SELECTIVE NEURAL AND COGNITIVE ALTERATIONS IN THE PREFRONTAL CORTEX WITH HEALTHY AGING AND ALZHEIMER'S DISEASE

A Dissertation

by

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Presented to the Department of Behavioral Neuroscience
and the Oregon Health Sciences University School of Medicine
in partial fulfillment of the requirements for the degree of Doctor of Philosophy

March 2000

School of Medicine

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GENERAL INTRODUCTION

ACKNOWLEDGEMENTS

I gratefully acknowledge the support of my mentor Dr. Jeri Janowsky in the development of this Dissertation.

I would like to thank Dr. Jeffrey Kaye for his guidance on these projects as well as the rest of my Dissertation Committee, Drs. John Crabbe, Barry Oken, and Martha Neuringer. I would also like to thank the research staff of the Oregon Brain Aging Study and the Oregon Alzheimer's Disease Center for assistance with these projects. Finally, I am thankful to the National Institute of Mental Health for support of these projects.

ABSTRACT

The prefrontal cortex (PFC) is vulnerable to age-related degeneration in both structure and function. The tissue and regional specificity of this degeneration is unclear. The Dissertation presented examined the specificity of PFC degeneration with aging, how this degeneration differs from degeneration in the PFC with Alzheimer's disease, and how the volume of specific structures in the PFC relates to performance on tasks of PFC function. The results of the three Experiments (five studies total) showed that there was a greater loss of PFC white matter compared to gray matter with healthy aging. There was a marginal loss of gray matter volume but orbital PFC volume was preserved relative to other PFC subregions. In Alzheimer's disease, there was a significant loss of PFC gray matter that was greatest in the inferior PFC subregion. Normal older subjects performed worse than younger subjects on numerous measures of PFC cognitive function, yet measures of working memory were particularly affected with age. PFC subregion volume was related to performance on cognitive tasks supported by those PFC subregions. Orbital PFC volume predicted working memory performance and changes in this region could be related to cognitive decline with healthy aging. Potential neural, physiological, and cognitive mechanisms of age-related cognitive decline are discussed.

1. INTRODUCTION

1.1 Specific aims. The goal of the research presented was to perform a detailed analysis of age-related changes in the prefrontal cortex (PFC) in both structural and functional domains. Specifically, the goals of the three experiments (five studies total) were to determine: *(a)* if degeneration in the PFC is greater in white matter compared to gray matter in the PFC with healthy aging; *(b)* if degeneration is greater in dorsolateral PFC subregions (middle and inferior gray matter) compared to gray matter of other prefrontal subregions with healthy aging; *(c)* if degeneration is greater in basal PFC subregions (inferior and orbital gray matter) compared to gray matter of other prefrontal subregions with Alzheimer's disease (AD); and *(d)* if attenuation of performance is greater on tasks of working memory compared to tasks of other prefrontally supported cognitive functions with aging. Volumetric measurements of prefrontal subregions were subsequently used to determine how regional volumes were related to cognitive function.

1.2 Summary of experiments presented. In Experiment 1, total, gray, and white matter tissue volumes were measured from the PFC in younger healthy elderly, older healthy elderly ('oldest old'), and subjects with AD. Experiment 2 examined subregional PFC changes in gray matter in similar yet larger groups of subjects. Experiment 3 examined the functional consequences of PFC degeneration by testing older and younger subjects on a battery of tasks

supported by subregions within the PFC and related subregional volumes to cognitive performance.

This cognitive and morphological analysis of the PFC contributes new knowledge of the primary degenerative features in healthy aging and AD. It also further guides research in the development of diagnostic procedures for very early or preclinical dementia and in the development of therapeutic interventions to prevent cognitive decline and neural degeneration with healthy aging and AD.

1.3 A note on study design. The three experiments presented used cross-sectional designs for data collection. Cross-sectional designs infer that differences in a measure among different groups represents a dynamic change with the progression across time from one group to another. For example, comparing a group of younger subjects to a group of older subjects assumes that any differences found between groups would no longer be apparent when the younger subject group reaches the age of the older subject group. This is not always necessarily true as cohort effects (effects specific to a certain group yet independent of the classification variable) can influence the data. For example, differences brain volumes between younger and older subjects could be due to different developmental dietary habits in the older subjects compared to younger subjects and not due to degeneration with age, the true factor of interest. Similarly, lack of differences could be due to 'selective mortality', the fact that the older group of subjects represents a group that is biologically

superior compared to the general population. Thus, this limitation should be considered when reviewing the studies presented. Longitudinal studies of aging (studies examining changes in the same subjects across time) will be useful in further examining the findings presented here.

1.4 Summary of background presented. The following sections provide a brief background for the studies presented. First, the anatomy and function of the PFC is discussed along with a brief discussion of the method used for imaging the PFC in the living human being. The second section discusses the phenomenon of aging including the significance of studying the aging population in the United States and a review of age-related degeneration of the brain and in cognitive function with special emphasis on changes related to the PFC.

2. THE PREFRONTAL CORTEX

2.1 Introduction to the prefrontal cortex. The PFC encompasses the anterior portion of the frontal lobe, lying just anterior to the premotor cortex and is separated from the rest of the frontal lobe by the precentral sulcus in primates (Figure 1; Kaufer and Lewis, 1999). This region is greatly expanded in the primate and especially hominid lineage (Rilling and Insel, 1999) and is quite distinct from the remainder of the frontal lobe. Hence, some have suggested that the PFC supports those cognitive processes that are uniquely human [or, uniquely primate for that matter; (Banyas, 1999)]. This great expansion has occurred fairly recently in evolutionary history along with expansions of the temporal and parietal lobes (Banyas C.A., 1999). The PFC is quite heterogeneous in both structure (Kaufer and Lewis, 1999) and function (Petrides, 1995; Fuster, 1997). The experiments presented used this heterogeneity to 'dissect' the PFC both structurally and functionally in the living human being.

[Figure 1 about here]

2.2 Dissecting the PFC structurally in vivo. The PFC is heterogeneous in cytoarchitecture, anatomical connections with posterior cortical structures, and function. The PFC is made up of approximately nine distinct cytoarchitectonic regions in modern interpretations of the classical Brodmann map (Figure 1;

Brodmann K., 1994/1909; Kaufer and Lewis, 1999). The lobe is typically divided into two main components, the 'dorsolateral' and 'orbital' regions which are often used to examine PFC structure and function. In this anatomical scheme, the dorsolateral region encompasses all of the lateral PFC from the superior frontal gyrus to the orbitofrontal gyrus, and the orbital region extends ventromedially to the interhemispheric fissure excluding the anterior cingulate gyrus (which is typically considered paralimbic; Figure 1a). Cortically, dorsolateral regions share information with inferior temporal and parietal visual association areas [(Kaufer and Lewis, 1999); the so called 'dorsal' and 'ventral' visual association streams (Ungerleider and Haxby, 1994)]. Subcortically, dorsolateral regions project to the dorsal thalamus, the pars compacta of the substantia nigra, and the dorsal raphe in the brainstem. Basal and orbitomedial regions have reciprocal cortical connections with limbic regions such as the hippocampal formation (Barbas and Blatt, 1995; Figure 1b) and amygdala (Kaufer and Lewis, 1999). Orbitofrontal regions project to caudate, globus pallidus, and thalamus subcortically with more ventral striatal projections from the more medial orbitofrontal regions (Chow and Cummings, 1999; See figure 1b for a description of PFC connections with medial temporal lobe structures).

