formation and the entorhinal
cortex) was undertaken in several steps. A literature review of studies that measured the hippocampal formation and entorhinal cortex using coronal MR images was conducted. Several groups of researchers were identified who had established measurement protocols for examining these structures in a variety of populations including alcoholics, amnestic, seizure patients, and schizophrenics (Jack, et al., 1990; Shenton et al., 1992; Press et al., 1989; Squire et al., 1990). Anatomical landmarks used to identify structures and measurements procedures were reviewed in all articles obtained.

Concurrently, this researcher reviewed anatomy texts to establish location and size of limbic system structures in relation to other structures in the region. This process was coupled with time spent evaluating the MR images obtained from the Burns et al., (1987) study as described in Section 1. Images were viewed using both a light box and computer screen to determine ability to identify brain regions and to begin to test out various rule sets for isolating structures.

Several experts in medial-temporal lobe brain anatomy were consulted to validate this researcher's ability to identify correctly target structures as well as to determine variability of structure size and shape in sequential MR slices. Consultation was provided by several psychiatrists and two neuroradiologists for this aspect of the study.
Next, this researcher and a research assistant from the larger study spent time viewing sets of 15-20 coronal MR images per subject to establish a reliable rule set for choosing the appropriate slices on which to conduct structure measurement of the HF and EC. Although the HF and EC were visible on 3-4 slices, it was determined that other structures, most notably the amygdala, interfered with isolation and measurement of the HF on one to two slices. The HF and EC could be seen in two slices, 5 mm apart, as separate, measurable structures. It was therefore decided to obtain sectional measurements of the HF and EC on the right and left side of two slices per subject. A protocol for choosing the two slices to measure was developed. The more anterior slice (coronal MR images were obtained posterior to anterior) containing the HF and EC was identified to occur one slice anterior to the pons. The next slice posterior to the pons occurred one slice anterior to the slice in which the lateral ventricles and third ventricle meet. The slice containing the pons was always measured. The other slice to be measured was either one slice posterior or one slice anterior to the pons. If the first slice anterior to the pons appeared to contain amygdala, it was rejected and the slice posterior to the pons was chosen. Practice sessions were conducted until 96% agreement between two researchers was reached on which two
slices to measure. Random checks on this phase of the research have reached inter-rater agreement of 100%.

Based on the above information, a protocol was established for identification and measurement of the hippocampal formation and the entorhinal cortex. The hippocampal formation (HF) was defined to include the dentate gyrus, Ammon's horn, and subiculum. The landmarks for tracing the hippocampal formation included:

- lateral-inferior tip of the lateral ventricle
- medial-apex of the parahippocampal gyrus
- superior-follow the contrast between the lateral ventricle (CSF) and the fimbria (white matter)
- inferior-follow the contrast at the inferior border of the subiculum

The landmarks for tracing the entorhinal cortex included:

- lateral-tip of the collateral sulcus
- medial-apex of the parahippocampal gyrus
- superior-follow the contrast between gray matter of superior border of EC and adjacent white matter
- inferior-follow contrast of gray matter of EC and adjacent CSF

**Measurement method**

In preparation for measurement all coronal slices for each subject were transferred to an optic disc to be used in the SUN-III-160 Expandable Workstation. Images were then
displayed on the imager and computer "normalized" to make each slice of equal brightness based on white matter pixel count. The above protocol for slice identification was used to identify two normalized slices for measurement.

Measurement began with the researcher using the SUN-III and Pixar II Image Computer software to magnify the chosen image on the Pixar. Using a mouse driven drawing cursor, the left cerebral half was circumscribed to isolate it and to obtain a sectional measurement (surface area x thickness of slice = volume of slice) of the total gray matter and total white matter in that cerebral half. This step was a necessary precursor in the software program to obtaining measurements of the HF and EC.

Manual tracing using the mouse driven drawing cursor was then undertaken to outline the HF based on the above anatomic landmarks. Next, a box was manually drawn around the HF which included surrounding white matter. The computer, using a combination of manual tracing and thresholding then provided a measurement of the gray matter, white matter, and CSF within the confines of the box. The gray matter number generated by the computer represented the sectional measurement of the HF for that MR slice.

Finally, the entorhinal cortex (EC), also a medial-temporal gray matter structure, was outlined based on the above anatomic rule set and a computer generated reading of the gray matter, white matter, and CSF within the box drawn
around the EC was obtained. The gray matter number generated by the computer from this boxed area represented the sectional measurement of the EC for that MR slice. This procedure was repeated for the right side of this slice, and for the left and right sides of the second slice for each subject. Sectional measurements for the two slices were summed and an average was computed. All measurements were recorded on a data sheet coded by the subject's 9 digit hospital number.

**Inter-rater reliability**

The first step in data analysis was the establishment of inter-rater reliability. At the beginning of the measurement process, 10 subjects were chosen at random and measured independently by each of two trained researchers (this researcher and a researcher from the larger study). Measurements were made over a 10 day period of time with each researcher working in isolation and recording measurements in her own notebook on a coded data sheet. Once all measurements had been obtained, they were given to a statistician for analysis. Inter-rater reliabilities were computed using a statistical program based on inter-class correlations developed by a statistician in the Department of Psychiatry at The Ohio State University.