There is currently no way to delineate cytoarchitectonically distinct brain regions in vivo. A combination of metabolic, neurochemical, and structural techniques would likely further development of *in vivo* cytoarchitectonic analysis, yet few if any studies like this exist. However, a much simpler, purely structural method has been used for decades in research to describe the

location of damage to the brain using Brodmann's map and probable relationships between gyri and cytoarchitectonic regions. For example, Brodmann's map shows that the majority of region 8 lies on the superior frontal gyrus, the majority of region 46 lies on the middle frontal gyrus, the majority of regions 45 and 47 lie on the inferior frontal gyrus, and the majority of regions 11 and 12 lie on the orbitofrontal gyrus. Thus, obtaining differential measurements of each of the frontal gyri could provide indirect information about degeneration of a particular cytoarchitectonic region.

Cytoarchitectonic boundaries are signals of functional subregions in the brain. Thus, degeneration of a particular cytoarchitectonic region would be expected to result in the attenuation of cognitive functions associated with that region and knowledge of patterns of degeneration would be useful for generating hypotheses about the cognitive consequences of such degeneration. Also, data from structural measurements using classic cytoarchitectonic regions could be related to prior anatomical and functional studies using similar procedures. Thus, knowledge of the probable gyral site of cytoarchitectonic regions would permit morphological analysis of the brain based on a surrogate measure of cytoarchitecture which would be useful for studying the aging brain.

2.3 Use of magnetic resonance imaging to examine the human brain in vivo. Current techniques using magnetic resonance imaging (MRI) allow for the study of the structure (volumetric MRI), function (functional MRI), and chemistry (MRI

spectroscopy) of the human brain in vivo. All of these techniques take advantage of differences in tissue properties at the biochemical and molecular levels to obtain images with different intensity for the tissues of interest. More specifically, MRI uses differences in the amount of time that it takes different constituent atoms to align with a magnetic field after an induced disruption (a radio frequency pulse that changes the alignment of a large number of atoms). This difference in 'relaxation time' between tissues is used to produce a signal that is then represented as image intensity. Differences in tissue intensity are greatest at different times after the disruption of the atoms depending on the properties of the particular tissues. For example, the experiments presented used two different types of images, one that differentiates gray matter from cerebrospinal fluid (the T2 weighted image), and another that differentiates gray matter from white matter (the proton density weighted image). Gray matter can be distinguished from cerebrospinal fluid because its atoms align with a magnetic field relatively quickly after the radio frequency pulse compared to cerebrospinal fluid. Thus, sampling the MR signal when gray matter and cerebrospinal fluid are at their greatest difference in alignment with the magnetic field would give the greatest image contrast between the tissue types. This sampling is between the time when there is no alignment in both tissue types due to the radio frequency pulse and before saturation, when both gray matter and cerebrospinal fluid are fully aligned with the magnetic field. Differentiation between gray matter and white matter is obtained in a similar manner (Sanders, 1995; Cohen, 1996). The studies presented here used the

procedures described above to obtain 'T2' weighted images to contrast brain matter and cerebrospinal fluid and 'proton density' weighted images to contrast gray matter and white matter.

2.4 Dissection of the PFC functionally. Another way of dissecting the PFC to examine age-related decline is functionally. Data from functional imaging and human and animal lesion studies demonstrate the subregional nature of cognitive processing within the PFC. Different tasks are supported by different regions, and these functional regions are potentially confined to cytoarchitectonic boundaries. Thus, attenuation of specific functions with aging could be related to dysfunction of the specific subregion supporting that function. Experiment 3 examined tasks supported by different PFC subregions to determine if there was a decline in PFC function that was selective to certain cognitive processes with aging or if there was a general decline in PFC function affecting numerous PFC cognitive processes.

Working memory, the process of holding information in mind for a brief period of time to perform a task, has been studied in detail with regard to PFC function. A number of studies have been performed showing the importance of dorsolateral PFC to performance of working memory tasks (in particular, Brodmann areas 9/46 (Petrides, 1995; Braver et al., 1997; Cohen et al., 1997). The neural and cognitive specificity of the PFC contribution to WM has also been examined. Deficits in performance of WM tasks are often seen with lesions to prefrontal areas as compared to other areas of the brain. For example,

subjects with lesions of the frontal lobe had greater deficits in WM performance than subjects with lesions of the temporal lobe in a study of patients with surgical transection due to intractable epilepsy (Petrides and Milner, 1982). Unilateral left PFC lesions caused the most severe WM impairments and patients with lesions of the temporal lobe were not impaired unless extensive lesions of the hippocampus were evident (Petrides and Milner, 1982). In monkeys, lesions of the dorsolateral PFC (Brodmann 9 and 9/46) cause deficits in the performance of WM tasks, while lesioning the directly adjacent periarcuate region leaves performance of this same WM task unimpaired (Petrides, 1995). Specific cognitive components of WM tasks have also been examined. Functional imaging studies show that dorsolateral PFC regions contribute to mental rehearsal, monitoring, and manipulation of online information (Petrides, 1991b; Braver et al., 1997; Cohen et al., 1997). Cell electrophysiological studies in macaque monkeys support localization of WM components to the dorsolateral PFC and have described populations of neurons that selectively increase firing when stimuli to be kept in mind are removed from view [the WM delay; (Goldman-Rakic, 1995; Fuster, 1997; Goldman-Rakic, 1999)]. Cells have also been identified that differentially respond in working memory for object versus location tasks (Rao et al., 1997).

Imaging studies have also been useful in determining the domain specificity of WM processing. For example, studies of spatial versus object WM [right mid-dorsolateral versus bilateral mid-dorsolateral and left inferior frontal, respectively; (Belger et al., 1998)] and mnemonic versus executive processing

in WM [left perisylvian versus dorsolateral, respectively PFC; (Postle and D'Esposito, 1999)] have been performed. Also, mnemonic processes have been dissociated from perceptual processes in WM [prefrontal versus visual regions, respectively; (Belger et al., 1998)], and WM has been dissociated from decision making [dorsolateral PFC versus orbital/ventromedial PFC respectively; (Bechara et al., 1998)] and task difficulty [dorsolateral PFC versus other regions in the PFC; (Barch et al., 1997)]. These studies have all supported the importance of particular regions in the PFC in specific aspects of WM task performance. Most recently, the dorsolateral PFC has been shown to activate to a greater degree when distractor stimuli are presented during working memory task performance (Jiang et al., 2000) or inhibitory processes are necessary to perform the task (D'Esposito et al., 1999). Experiment 3 examined age-related changes in working memory to determine if working memory is more affected by aging other tasks of PFC function.

Other cognitive processes have been localized to regions of the PFC that are not typically associated with working memory. For example, tasks of conditional association learning depend on superior and posterior PFC regions (Petrides, 1985a; Petrides, 1985b; Petrides et al., 1995) while response alternation tasks depend on orbital and ventromedial PFC regions (Mishkin and Manning, 1978; Freedman et al., 1998). Differential degeneration of subregions within the PFC could be manifested in a selective decline in cognitive function. Consequently, subregionally supported tasks of PFC function were used to probe the integrity of those regions in Experiment 3.