**Variables analyzed**

Once acceptable reliabilities were obtained, the remaining subjects were measured by this researcher and the
trained research assistant from the larger study. Upon completion of measurement, data analysis was begun. Data for each subject included measurements from two MR slices for right and left cerebral hemispheres on two medial-temporal lobe structures, the hippocampal formation and entorhinal cortex. Measurements from the right and left sides of the brain for each structure were analyzed separately. Each structure's final measurement score consisted of the average of that structure's sectional measurement from two MR slices.

The above data were entered into a database and the statistical program Minitab was used to obtain descriptive statistics including mean age and education for each group. Data were transferred to the SAS system for further analysis. Four analyses of co-variance were run using each of the four sectional measurements separately as the dependent variables (R-HF, R-EC, L-HF, L-EC) and the at-risk variable, along with sex, age, height, subject's alcohol use, subject's parent's alcohol use, and all logical two way interactions thereof as independent variables entered as co-variates in the model. Height was used as a co-variante in the model to correct for variation in individual body size (Andreasen, et al., 1993)

Information on the five independent variables was obtained from a variety of sources. As reported above, a subject was considered to be "at-risk" for AD if a
biological parent had been diagnosed with definite, probable, or possible AD confirmed by chart review or a letter from a physician. Controls had no family history of AD. The subject's sex, age, and height were obtained from self-report. Subject's alcohol use was taken from section E.1 of the SCID (Appendix A) that queries alcohol dependence or abuse (lifetime). Use was then categorized as:

1. rare-never= those who drank 1-2 drinks or less per month
2. social= one to two drinks once or twice per week
3. heavy= more than two drinks per occasion and alcohol use more than twice per week.

Parental alcohol use had been recorded on the Family History questionnaire and represented two categories:

1. rare-never= those who drank 1-2 drinks or less per month
2. moderate-heavy= those who drank several times per week or who were reported as "alcoholic."

**Human Subjects Concerns**

The Biomedical Sciences Review Committee at The Ohio State University reviewed and approved the original research used in this dissertation. An expedited review was requested for this dissertation research and it was approved. All subjects signed an approved consent form after receiving a verbal explanation of the research by a trained research assistant. It was not expected that
completion of any part of this research would represent any psychological, social, or legal risk to the subject. Time was alloted during several phases of the study to talk to subjects to determine if any aspect of the research study had a significant emotional impact upon them. Information from all aspects of the study was kept in a confidential file and all data analysis was done anonymously.
CHAPTER IV
RESULTS

This chapter will report the results of the statistical analyses used in this study. Four research objectives guided this research:

1. To establish reliable sectional measurements of the anatomic landmarks visible on MR images to be used as a guide for outlining and quantifying the hippocampal formation and entorhinal cortex.

2. To compare sectional measurements of the hippocampal formation and entorhinal cortex in two populations: offspring of AD patients and offspring with no known family history of dementing illness.

3. To assess the relationships between sectional measurements of the hippocampal formation and entorhinal cortex and known risk factors for AD.

4. To assess the relationship between sectional measurements of the hippocampal formation and entorhinal cortex and hypothesized risk factors for AD.

Objective 1: Establishment of reliable sectional measurements

The first objective involved establishing inter-rater reliability based on the protocol developed by this
researcher to measure the hippocampal formation and entorhinal cortex in each hemisphere of the brain from MR images. Table 1 shows the results of the inter-rater reliabilities for the two structures on each side of the brain. Reliabilities ranged from .988 for the right entorhinal cortex to .922 for the right hippocampal formation.

**TABLE 1**

<table>
<thead>
<tr>
<th>Inter-Rater Reliability</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-HF</td>
<td>0.985</td>
</tr>
<tr>
<td>L-EC</td>
<td>0.971</td>
</tr>
<tr>
<td>R-HF</td>
<td>0.922</td>
</tr>
<tr>
<td>R-EC</td>
<td>0.988</td>
</tr>
</tbody>
</table>

**Description of the sample**

The sample consisted of 39 subjects at-risk for development of Alzheimer's disease (parent with possible, probable, or definite AD diagnosis) and 23 controls (no known family history of dementia). The only left handed subject in the study was removed before data analysis began leaving an all right handed sample. The at-risk group
consisted of 29 females and 10 males. In the control group 7 were male and 16 were female. Table 2 shows the mean age and mean education for this group. Mean age for the at-risk group was 53.3 (SD=3.8) and for the controls was 52.7 (SD=4.7). Both groups were similar in education with the at-risk group reporting 15.8 years (SD=2.6) and the controls 15.5 years (SD=3.1) (Table 2).

<table>
<thead>
<tr>
<th></th>
<th>Mean Age</th>
<th>Mean Education</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(SD)</td>
<td>(SD)</td>
</tr>
<tr>
<td>At-Risk</td>
<td>53.3</td>
<td>15.8</td>
</tr>
<tr>
<td></td>
<td>(3.8)</td>
<td>(2.6)</td>
</tr>
<tr>
<td>Control</td>
<td>52.7</td>
<td>15.5</td>
</tr>
<tr>
<td></td>
<td>(4.7)</td>
<td>(3.1)</td>
</tr>
</tbody>
</table>

From medical chart data available on the affected parent of at-risk subjects, an assignment to a diagnostic group of possible, probable, or definite AD was made. Definite AD was diagnosed based on the report of a pathologist from autopsy data. To date, autopsy data
confirming AD has been obtained for four parents of at-risk subjects. Probable AD was the chart diagnosis in 21 cases and the remaining 14 were listed as possible AD by the reporting physician.

As noted in the Procedures section, alcohol use was obtained and categorized for both subjects and parents. Subject's alcohol use was coded 1, 2, or 3: 1=rare/never, 2=social (weekly or less), 3=heavy (three or more times per week). Table 3 shows the distribution of alcohol use by group. Most subjects in both groups (twenty-four at-risk and thirteen control) fell into the rare to never category. Eleven at-risk and six controls drank socially. Four from each group were heavier drinkers but failed to meet exclusion criteria for alcohol dependence or abuse.
### TABLE 3

**Distribution of Alcohol Use by Group**

<table>
<thead>
<tr>
<th>Level of Alcohol Use</th>
<th>Definite</th>
<th>Probable</th>
<th>Possible</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rare/Never</td>
<td>0</td>
<td>16</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>Weekly</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>&gt; 3 x Per Week</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

**Objective 2-4: Assessment of factors associated with variation in sectional measurements**

Research objectives 2, 3, and 4 involved comparison of surface area means in relationship to known and hypothesized risk factors for development of AD. After discussion with the Department of Statistics Consultation Service at The Ohio State University, Analysis of Co-Variance was chosen as the appropriate method for data analysis. Plots of residuals versus predicted values for each of the dependent variables and residuals were found to be normally distributed. All assumptions for ANCOVA were met.
ANCOVA models were tested specifying each of the four surface area measurements separately as the dependent variables (R-HF, R-EC, L-HF, L-EC) and subject group code (three levels of at-risk and one level of control), sex, age, height, subject's alcohol use, and parent's alcohol use were entered as independent variables which are treated as covariates in this model. All logical two way interactions were also entered into this analysis (code & age, code & sex, code & subject's alcohol use, code & parent's alcohol use, age & sex, age & subject's alcohol use, age & parent's alcohol use, and subject's alcohol use & parent's alcohol use).

None of the four analyses of covariance was significant. P-values for the overall models were: L-HF, .3258; L-EC, .1513; R-HF, .0982; R-EC, .1273. The covariate height was significant for two of the models: L-EC, 0.01 and R-HF, 0.003. The interaction between age and sex had a p-value of 0.01 for the R-EC. None of the other direct or interaction effects had p-values at the 0.01 level or below.

Because several of these variables were found not to contribute to the explanatory power of the model, they were removed from the model. Most of the subjects fell between age 45 and 58 suggesting little variance in age range, so age was removed as a variable. Since body size was controlled for by the covariate height, sex was removed from
the model. Parental alcohol use did not contribute significantly to the model and it was removed.

Scatter plots of measurements of the HF and EC revealed two subjects as outliers based on the values obtained for their L-HF. These outliers, one at-risk and one control subject, had L-HF values >100 mm larger than the next largest L-HF value. These subjects were identified and their brains were re-measured. Upon re-measurement it was determined that these two subjects had been imaged so that the first slice that contained the hippocampus was significantly rostral to the first slice in the other subjects. That meant that the second slice for these two outliers contained the amygdala, a larger structure not included in this analysis. It was decided to remove these two outliers from the study as their MR images did not contain two measureable slices.

During consultation with the Department of Statistics, it was determined that defining three categories (definite, probable, and possible AD in the parent) within the code at-risk did not contribute to the model and resulted in too few subjects in several categories (4 definite, 21 probable and 14 possible). These three categories were collapsed into one category, at-risk. Level of subject's alcohol use was reduced from three categories to two: rare-never (less than once a month) and social use (greater than once per month but not meeting criteria for alcohol abuse or dependence).
A series of ANCOVA models were run using this adjusted specification and containing 38 at-risk subjects and 22 controls. The covariate height, along with group (at-risk versus control), and subject's alcohol use were used in the analyses of direct effects and the interaction between group and subject's alcohol use was assessed as the most relevant. The following model p-values were obtained: L-HF, 0.05; L-EC, 0.0002; R-HF, 0.0001; R-EC, 0.0009 (Table 4).

### TABLE 4

Model Statistics for ANCOVAs by Brain Structures

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>SS</th>
<th>MS</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-HF</td>
<td>4434</td>
<td>1109</td>
<td>4</td>
<td>2.56</td>
<td>0.048</td>
</tr>
<tr>
<td>L-EC</td>
<td>32250</td>
<td>8063</td>
<td>4</td>
<td>6.48</td>
<td>0.0002</td>
</tr>
<tr>
<td>R-HF</td>
<td>11116</td>
<td>2779</td>
<td>4</td>
<td>6.91</td>
<td>0.0001</td>
</tr>
<tr>
<td>R-EC</td>
<td>20792</td>
<td>5198</td>
<td>4</td>
<td>5.48</td>
<td>0.0009</td>
</tr>
</tbody>
</table>

Model: Covariates = Group (At-Risk vs Control), height, subject's alcohol use, and two-way interaction code by alcohol use.
For L-HF, height was the only significant variable with a p-value of 0.003. Three variables demonstrated significant p-values for L-EC, subject's alcohol use (p=0.009), height (p=0.002), and group by subject's alcohol use (p=0.001)(Table 5 & 6).