3. AGING

3.1 Significance. Age-related neurodegeneration and subsequent cognitive decline is important to study from both a public health and a cognitive neuroscience perspective. Recent census estimates project a significant increase in the population of seniors with the aging of the 'baby boom' generation (Administration On Aging, 1999). By the year 2030, an expected 70 million older persons (≥65 years of age), representing 20% of the population, will live in the United States. Another segment of the population, the so-called 'oldest-old' (people ≥85 years of age), is also rapidly growing and it is estimated that this population will more than double to 8.5 million by 2030. Neurologic changes seem inevitable, even with optimal aging (Kaye et al., 1994), and these neurologic changes can lead to a loss of functional independence. Thus, understanding the neural basis of cognitive decline in this rapidly growing population is a national health-care interest. Similar to lesion models, healthy aging provides an understanding of how neural structures contribute to cognition by examining how degeneration of a particular structure relates to cognitive performance. The studies presented examined how morphologic changes in the brain relate to cognitive decline with aging.

3.2 Classifying Aging. Aging is a multifactorial and heterogeneous process. Thus there is uncertainty as to how to describe the process. In its simplest form, aging has traditionally referred to biological changes that accompany the

progression of chronological age as well as the separation of pathologic from normal change. Still, the process of 'normal' aging is extremely heterogeneous. More recent work has emphasized *preservation* of function and the heterogeneity of the aging process (Rowe and Kahn, 1987). These studies have defined a demographic of 'successful agers', older people with minimal or no physiologic loss compared to the average of a similar *younger* cohort. With this model of aging, 'age' itself is not a sufficient variable to explain decline in function as other age-extrinsic parameters likely contribute to a great degree.

This concept is illustrated well in a review of human, monkey, and rat studies by Rapp and Amaral (1992). Their meta-analysis showed that aging does not necessarily lead cognitive decline. More importantly, cognitive decline in older animals is related to alterations in the brain (Rapp and Amaral, 1992). Although not the average, successful aging provides a reference for examination of the heterogeneity of the aging process in addition to the previous focus on disease-related aging. The studies presented examined age-related changes in groups of subjects originally selected to be optimally healthy (See Subjects, Experiments 1,2,3). Thus, changes in the PFC or cognition related to medical comorbidities were expected to be minimal. Thus, it was expected that neural structures could be more reliably related to cognitive function.

In contrast to successful aging, a number of degenerative diseases accompany the aging process. Age-related diseases are characterized by profound changes in physiological function, beyond what is expected with

normal aging. It is not clear if certain disease-related processes are distinct from normal aging or are simply a more rapid progression of normal age-related change. Still, the rapid progression of physiologic and cognitive changes and the decline in function below a cohort of normal elderly is useful for distinguishing between disease and normal age-related change.

3.3 Alzheimer's disease. Age-related neurodegenerative disease contributes to a severe loss of independence in older people. AD is the most common form of age-related dementia and it is estimated that over 4,000,000 people in the United States currently have the disease (National Institute on Aging, National Institutes of Health, 1995). The percentage of people over 85 years of age with the disease has been estimated at up to 47% (National Institute on Aging, National Institutes of Health, 1995).

Cognitive manifestations early in the disease process include a profound decline in verbal and visual memory as measured by delayed recall tasks (Bondi et al., 1994). Cognitive deficits can be recognized in the preclinical stages of the disease (Howieson et al., 1997). Regions of the brain that support the formation of memories (e.g. medial temporal lobe structures such as the hippocampus and entorhinal cortex) show the earliest and most profound degeneration in the disease (Hyman and Gomez-Isla, 1994; Gomez-Isla et al., 1997). The pathology of AD includes a characteristic profile of amyloid plaque and neurofibrillary tangle deposition and granulovacuolar degeneration of neurons (Kemper, 1994). Although degenerative changes with AD are likely

different from those of physiologic aging, conclusive diagnosis of AD can only currently be obtained by histological examination of the brain. Still, longitudinal studies of brain aging suggest that there is not an increase in the rate of degeneration of the brain with increasing age in healthy subjects (Mueller et al., 1998). Thus, accelerated rates of degeneration in cognitively healthy subjects could potentially represent early disease-related change. *In vivo* techniques for differentiating between healthy aging and AD will be necessary for the administration of therapeutic medication early in the disease process. The present studies compared age-related degeneration in the brain to degeneration with AD to determine if there were differential patterns of degeneration that are informative for differentiating the pathophysiology of aging from AD and could potentially be useful for *premortem* diagnosis of the disease.

3.4 Aging and Alzheimer's related changes in gray and white matter. There is general agreement between *post-mortem* (Kemper, 1994) and *in vivo* structural imaging (Jernigan et al., 1991; Raz et al., 1997; Raz et al., 1998) studies that the brain degenerates, even with 'optimal' aging (Mueller et al., 1998). The degree and selectivity of this atrophy is of much greater debate (Kemper, 1994; Raz et al., 1997; Salat et al., 1999). The examination of age-related changes in gray and white matter is a first step in understanding the selectivity of this degeneration. Because these tissue types differ in ultrastructural and cellular components and have different molecular composition, pathological processes

could selectively target one tissue type. Gray matter is composed of cell bodies and this tissue is involved in regional processing of neural information. White matter is composed of the myelinated axonal projections among neural regions and is important in the integration of information from various neural regions. Although these two tissue types are interdependent, the functional consequences of degeneration would be expected to differ as there are differences in their functional properties. Thus, understanding the pattern of tissue degeneration could be useful in explaining functional decline.

Many studies of aging report a predilection for degeneration in one tissue type, yet these studies are not always consistent. Some studies show greater loss of gray matter than white matter whereas other studies demonstrate the reverse. For example, *post mortem* human and monkey studies suggest that white matter and myelin degenerate possibly to a greater degree than gray matter with aging (Kemper, 1994; Double et al., 1996). The loss of white matter can be particularly great in the frontal lobes (for review see Kemper, 1994). Degeneration of myelin fibers correlated well with cognitive performance in aged monkeys suggesting that white matter degeneration was at least partially responsible for age related attenuation in cognition (Peters et al., 1996). The present studies examined whether one tissue degenerates more than the other tissue in healthy aging or AD (Experiment 1).

Although age-related loss of white matter has been reported in *in vivo* magnetic resonance imaging (MRI) studies (Raz et al., 1997; Guttmann et al., 1998), other studies found only minor or no age-related loss of white matter

(Sullivan et al., 1995) and greater propensity for gray matter deterioration (Raz et al., 1997). It is clear that there is age-related thinning of gray matter (Esiri, 1994), at least in some regions of the brain [e.g. layer 1 of area 9/46 of the PFC; (Peters et al., 1994; Peters et al., 1998)]. This thinning is likely due to loss of synapses and dendritic spines and not neuronal death (Haug et al., 1984; Peters, 1993; Morrison and Hof, 1997; Peters et al., 1998). Differences between the post-mortem and structural imaging studies could have been due to a number of factors including the morphometric methods used. For example, older histological procedures biased studies towards finding significant cell death while newer unbiased stereological procedures correct for this. Similarly, past imaging studies could have been less accurate as early automated image segmentation procedures potentially misclassified tissue volumes due to imprecise discrimination and image intensity inhomogeneity. It is also possible that tissues degenerate differently in different regions of the brain and thus the region examined could contribute to these discrepancies.