**TABLE 5**

ANCOVA For L-HF by Variables

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>SS</th>
<th>MS</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(At-risk vs Control)</td>
<td>346</td>
<td>346</td>
<td>1</td>
<td>0.80</td>
<td>0.375</td>
</tr>
<tr>
<td>Alcohol Use</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(rare/never vs social)</td>
<td>317</td>
<td>317</td>
<td>1</td>
<td>0.73</td>
<td>0.396</td>
</tr>
<tr>
<td>Height</td>
<td>4096</td>
<td>4096</td>
<td>1</td>
<td>9.47</td>
<td>0.003</td>
</tr>
<tr>
<td>Interaction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(code vs alcohol use)</td>
<td>98</td>
<td>98</td>
<td>1</td>
<td>0.23</td>
<td>0.636</td>
</tr>
<tr>
<td>Variable</td>
<td>SS</td>
<td>MS</td>
<td>df</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----</td>
<td>-----</td>
<td>----</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td><strong>Group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(At-Risk vs Control)</td>
<td>867</td>
<td>867</td>
<td>1</td>
<td>0.70</td>
<td>0.407</td>
</tr>
<tr>
<td><strong>ALCOHOL USE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Rare/Never vs Social)</td>
<td>8890</td>
<td>8890</td>
<td>1</td>
<td>7.14</td>
<td>0.009</td>
</tr>
<tr>
<td><strong>HEIGHT</strong></td>
<td>12869</td>
<td>12869</td>
<td>1</td>
<td>10.34</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>INTERACTION</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Code vs Alcohol Use)</td>
<td>14027</td>
<td>14027</td>
<td>1</td>
<td>11.27</td>
<td>0.001</td>
</tr>
</tbody>
</table>

On the right side of the brain R-HF had one variable, height, with a significant p-value of 0.0001. R-EC had three model variables that achieved significance: subject's alcohol use (p-value 0.046), height (p-value 0.0004), and
the interaction between group and subject's alcohol use (p-value 0.037) (Table 7 & 8).

TABLE 7

ANCOVA for R-HF by Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>SS</th>
<th>MS</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(At-Risk vs Control)</td>
<td>396</td>
<td>396</td>
<td>1</td>
<td>0.98</td>
<td>0.356</td>
</tr>
<tr>
<td>Alcohol Use</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Rare/Never vs Social)</td>
<td>79</td>
<td>79</td>
<td>1</td>
<td>0.20</td>
<td>0.66</td>
</tr>
<tr>
<td>Height</td>
<td>11016</td>
<td>11016</td>
<td>1</td>
<td>27.41</td>
<td>0.0001</td>
</tr>
<tr>
<td>Interaction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Code vs Alcohol Use)</td>
<td>95</td>
<td>95</td>
<td>1</td>
<td>0.21</td>
<td>0.628</td>
</tr>
</tbody>
</table>
### TABLE 8

**ANCOVA for R-EC by Variables**

<table>
<thead>
<tr>
<th>Variable</th>
<th>SS</th>
<th>MS</th>
<th>df</th>
<th>F</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(At-Risk vs Control)</td>
<td>1888</td>
<td>1888</td>
<td>1</td>
<td>1.99</td>
<td>0.164</td>
</tr>
<tr>
<td><strong>Alcohol Use</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Rare/Never vs Social)</td>
<td>3942</td>
<td>3942</td>
<td>1</td>
<td>4.16</td>
<td>0.046</td>
</tr>
<tr>
<td><strong>Height</strong></td>
<td>13407</td>
<td>13407</td>
<td>1</td>
<td>14.15</td>
<td>0.0004</td>
</tr>
<tr>
<td><strong>Interaction</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Code vs Alcohol Use)</td>
<td>4319</td>
<td>4319</td>
<td>1</td>
<td>4.56</td>
<td>0.037</td>
</tr>
</tbody>
</table>

As can be seen from Tables 5-8, height was significant in all four models. A multiple regression was run to determine the direction of the correlation for height. The correlation was determined to be negative with each one millimeter increase in height associated with a decrease in the size of the specified brain structure.

An interaction table was constructed to assess mean size of the dependent variable covaried with height and two levels of the variables, group and subject's alcohol use.
As Table 9 shows, little difference is seen in the mean value of L-HF among the four levels of variables. Mean values for the L-EC show a decrease in size for controls categorized as social drinkers (mean = 81, SD = 13) when compared to controls in the rare-never category of alcohol use (mean = 139, SD = 47). The reverse occurred in the at-risk group with heavier drinkers showing larger L-EC (mean = 115, SD = 39) when compared to lighter drinkers (mean = 105, SD = 39).