Differential tissue degeneration could also occur at different ages and thus the subject sample examined is important to consider. Prior studies of aging have also included subjects with health conditions that could confound volumetric studies and it has been suggested that some even contain subjects in the preclinical stages of AD (see Mueller, 1998 for discussion). It is also possible that studies examining changes across the lifespan [e.g. 21-70 years of age (Sullivan et al., 1995); 18-77 years of age; (Raz et al., 1997)] could miss more profound changes that occur in late aging. This would be particularly true

if there were few subject in the later decades. Experiment 1 examined tissue volumes with late aging (Salat et al., 1999). Prior studies examining age-related change in the PFC had few subjects in the later decades (e.g. Raz et al., 1997) and differences between the findings of this study and prior studies could be due to the age-range examined as well as the other factors mentioned above. A tentative conclusion is that both tissues deteriorate, but differences in tissue susceptibility are still unknown. Thus, an analysis of tissue volumes within a structure that degenerates more than other structures with aging (e.g. the PFC, see below) could help determine if there is a greater degeneration of one tissue over another.

The pattern of prefrontal degeneration could be different in healthy aging compared to AD. In particular, although degradation of both gray and white matter is likely with AD, studies have noted the significant amount of neuronal death with AD (Kemper, 1994). Significant neuronal death does not occur with healthy aging (Peters, 1993; Peters et al., 1998). Experiment 1 compared gray and white matter with healthy, *late* aging (i.e. oldest old) and AD, to contrast changes in tissue volume related to these conditions.

3.5 Regional degeneration in the brain with aging and Alzheimer's disease. Lobar measurements provide the next level of analysis of brain aging. The greatest healthy age-related degeneration seems to occur in multimodal association cortices as opposed to primary sensory or motor cortex. Thus, the PFC, which is association cortex, could be substantially more vulnerable to

age-related degeneration (Kemper, 1994; Raz et al., 1997). Greater prefrontal degeneration has been discovered at both the neuronal (Esiri, 1994; Liu et al., 1996) and MRI volumetric levels (Coffey et al., 1992; Cowell et al., 1994; Murphy et al., 1996; Raz et al., 1997) compared to other neural regions. For example, there are greater differences in PFC volume between old and young subjects (Coffey et al., 1992; Cowell et al., 1994; Murphy et al., 1996) and the volume of the PFC is more closely related to age (Raz et al., 1997) compared to the temporal lobe and other brain regions. Greater age-related change in the frontal lobe compared to other regions of the brain have also been found for cerebral metabolism and blood flow (Moeller et al., 1996; Petit-Taboue et al., 1998). Neurotransmitter systems in the frontal lobe also change with age (e.g. D1 receptors, de Keyser et al., 1990; alpha-2 adernoceptors, Pascual et al., 1991; muscarinic receptors, Lee et al., 1996). Thus, the PFC is a hallmark region for the examination of age-related neurodegeneration.

In contrast to healthy aging, the earliest and most severe degeneration with AD occurs in medial temporal lobe regions such as the hippocampus and entorhinal cortex (Gomez-Isla et al., 1996; Esiri, 1994; Kemper, 1994). Degeneration of the frontal lobe is also apparent with AD at both the histological (Masliah et al., 1993) and structural imaging level (Laakso et al., 1995; Fama et al., 1997; Pantel et al., 1997; Pantel et al., 1998), and synapse loss in the prefrontal cortex is a major correlate of cognitive deficiency in AD (Masliah et al., 1993). Experiment 1 examined tissue volumes in the PFC with healthy aging and AD to determine if there is differential tissue loss in these conditions.

3.6 Degeneration within the PFC with aging. Although many *in vivo* neuroimaging studies have examined age and AD-related degeneration of the hippocampus (Geinisman et al., 1995; Lehtovirta et al., 1995; Kaye et al., 1997), much less attention has been paid to cortical subregions of the brain. Whether subregions within the PFC are differentially vulnerable to degeneration with aging or AD is not known. Few studies of aging, if any, have attempted to parcel the PFC via structural imaging based on anatomical or functional criteria. Neuropathologic studies suggest that this could be a fruitful line of exploration. For example, age-related degeneration is more robust in the pyramidal neurons of layer V of the middle frontal gyrus than pyramidal neurons of layer IIIc (de Brabander et al., 1998). This and similar findings shows that cell layers *within* a cortical region are differentially affected by aging. Thus, degeneration can follow cytoarchitectonic boundaries. It is possible that this cytoarchitectonic specificity is not limited to cortical layers but is regional. Similarly, neuropathological changes with AD are regionally distributed with greater and earlier degeneration in limbic regions compared to primary sensory regions (For review see Kemper, 1994). Thus, an examination of the PFC based on cytoarchitectonic regions would help to determine if degeneration within the PFC is selective to certain regions. One MRI volumetric study divided the PFC into a 'dorsolateral' region and an 'orbitofrontal' region and found that age-related degeneration was greater in the dorsolateral region compared to a number of other regions of the brain (Raz et al., 1997). The volume of the

dorsolateral region was related to cognitive function (Raz et al., 1998) suggesting that degeneration of the region with age could be responsible for cognitive decline. However, the dorsolateral region examined combined a number of functionally and anatomically distinct regions into one measurement; thus, more specific regional changes were not examined.

Brodmann's classic 1909 map of the cellular subregions of the brain can be used to determine PFC subregions. Brodmann developed his map by Golgi staining the entire brain and delineating cortical regions by differences in neuronal densities (Brodmann K., 1994/1909). Support for the existence of these cytoarchitectonic regions in both human and nonhuman primates and their functional significance was elucidated in numerous subsequent anatomical and behavioral studies [(Vogt and Vogt, 1919; Von Bonin and Bailey, 1947; Petrides and Pandya, 1999) and others]. Experiment 2 examined age and AD related changes in specific regions of the PFC *in vivo* using volumetric MRI procedures. Although this method was not histological and thus did not truly follow cytoarchitectonic boundaries, cellular regions were approximated from prior histological studies.

A detailed analysis of prefrontal regions based on probable cytoarchitectonic and functional areas could also be especially useful in differentiating healthy aging from AD. For example, studies of neurofibrillary tangle deposition in AD brains suggest that regions with anatomical connectivity to primary degenerating structures exhibit greater degeneration compared to unconnected regions. Studies show that secondary amyloid deposition seems

to occur in structures connected to the primary degenerating structures such as hippocampal regions and the amygdala (Mann and Esiri, 1989; Esiri et al., 1990; Braak and Braak, 1991). In the frontal lobe, this is exemplified by a considerable amount of neurofibrillary pathology in inferior frontal regions (Braak and Braak, 1991), areas connected to medial temporal lobe structures (Barbas, 1992; Barbas, 1993; Barbas, 1995). Thus, although dorsolateral PFC may degenerate to a greater degree with healthy aging (Raz et al., 1997) inferior and orbital regions of the PFC could degenerate in AD due to retrograde or anterograde degeneration from anatomical connections with the degenerating medial temporal lobe. Thus, a detailed analysis of degeneration of the PFC in healthy aging and AD using volumetric data from regions based on probable cytoarchitectonic regions was performed in Experiment 2. This Experiment sought to determine if degeneration is greater in specific subregions of the PFC and if subregional changes in the PFC are different in aging compared to AD.

3.7 Cognitive changes with aging. Although cognitive decline is common in healthy aging (Kaye et al., 1994), this decline can be a distressing and continuous event throughout the later decades of life. Worse yet, decline in cognitive function could be the harbinger of the early stage of an age-related neurological disease such as AD (Howieson et al., 1997). It is necessary to understand the quality of cognitive decline in order to predict who will develop neurodegenerative disorders versus those who will remain cognitively intact

and to develop therapeutic strategies to prevent such deterioration.

Age-related cognitive decline is not a uniform process, affecting all domains of cognition equally, but a more specific process that affects certain domains to a greater degree (Albert and Moss, 1996). Memory has been examined in most detail in studies of aging. Memory is a cognitive function with neurally separable subdomains. There are profound changes in declarative memory, conscious memory for facts and events as demonstrated in tasks of delayed recall, in the early stages of AD. More mild yet significant changes in declarative memory has also been shown in healthy older people (Albert and Moss, 1996). Healthy older subjects are much less impaired on tasks of implicit memory (Verhaeghen and Marcoen, 1993), nonconscious memory processes including habituation, conditioning, and repetition priming. Understanding whether specific functions are at the root of the more generalized cognitive decline manifested is a central theme in the cognitive neuroscience of aging.

Recent studies have attempted to explain much of cognitive aging in terms of a decline in prefrontal function and particularly working memory capabilities (Daigneault and Braun, 1993; Salthouse, 1993; West et al., 1998), cognitive slowing (Salthouse, 1992; Salthouse, 1994; Salthouse, 1996), and defective inhibitory processes (Hasher et al., 1991; Hasher et al., 1997; Hasher et al., 1999). For example, much of the variance in working memory performance can be explained by potentially more simple measures such as inhibition and processing speed (Salthouse and Meinz, 1995).

Working memory and behavioral inhibition are both supported by

prefrontal regions (Petrides and Milner, 1982; Petrides, 1991a; Petrides, 1995; Braver et al., 1997; Cohen et al., 1997; D'Esposito et al., 1999) and decline in these cognitive domains coupled with the neural changes described in the previous section support the idea that cognitive aging is greater for processes supported by the PFC. Cognitive processing speed on the other hand, could be adversely affected by white matter damage as has been previously demonstrated (Schmidt et al., 1993). Thus, an examination of performance on tasks of cognitive processes supported the PFC would provide information on how prefrontal function is affected by aging. Further, the use of tasks supported by different subregions within the PFC would provide additional support to structural data implicating greater degeneration of certain subregions compared to others within the PFC. Experiment 3 examined performance on cognitive tasks that are supported by the PFC to ascertain whether there is a selective decline in certain PFC cognitive functions with aging or if PFC cognitive decline is a more general process affecting all domains of PFC cognition equally.

3.8 Relationship between structural and functional changes with aging. One way to study the relationship between brain regions and cognitive function is by using *in vivo* imaging techniques to obtain a structural measurement of the brain in close temporal proximity to cognitive testing, permitting an analysis of the relationship between structure and function. AD pathological changes in medial temporal lobe structures such as the hippocampus have been related to memory function using this method (Kohler et al., 1998). Whether or not such

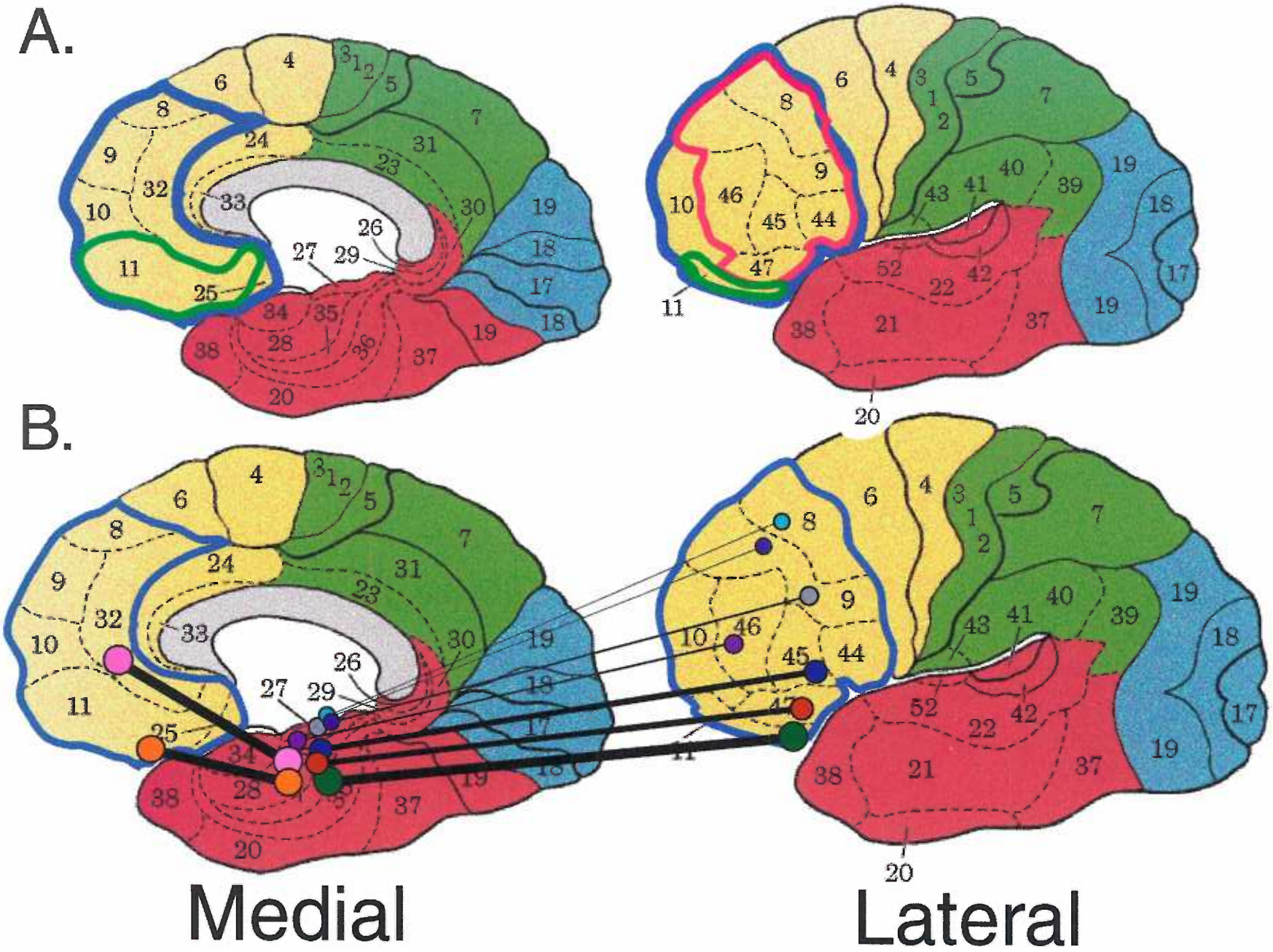
relationships can be made with the more subtle degenerative changes of healthy aging is not known and is the subject of Experiment 3 presented here. Structural and functional measurements have been related in healthy subjects (Almkvist et al., 1992; Raz et al., 1998). For example, studies have found a relationship between the volume of the hippocampus and memory in subjects with age associated memory impairment (Soininen et al., 1994) and in healthy older subjects (Golomb et al., 1994a; Golomb et al., 1994b; Golomb et al., 1996). Also, changes in white matter (white matter abnormalities) have been related to declines in cognition (Boone et al., 1992; DeCarli et al., 1995) and electrophysiological (Oken and Kaye, 1992) function in older subjects.