<table>
<thead>
<tr>
<th>Group</th>
<th>Alcohol</th>
<th>N</th>
<th>L-HF</th>
<th>L-EC</th>
<th>R-HF</th>
<th>R-EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Rare/Never</td>
<td>12</td>
<td>109</td>
<td>139</td>
<td>100</td>
<td>121</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Social</td>
<td>10</td>
<td>107</td>
<td>81</td>
<td>101</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At-Risk</td>
<td>Rare/Never</td>
<td>24</td>
<td>110</td>
<td>105</td>
<td>103</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At-Risk</td>
<td>Social</td>
<td>14</td>
<td>104</td>
<td>115</td>
<td>101</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
On the right side, the R-HF was essentially unchanged among the four categories. For the R-EC the control group again had smaller mean sectional measurements in those who drank socially (mean = 86, SD = 20) when compared to the rare-never category of alcohol users (mean = 121, SD = 46). For the at-risk group the reverse occurred with the social drinkers having a mean of 101 (SD = 27) and the rare-never group a mean of 97 (SD = 34) (Table 6).
CHAPTER V

DISCUSSION

It is currently estimated that Alzheimer's Disease affects 4 million Americans at a total annual cost to the Nation of $90 billion (Progress Report on Alzheimer's Disease). Definitive diagnosis of AD is made based on neuropathological study of brain tissue at autopsy. Presumptive diagnosis of AD is based on clinical symptoms and the absence of other treatable pathology. By the time the clinical symptoms of AD appear, significant cell loss has occurred in the association neocortex and limbic system structures including the hippocampus, entorhinal cortex, and the cholinergic forebrain basal nucleus of Meynert (Katzman & Saitoh, 1991). The etiology of AD remains unknown.

First degree relatives of patients diagnosed with probable AD are believed to be at increased risk for development of the disease (Breitner et al., 1988; Huff et al., 1988). A second risk factor for the development of AD is age with the prevalence reported to be 3 per cent for those ages 65-74 compared to 47.2 per cent for those over 85 years of age (Evans et al., 1989).

Several studies have looked at the risk of developing AD for relatives of AD probands. Five studies used
cumulative incidence data for development of AD-like illness or dementia in relatives of AD cases (Breitner et al., 1988; Heston et al., 1981; Huff et al., 1988; Martin et al., 1988; Mayeux et al., 1991). Using cumulative incidence of AD in 366 relatives of 79 AD cases (Kaplan-Meier technique) based on age as opposed to time, Breitner et al. (1988) calculated the probability of AD relatives developing AD to be 1.03 out of 100 by age 60 (SE 0.60) increasing to 49.33 out of 100 by age 87 (SE 8.38). Controls had a cumulative incidence of AD-like illness at age 72 of 1 out of 100 (SE 0.99) rising to 9.81 out of 100 by age 85 (6.06). Martin et al. (1988) also looked at cumulative incidence of AD among offspring using the Kaplan-Meier technique and reported a cumulative incidence of AD in 130 first degree relatives of 22 AD patients to be 1.39 out of 100 (SE=1.38) at age 65 increasing to 40.82 per 100 by age 83 (SE=9.42). Control relatives (n=144) had a cumulative incidence of AD of 1.64 out of 100 (SE=1.63) at age 69 and 23.23 (SE=10.80) at age 85. Heston et al. (1981) found no difference in cumulative incidence of dementia in AD relatives versus controls until after age 65 while Mayeux et al. (1991) reported no difference until after age 70.

These data suggest that although risk of developing AD is increased in offspring of AD probands, the increased risk is not evident until after age 65 or even age 70 in some cases. Three of these studies reported the age of onset of
AD in offspring to be from 5 years to 13 years later then age of onset in AD probands (Breitner et al., 1988; Heston et al., 1981; Huff et al., 1988). The establishment and reporting of age of onset in AD has raised many as yet unanswered validity questions.

Both AD and Alcoholic Dementia produce similar clinical and neuropathological changes leading to the suggestion that the two disease states may share a common neurochemical origin and that the possible interaction between these two forms of dementia should be investigated (Freund & Ballinger, 1992; Lishman, 1986).

Exploration of quantitative and qualitative in vivo brain changes has become possible through application of magnetic resonance imaging technology coupled with computer based software programs. MR imaging has potential for use as a screening tool in early diagnosis of neurological conditions including AD. Using MR imaging, those at higher risk for development of AD, such as first degree relatives, could be identified and routinely followed for evidence of early brain changes indicative of AD. Intervention with medication during early stages of cell loss before behavior changes occur has greater potential of retarding or reversing the pattern of cell loss. Several of the medial-temporal lobe structures implicated in AD-type pathology and in alcoholic dementia have been reliably measured from MR images.
This study used brain MR images of subjects at-risk for AD and controls with no family history of dementia to develop a reliable protocol to measure selected sections of the hippocampal formation and entohrinal cortex. The protocol established anatomic landmarks for the lateral, medial, superior, and inferior aspects of each structure that, when coupled with computer generated edge detection, yielded inter-rater reliabilities of .922 and above. This protocol is currently being used in the Department of Psychiatry at The Ohio State University to measure the HF and EC in schizophrenic subjects.

Results for Objectives 2-4 were mixed. Known risk factors tested included family history of AD and age. No difference was found between measurements of the HF and EC in the at-risk group when compared to controls based on family history and age. Assessment of alcohol use as an hypothesized risk factor revealed the control subjects who drank alcohol socially had decreased sectional measurements of the R-EC and L-EC when compared to the controls who drank rarely or never. There was no difference in the sectional measurements of the HF and EC between at-risk subjects who drank socially versus those who drank rarely to never. Height showed a significant inverse relationship to brain structure size with taller subjects having smaller brain structure measurements.
Some observations about the MR imaging protocol used for this study are necessary prior to discussion of study results. The images available to this researcher from the Burns et al. study were based on an MR imaging protocol developed in 1986. That protocol obtained coronal images 5mm thick with a skip interval of 5mm. Although a smaller skip interval (2.5mm) was used for axial and sagittal slices, the HF and EC are best visualized on coronal slices. Imaging protocols developed after 1986 for the purpose of providing surface area or volume measurements of smaller structures such as the HF and EC use a skip interval of from 2.5mm to no skip in some cases. The HF is a sea-horse shaped structure comprised of a head, body, and tail with an estimated total length of about 40mm (Squire et al., 1990). To image the hippocampus completely special positioning of the subject's head with hyperextension of the neck is necessary.

The computer program used to obtain surface area measurements in this study established "volume estimates" based on an equation that obtained pixel counts for the surface area measured and then factored in the skip interval of 5mm resulting in an estimated volume measurement for each slice. Because this MR imaging protocol did not target the HF and EC specifically but rather measured these structures as part of the total brain producing a variable number of slices containing HF and EC and used a large skip interval.
when imaging these fairly small structures, it was not possible to image each subject's HF and EC uniformly. The first slice containing the HF or EC could vary by 5mm in either direction with the last slice of the HF possibly including up to 4mm of the next anterior structure, the amygdala. Based on the skip interval and the imprecise imaging of the HF and EC, it was not possible to obtain total surface area or total volume of either structure. Therefore, it was decided to choose two slices containing the best images of the HF and EC for measurement. It was hypothesized that cell loss could be detected using this method.

The remaining objectives in this study concerned assessment of factors associated with variation in sectional measurements of the HF and EC in those at-risk for AD when compared with controls with no family history of dementia. Using ANCOVA a model was developed to assess the relationship of selected sectional measurements of four structures, L-HF, L-EC, R-HF, and R-EC, to the variables group (at-risk versus control) and subject's alcohol use. Height was entered into the model as a covariate to control for variation in body size (Andreasen, et al., 1993). Three of the overall models, L-EC, R-HF, and R-EC, were highly significant at 0.0009 level or above. The model for the L-HF had a p-value of 0.048.
Investigation of individual model statistics revealed that the two groups, at-risk versus controls were not different when compared based on measurements for any of the four brain structures. There may be several explanations for this result. Subjects in the study were relatively young with a mean age of 53.3 for the at-risk group and 52.7 for the control group with the subject pool ranging in age from 45-60. These subjects were studied a decade or more before the age at which their parent reportedly developed clinical symptoms of AD.

Failure to detect differences in size of selected brain structures between these two groups may reflect the fact that no detectable cell loss has yet occurred. This finding could be the result of subjects in this study being too young to have experienced cell loss. Another factor mitigating against identifying cell loss in the at-risk group is the finding of Heston et al. (1981) that in 51 families with an AD proband, only about one-third of the families had any secondary cases. It may be that none of these at-risk subjects are destined to develop AD. A third explanation for lack of detection of cell loss may lie in the MR protocol used. As discussed above, the available MR images were not capable of producing total volume or total surface area for either the HF or EC. It could be that cell loss is occurring in sections of the structures not available for measurement in this study.
Alcohol use was found to have a significant effect ($p < 0.05$) in two of the models, the L-EC and R-EC. Subjects entered into this study were screened based on alcohol use and were excluded if they currently or in the past met criteria for alcohol abuse or alcohol dependence. Those remaining in the study were categorized as either social drinkers or rare/never drinkers. Based on the literature reporting the effects of alcohol on memory structures, alcohol was entered into the model as an hypothesized risk factor contributing to the cell loss seen in AD. Further analysis of these two models (Table 9) revealed control subjects categorized as social drinkers showed a significant decrease in mean sectional measurements of the L-EC and R-EC when compared to those in the rare/never drinking category. There was no significant difference in mean brain structure size of at-risk subjects based on alcohol use.

The distribution of alcohol use by group in Table 3 shows that 13 control subjects were categorized as rare to never drinkers and 10 were social drinkers (categories "weekly" and ">3 times per week" were collapsed into the category "social drinker"). In terms of per cent, 44 per cent of the control versus 39 per cent of the at-risk subjects were classified as social drinkers. During interviews for this study, at-risk subjects often revealed their worry about developing AD. Some of them even talked about their efforts to "eat right", exercise, and not get
"too stressed." It may be that consciously or unconsciously these at-risk people also modified their alcohol intake as part of their concern for the health of their brains.