Few studies have examined the relationship between prefrontal structure and cognition with healthy aging. A single study found that measures of perseveration were related to the volume of the PFC (Raz et al., 1998). There were few significant cognitive correlates of regional volumes in this study. Although the authors divided the PFC into two anatomical domains (dorsolateral and orbital), functional regions within the PFC are more circumscribed and thus relationships between performance and volume may have been masked in this study. An examination of the relationship between anatomically distinct regions of the PFC and cognitive processes supported by those subregions would be useful in understanding both the selectivity of degeneration and the relationship between degeneration and cognitive decline. Experiment 3 examined the relationship between the volume of subregions within the PFC and performance on cognitive tasks supported by those specific

subregions to determine how changes in subregions of the PFC are related to cognitive decline.

3.9 Rationale for the studies proposed. Converging evidence from structural, metabolic, and neuropathologic studies point to the PFC as a particularly vulnerable neural region for age-related deterioration. A literature using functional imaging techniques such as positron emission tomography and functional magnetic resonance imaging is building in support of these findings (Grady et al., 1995; Cabeza et al., 1997; Grady et al., 1998), yet these studies are still in the preliminary stages as it is not clear that standard analysis procedures are useful in analyzing brains that differ in cortical morphology. Thus, a detailed analysis of alterations in the structure and function of the PFC would be useful for potentially understanding the most profound alterations in brain morphology and cognition with aging and how changes with aging differ from those with Alzheimer's disease.

FIGURE 1 Anatomy of the PFC. The medial and lateral surfaces of the cerebral cortex with cortical lobes highlighted in color. A. The prefrontal cortex is the anterior portion of the frontal lobe (yellow lobe) and the prefrontal portion is highlighted by a blue tracing. Dorsolateral PFC regions are highlighted with a red tracing and orbital PFC regions are highlighted by a green tracing. B. Orbital, medial, and inferior PFC regions have stronger connections with the medial temporal lobe (red lobe) than more dorsal regions (see text for details).


A.
B.
Medial
Lateral

4. EXPERIMENT #1

Prefrontal Gray and White Matter Volumes in Healthy Aging and Alzheimer's Disease

Published in Archives of Neurology (1999) 56:338-344.

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Supported by National Institutes of Health grants AG12611 (JSJ), AG08017 (JAK) and a Department of Veterans Affairs Merit Review Grant (JAK).

Acknowledgments:

The authors gratefully acknowledge Tamara Karnos and Milar Moore for assistance with data retrieval and image and technical matters.

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4.1 ABSTRACT

Objective: To quantify the contribution of gray and white matter volume to total prefrontal volume in healthy aging and to determine if prefrontal tissue volumes distinguish healthy aging from Alzheimer's Disease (AD).

Design: Volumes of total prefrontal cortex, prefrontal gray matter, and prefrontal white matter were compared among young healthy elderly (YHE; n=14, mean age 70 years), old healthy elderly (OHE; n=14, mean age 90 years), and AD (n=14, mean age 70 years) by analysis of variance. Additionally, Pearson's correlations were performed between volumes and age.

Results: OHE and AD had significantly less total prefrontal volume (approximately 15% less in both groups) and prefrontal white matter volume (approximately 30% less and 20% less in the OHE and AD groups respectively) than YHE, but there were no differences between the OHE and AD groups. There was a significant difference in gray to white matter volume ratio with OHE having a higher ratio than YHE. AD patients did not differ from YHE or OHE in this ratio. There were significant negative correlations between age and total prefrontal volume and age and prefrontal white matter volume in the healthy subjects.

Conclusions: In the very old, the decline of white matter volume is disproportionately greater than the decline of gray matter volume. In AD both gray and white matter loss contribute to the decline of prefrontal volume. This is demonstrated by the gray to white matter ratio which does not differ between

YHE and AD. Thus, it is likely that AD is different from accelerated aging.

4.2 Introduction

Studies of frontal lobe cognition and morphology suggest that the frontal lobe, and specifically the prefrontal cortex, may be disproportionately sensitive to changes with aging compared to other areas of brain [1,2,3,4]. The prefrontal cortex is involved in mediating certain cognitive processes including metamemory [5], source memory [6], working memory [7,8], behavioral inhibition [9], and the nonconscious biases that guide decision making [10]. These and other frontal lobe cognitive functions are often the first to decline in early senescence [2,3].

Cognitive changes with aging may be related to age-related decreases in volume of the frontal lobe demonstrated in studies measuring volume *in vivo* through magnetic resonance imaging (MRI) [1,4,11,12]. Age-related decline in regional brain volume is an indirect measure of atrophic degeneration. Many areas of the brain, such as the hippocampus [13], temporal lobe [1,12], and corpus callosum [14,15] show age-related declines in volume. It is of note that the frontal lobe shows a greater age-related degeneration than the temporal lobe in MRI studies of both structures [1,4,11]. Thus, disproportionate degeneration of the frontal lobe could be responsible for the cognitive changes observed with aging.

Within the prefrontal cortex white matter as compared to cortical gray matter may be particularly susceptible to age-related degeneration and

contribute to cognitive decline. In humans, myelin lipid concentrations in the brain begin to decrease from as early as 20 years of age and show a progressive decrease to 100 years of age examined post-mortem [16]. Similarly, the aged rhesus monkey exhibits a breakdown in the integrity of myelin around axons which correlates with cognitive decline [17]. Various mechanisms of white matter damage, including strokes [18] and oxidative stress [19,20,21], could cumulatively contribute to the atrophic loss of white matter volume with healthy aging. Thus, age-related prefrontal white matter degeneration may make a significant contribution to cognitive and behavioral changes in healthy aging [22].

In contrast to healthy aging, a combination of both white and gray matter degeneration may contribute significantly to Alzheimer's disease (AD). Studies of brain degeneration in AD have demonstrated pathology in cortical gray matter [23]. Other studies have additionally demonstrated a relationship between white matter degeneration and clinical measures in AD. For example, abnormal white matter volume is related to poor cognitive performance independent of cortical gray matter volume in AD [24]. Thus, alteration of both gray and white matter in AD may lead to cognitive dysfunction.

Using quantitative MRI, we examined gray and white matter volume differences in young healthy elderly (YHE; mean age 70 years), old healthy elderly (OHE; mean age 90 years), and a group of AD patients (mean age 70 years) age matched to YHE. This study is in contrast to most MRI studies of frontal lobe volume which have looked only at the total brain volume of the

region [1,11,12] or MRI white matter hyperintensities (a measure of putative white matter lesions most commonly attributed to ischemia [25]). Instead, we measured separately total gray and white matter volume in the prefrontal cortex in addition to total prefrontal volume. Finally, previous studies of frontal lobe volume have not examined significant numbers of healthy subjects in the 'oldest old' age-range (subjects ≥85 years of age)[26]. Thus, this study provides new information on changes in prefrontal tissue volume in healthy oldest old and AD.