Freund (1982) suggests that chronic alcohol consumption could potentially retard, accelerate, or have no effect on normal or pathological aging. AD, one form of pathological aging, may be affected by chronic alcohol consumption. It is possible that being at-risk for AD is a protection against brain changes sometimes linked to alcohol use when that use is only at the social level. Further study of subjects who do develop AD along with rigorous quantification of their past alcohol use will be needed to answer this important question.

Those in the control group who fit the category for social drinking showed decreases in R-EC and L-EC measurements while the HF on both sides remained unaffected. Integrity of the EC is essential for normal hippocampal function. Superficial cell layers II and III of the EC are the origin for the axons that form the perforant pathway which perforates the gray matter of the subiculum on the way to termination sites in the HF. The EC receives input from the visual, somatosensory, auditory, and olfactory cortices as well as other sub-cortical input. Nearly all the cortical connections in the HF are not direct but relay via the EC and the perforant pathway. The EC and HF are major sites of neuropathology in AD with the superficial layers of
the EC which give rise to the perifornat pathway selectively involved in NFT's characteristic of AD (Hyman et al., 1986). In a study of non-demented elderly Arriagada et al. (1992) report that the accumulation of NFT's in this non-demented group followed a progressive pattern that increased with age with neurons in the EC being one of the earliest sites of NFT formation.

No ethanol-specific morphologic changes identifiable in a given brain have been acknowledged. Rather it is believed that chronic ethanol abuse may act to modify already ongoing processes including normal aging and other forms of dementia (Freund, 1985). Lishman (1990) postulates the brain of an alcoholic to be vulnerable to two distinct forms of pathology. The first includes cortical changes with possible neuronal loss as the result of alcohol neurotoxicity while the second involves the basal brain regions as a result of thiamine deficiency. Both he believes may occur before clinical evidence is available. The idea that cortical and subcortical structures are differentially vulnerable to the neurotoxic effects of alcohol has been raised by Freund and Ballinger (1989, 1992).

Several processes may be contributing to the decrease in size of the EC in those controls who drank socially. Most AD is the sporadic type with no known familial genetic link. It is possible that some of the controls in this study may have early neuropathological changes indicative of
AD. Changes in the EC could be exaggerated due to current and/or past alcohol use in these subjects. It may be that the EC is differentially susceptible to the effects of alcohol and the changes seen here are alcohol related in nature.

Height was entered into the ANCOVA model to adjust for differences in body size (Andreasen et al., 1993). In all four models height contributed highly significantly to the model. Further analysis of this relationship revealed that height and sectional measurements of the HF and EC were inversely correlated suggesting that taller individuals had smaller structures. There are several explanations that may account for this finding.

Andreasen's group reported using height to adjust for individual differences in body size in a study that looked at volumes of specific brain structures (cerebrum, temporal lobes cerebellum, hippocampus, caudate, and lateral ventricles). It may be that height is an appropriate covariate when looking at larger structures and when using volumetric measurement of an entire structure. Height may be more closely correlated with total brain volume and less correlated with individual brain structure sectional measurements as used in this study.

Several studies using MR imaging have adjusted for individual differences in body size by using head circumference, a measure not available for all subjects in
this study. Head circumference may be more closely correlated with brain size as growth in brain size precedes and ultimately dictates head circumference. Anatomically, head circumference is more closely tied to brain size. Height may be a less close fit. In a disease process such as AD that involves progressive neuronal loss in several structures, use of height or head circumference to adjust for variations in body size is probably more valid then the use of brain structure measurements such as ventricle-brain ratio or total gray-white matter that rely on proportions or ratios which could be affected by the cell loss in AD (Arndt et al., 1991).

It may be that growth of individual brain structures like the HF and EC is mediated more by the development of neuronal connections and synaptic input very early in neurodevelopment than by the overall height of the structure in which they are housed. There may be a narrow range of size for these phylogenetically older, smaller structures that does not vary as much as height within individuals and would tend to make tall individuals appear to have smaller structures. Newer structures such as the neocortex may vary more based on overall brain size. Finally, it may be that the association with height is a spurious effect.

The association of alcohol use with brain structure size differed according to group. Significant findings occurred in the model for L-EC (p-value 0.0014) and R-EC (p-
value 0.03). The association was not significant for either L-HF or R-HF. Plots of the interaction effects revealed that the association of brain structure size with alcohol use differed in the control group but not in the at-risk group. As discussed above, these results may be reflective of other processes. It is possible that control subjects were heavier drinkers than the at-risk subjects and are showing early signs of greater vulnerability of the EC to the toxic effects of alcohol. In conducting the SCID and Family History Interview with both groups of subjects in this study, it was this researcher's impression that at-risk subjects were more invested in giving accurate, complete answers to questions while some control subjects appeared to give shorter, more perfunctory replies. A high percentage of controls screened for this study were excluded for conditions including possible psychiatric diagnosis and history of or current heavy alcohol use. Some controls with heavy drinking histories may have survived the screening process. Further study will be necessary to establish if this interaction finding is stable.