4.3 Subjects and Methods

Subjects. Three groups of subjects were studied, young healthy elderly (YHE; 65-76 years), old healthy elderly (OHE; ≥85 years), and patients with Alzheimer's disease (AD; 61-75 years; see table 1 for subject characteristics). MR images from OHE and YHE subjects (n = 14 in each group) were examined as part of a longitudinal study of brain aging and cognition (Oregon Brain Aging Study; OBAS [27,28]) at the Oregon Health Sciences University (OHSU) and Veterans Affairs Medical Center in Portland Oregon. OHE subjects were 85 years of age or older. AD subjects (n=14) were studied as part of a National Institute on Aging Alzheimer's Disease Center (ADC) clinical protocol at OHSU. AD subjects were age matched to the YHE group. All groups were matched for education and there was an equal number of men and women within and among groups (7 male, 7 female). Detailed descriptions of the recruitment procedures and inclusionary and exclusionary criteria on the OBAS and ADC subjects, as well as extensive medical and cognitive data on all subjects has been published elsewhere [13,27,28]. Briefly, the OHE and YHE subjects were functionally independent, had English as their principal language, had not sought evaluation for cognitive impairment, scored well on a variety of clinical tests including the Instrumental Activities of Daily Living [29], the Mini-Mental State Examination [30], the Cornell Depression Scale [31], the Geriatric Depression Scale [32] and the Clinical Dementia Rating Scale [33]. They did not have significant medical disorders and did not use medicines that affect cognitive

function. No subjects had cerebral infarctions. Only minor degrees of periventricular white matter hyperintensity were present in these subjects' images. These subjects were examined biannually for signs of dementia or changes in their medical status and had annual neurologic, neuropsychologic (WAIS-R, Weschler Memory Scale [34], Verbal Fluency etc.) and MRI examinations. AD subjects met the same health and screening criteria except they met Alzheimer's Disease and Related Disorders Association (NINDS-ADRDA [35]) criteria for probable AD. Thus, these are medically healthy subjects so that cognitive or neural changes are not due to medical comorbidities. MR scans on both healthy elderly and AD subjects obtained as part of the annual examinations were those used for the present study. Scans chosen for analysis were randomly picked from those which met two criteria: a) the scan was taken at a time during which the subject was within the age range of the subject group and b) the scan was free from movement artifact which would disrupt the analysis. All subjects or subjects caregivers gave informed consent to participate in this project.

[Table 1 about here]

MRI Procedures. a. Scan Protocol. MRIs were performed as previously described [13] using a GE 1.5-T magnet. The imaging protocol used to image the entire brain consisted of continuous slice, multiecho, multiplanar image

acquisition, with 4 mm-thick coronal slices, a 24 cm^2 field of view using a 256 X 256 matrix, with two as the number of excitations. The brain was visualized using the following sequence: multi-echo coronal sequence, TR = 3000 msec, TE = 30 and 80 msec. T_1-weighted images centered in the midsagittal plane were used to orient the coronal plane. The coronal plane was determined as the plane oriented perpendicularly to a line drawn from the lowest point of the splenium to the lowest point of the genu of the corpus callosum on the midsagittal image. Analysis of MR images was performed with computer-assisted techniques utilizing a program called REGION, developed for use with any Macintosh series computing equipment [36].

b. Region of Interest Analysis. Coronal slices used in these analyses were those in which the superior frontal gyrus could first be visualized (the tip of the frontal pole) and continued posteriorly until the anterior tip of the corpus callosum was visualized. This process utilizes approximately eight slices per subject and the slices used encompass approximately 90% of the total prefrontal cortex. For the purpose of discussion this area will be referred to as 'prefrontal'.

Data were collected from three regions of interest (ROI; total prefrontal volume, prefrontal white matter volume, and prefrontal gray matter volume). Total prefrontal volume and prefrontal white matter volume for all subjects were determined, by the same analyst, by outlining the structures with a cursor directly on a computer display. Total prefrontal volume was defined by tracing

all gyri and sulci around the cortical ribbon of each hemisphere in the T_2-weighted image (Figure 1a). Prefrontal white matter volume was traced in the proton-density weighted image. This was performed by first enhancing the windows and levels of the image so that ambiguous pixels were reduced without changing relative pixel intensities. This procedure was used to augment differences between gray and white matter boundaries (Figure 1b). Prefrontal gray matter volume was calculated by subtracting prefrontal white matter volume from total prefrontal volume.

[Figure 1 about here]

Pixel area data were collected separately from each ROI for each coronal slice of the prefrontal cortex. Volumetric data for each ROI was calculated by summing the pixel area of each ROI to get a total pixel volume for each region. All regions were normalized by dividing volumes by the subjects' total intracranial volume. Total intracranial (supratentorial cavity) volume was defined as all non-bone pixels beginning with the first slice in which the frontal poles were present and ending at the occipital pole. This was determined with an automated technique called recursive segmentation built into the REGION analysis program. Within each image, tissue types are coincidentally sampled on the spatially registered multiecho images by selecting a predetermined number of sample points comprised of a 3 X 3 pixel sample within three tissue

types: bone, brain (gray and white matter combined), and cerebrospinal fluid. The recursive segmentation is completed automatically by successively applying a discriminant function to the established tissue type sample intensities and "peeling" away bone from the image leaving total intracranial contents for analysis [13]. At the base of the brain, brainstem structures were excluded from supratentorial structures by manually tracing those pixels to be excluded according to atlas-based rules. Left and right sides for each ROI were analyzed together as the total volume. The examiner was blind to all subject demographics including group status, sex, and age. All scans were analyzed by the same examiner and both intra and inter-rater reliability (intra and inter-class correlation coefficients) with this method for all regions was >0.80.

Previous studies have reported systematic alterations in signal intensity with aging [37,38]. This type of age-related change could affect data acquisition. We have examined this issue and it is not likely that age-related changes in signal intensity affect the current study. For example, we compared mean pixel intensities in multiple areas of both white and gray matter in a subset of 6 YHE and 6 OHE subjects (3 male and 3 female in each group) using NIH Image. No significant differences in mean pixel intensities of white or gray matter were found between YHE and OHE subjects in any area. In addition, images opened in REGION are auto-adjusted by the program to attain similar mean pixel intensities for all subjects. Thus, systematic alterations in signal intensity

with aging are not likely to affect data acquisition in the current study.

c Statistical Analysis. Group demographic characteristics at entry were compared by one-way analysis of variance (ANOVA). Separate analyses for total prefrontal volume, prefrontal white matter volume, prefrontal gray matter volume, and prefrontal gray to white matter volume ratio (calculated by dividing total prefrontal gray matter volume by total prefrontal white matter volume) were performed to determine differences in ROI volumes among OHE, YHE, and AD subjects. Differences were considered significant when $p < 0.05$. When significant differences were found, post-hoc Fisher's PLSD tests were used to determine which groups differ. Age-related volume decreases were also examined by calculating Pearson's correlations between each region and age with both factors as continuous variables in the combined YHE and OHE groups.

4.4 Results

Subject Characteristics. Clinical and demographic characteristics of the subjects are described in Table 1. WAIS-R vocabulary data were unavailable for the AD group. The subjects did not differ with respect to education, WAIS-R vocabulary score, socioeconomic status, or sex. There was a significant difference in age ($F(2,39) = 97.7$, $p < 0.0001$) which was expected as the OHE group was selected to be significantly older then the YHE and AD groups (post-hoc ps < 0.0001 for both YHE and AD). The YHE and AD groups did not differ in age. There was a significant difference in MMSE scores between the groups ($F(2,39) = 23.2$, $p = <0.0001$) with this difference due solely to the AD patients. YHE and OHE did not differ in MMSE score.