Implications for Nursing

As basic research, this study provides a foundation from which to develop indicators for potentially useful interventions based on assessments by nurses as well as other health care professionals. Results from this study indicate the importance of screening for early detection of
brain changes indicative of AD as well as for alcohol use with its potential for neurotoxic effects. As health care providers, nurses come into frequent contact with at-risk populations and may be the first to notice signs of personality change, depression, and confusion, or to hear reports from the family about changes in a patient's functioning.

Currently the etiology of AD remains unknown and no treatment exists. Basic research provides a window into brain function with the potential to explain disorders such as AD. A second potential contribution of basic research is to provide the link between environmental factors and disease states.

Implications for Future Research

The results of this study suggest several directions for future research. Further study is needed to determine standards to be used to adjust for variation in body size when measuring individual brain structures. Height, head circumference, and total brain volume have been variously used with little data to support the application of these measurements as standards against which to adjust measurement of brain structures. It may be that some brain structures vary with head circumference while others vary with total brain volume. Or it may be that no correlation exists between the volume or surface area of some brain structures and any overall brain or body size measurement.
Currently there exists only a beginning understanding of the interaction between environmental factors and the development of AD. One such factor, alcohol use, was explored in this study. Further study is needed using subjects who represent a range of alcohol use from abstinence to chronic alcoholism to determine if and how alcohol use contributes to the brain changes in AD. The study needs to include individuals at-risk for AD with a wider age range then this study used. It may be that those who drink more heavily develop AD sooner, or could have a more rapidly progressing dementia, or may in fact be protected from the ravages of AD.

The data in this study suggesting that control subjects categorized as social drinkers had smaller right and left entorhinal cortex sectional measurements when compared to subjects at-risk for AD indicates a need for further study of the neurotoxic effects of alcohol on selected brain structures. The condition referred to as Alcohol Dementia may represent several different processes within the brain or it may be characterized by a single, predictable pattern of cell damage. The effects of different levels of alcohol intake on the brain may vary based on factors such as age, nutrition, genetic make-up, as well as other physical conditions including vascular disease. All these questions warrant further study.
MR imaging offers a unique opportunity to visualize and measure the human brain in vivo. Studies of individuals at-risk for AD as well as those suspected to be in the early stages of AD need to be imaged with more advanced MR protocols such as the one developed by the Squire group. These new protocols with their enhanced imaging capacity and use of neck hyperextension to better capture the hippocampus in its entirety allow both volume and surface area measurements of whole structures. Given the cytoarchitectural pattern of cell loss in AD, the capacity to image complete structures is crucial to early identification of cell loss. Early identification of AD pathology enhances the possibility that pharmacological agents currently being developed and tested will be effective in halting cell loss.

Finally, recent reports that identified a gene for cholesterol in 42 families with familial AD through blood testing points up the ethical dilemmas in AD research. It appears it may soon be possible to identify those who will develop AD though no treatment for AD yet exists. Understanding the effects of factors like alcohol on the brain and the possible interaction effects of alcohol and a dementia such as AD may provide information that can guide life-style choices for those faced with the eventual prospect of developing AD. It may be possible to recommend alterations in eating, drinking, and exercise that reduce
the risk of developing AD or improve the quality of life of those who eventually develop AD.
References


SCID-NP (Version 1.0)

E. "Psychoactive Substance Use Disorders"

ALCOHOL DEPENDENCE OR ABUSE (LIFETIME)

What are your drinking habits like? (How much do you drink?)

Was there ever a period in your life when you drank too much? (Has alcohol ever caused problems for you?)

IF YES: What problems did it cause?

Has anyone ever objected to your drinking?

IF YES: Why?

IF NO SUGGESTION THAT EVER DRANK ALCOHOL EXCESSIVELY OR HAD ALCOHOL-RELATED PROBLEMS, CHECK HERE _______ AND SKIP TO *Non-Alcohol PSUD,* E.6.

IF HAS ACKNOWLEDGED HAVING PROBLEMS: When in your life were you having the most problems because of your drinking? (How long did that period last?)

IF HAS NOT ACKNOWLEDGED HAVING PROBLEMS BUT DRANK EXCESSIVELY: When in your life were you drinking the most? (How long did that period last?)

Now I am going to ask you several questions about that time.

How often were you drinking (then)? What were you drinking? How much?

ALCOHOL DEPENDENCE CRITERIA

A. At least three of the following:

Did you often find that when you started drinking you ended up drinking much more than you were planning to?

IF NO: What about drinking for a much longer period of time than you were planning to?

Did you try to cut down or stop drinking alcohol?

IF YES: Did you ever actually stop drinking altogether?

(How many times did you try to cut down or stop altogether?)

<table>
<thead>
<tr>
<th>A.</th>
<th>? 1 2 3</th>
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<tbody>
<tr>
<td>1</td>
<td>Alcohol often taken in larger amounts OR over a longer period than the person intended</td>
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<tr>
<td>2</td>
<td>Persistent desire OR one or more unsuccessful efforts to cut down or control alcohol use</td>
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? = inadequate information 1 = absent or false 2 = subthreshold 3 = threshold or true