MRI Region of Interest Analysis. A summary of all group mean ROI volumes is presented in Table 2. Total prefrontal volume differed among groups ($F(2,39) = 5.9$, $p = 0.006$). Post-hoc testing showed that YHE had significantly greater total prefrontal volume than OHE and AD groups (ps < 0.004 and < 0.007 respectively; Figure 2a). OHE and AD did not differ in total prefrontal volume. To further examine the age-related nature of these differences, analysis of covariance (ANCOVA) was performed with age as the covariate. The main effect of total prefrontal volume was no longer present in this analysis suggesting that age is the factor responsible for these differences.

There was a main effect of prefrontal white matter volume ($F(2,39) = 7.9$, $p = 0.001$). Post-hoc testing showed that YHE had significantly greater

prefrontal white matter volume than both OHE and AD (ps = 0.0004 and 0.012 respectively; Figure 2b). OHE and AD did not differ in prefrontal white matter volume. There was a trend toward a main effect of prefrontal gray matter volume ($F(2,39) = 2.76$, $p = 0.07$) with the trend showing the YHE group having a greater prefrontal gray matter volume than the AD group (Figure 2c). There was no difference in prefrontal gray matter volume between YHE and OHE groups.

There was a main effect of prefrontal gray to white matter ratio ($F(2,39) = 5.00$, $p = 0.01$) with OHE having a greater ratio than YHE ($p = 0.003$; Figure 2d).

[Table 2 about here]

[Figure 2 about here]

There were significant negative correlations between age and total prefrontal volume ($r = -0.467$, $p = 0.01$; Figure 3a) and age and prefrontal white matter volume ($r = -0.541$, $p = 0.002$; Figure 3b) when YHE and OHE groups were combined (AD patients were not included in these analyses). Prefrontal gray matter volume did not show a relationship with age when the YHE and OHE groups were combined ($r = -0.26$, $p = 0.18$; Figure 3c). Prefrontal gray to white matter volume ratio showed a significant positive correlation with age in YHE and OHE groups combined ($r = 0.469$, $p = 0.01$; Figure 3d). No significant

correlations with age were found for any ROI measure in the AD group or the YHE and OHE examined separately.

[Figure 3 about here]

4.5 Discussion.

There is a decline in volume of the prefrontal cortex in both the very old and in AD patients age matched to younger healthy elderly. In addition, there is an age-related decline in white matter volume which is disproportionately greater than the decline of gray matter volume in the OHE when compared to YHE. In AD, the decline is more proportional in both tissue types as their gray to white matter ratio does not differ from YHE.

Three findings in this study suggest that the decline in prefrontal volume in the oldest old is due in part to a selective loss of white matter. First, prefrontal volumetric measurements showed significant negative correlations with age. White matter volume was more closely related to age than total prefrontal volume. Prefrontal gray matter did not show a significant relationship with age suggesting that white matter loss is selective. Second, differences in prefrontal volume between the YHE and OHE groups were removed when data were adjusted for age suggesting that age is the critical factor that causes the differences in prefrontal volume between groups. Finally, the OHE group had a greater gray to white matter volume ratio compared to the YHE group. It is likely that this greater ratio represents the relative loss of white matter rather than an increase in gray matter with aging because neuronal density does not increase with aging [39]. These results add to recent work by Raz [26] demonstrating selective vulnerability of prefrontal gray matter with early aging (subjects 18-77 years of age with a mean age of 43.5 years younger than the OHE of the

present study).

The finding of disproportional white matter loss in OHE subjects is in contrast to what was observed in AD. Differences between AD and YHE in the gray to white matter volume ratio were not found. Although AD subjects showed an overall loss of total prefrontal volume, this decline seems to be a proportionate loss of white matter and gray matter volume together contributing to the decline of prefrontal volume. These results are similar to previous post-mortem findings showing an increase in the gray to white matter volume ratio with aging but not dementia [40]. The age matching of AD to YHE suggests that volume differences between these groups is secondary to disease processes of AD. These findings suggest that tissue loss in the prefrontal cortex with aging is qualitatively different than that of AD.

The decrease of total volume of the prefrontal cortex with increasing age is consistent with previous findings of age-related frontal lobe volumetric differences [1,4,24,41]. Additionally, the decline in white matter volume in the oldest old is consistent with previous findings suggesting that white matter degenerates with aging [42] and our data extend these findings through to the ninth decade of life.

Another interesting finding in the current study is the increasing heterogeneity in regional volumes with increasing age. This increase in intra-group anatomic variability may be related to physiological changes which occur primarily in late stages of aging. There is a wealth of literature demonstrating a

similar increase in variability with age when measuring a variety of parameters including volumetric measures [4] and cognition [43]. This variability in regional volumes among the OHE subjects may be useful in differentiating between subjects exhibiting successful aging and subjects with incipient dementia.

A multitude of pathologic mechanisms could contribute to the loss of white matter within the prefrontal cortex. It is of interest that free radicals are particularly damaging to myelin due to its composition of peroxidizable phosopholipids [21]. Plasma antioxidant levels (ascorbic acid and B-carotene) were significantly correlated with better memory performance in subjects aged 65 to 94 years [20] suggesting that free radical damage may have a negative consequence on cognition. Still, it is unknown if plasma antioxidant levels are directly related to white matter volume in the aged or with CNS oxidant stress in general.

Because this was a cross-sectional study of the healthy aged, it is difficult to directly attribute volume differences between the YHE and OHE to atrophic changes with aging. These results may reflect a cohort effect with younger subjects having greater brain volumes to begin with. We did not analyze subjects in the 76-84 year age-range which contributes to this uncertainty.

It is unclear how the present results showing a decline in white matter volume with age relate to other MRI measures of white matter changes such as white matter hyperintensities. Hyperintensities on MRI images are often

thought to represent ischemic insult to the tissue and thus, are an indirect measure of pathology. White matter hyperintensities show significant correlations with various frontal lobe functional measures [25,45,46]. Additionally, hyperintensities have been associated with a variety of cognitive and behavioral disorders including AD [42], depression [46], and psychosis [47]. Similar to the decline of white matter volume with age, hyperintensities have been found to increase with age [48,49,50]. Still, preliminary data collected in our laboratory suggest that white matter volume is not correlated with white matter hyperintensities. These results imply that white matter volume loss and white matter hyperintensities occur by different mechanisms in the prefrontal cortex of the aged. Alternatively, these results may be due to the very limited range of white matter hyperintensity in the frontal lobe of this sample. Data was available on 19 of the 28 healthy elderly and 7 of the 12 AD patients in this study showing that white matter hyperintensities occupied less than 1% of total frontal lobe volume. We are currently looking further into this relationship.

Future longitudinal studies of white matter atrophy and clinical cognitive correlates of white matter volume will be of interest to further understand the neural and behavioral changes which differentiate healthy aging from impending degenerative disease.

Table 1. Subject Clinical and Demographic Characteristics.

Group	Age	Sex	Education	SES	MMSE	WAIS-R Vocabulary†
YHE	70.9 64.6-75.8	7M 7F	14.6 9-19	48.4 25-66	29.0 28-30	53.7 32-63
OHE	90.0* 84.3-95.4	7M 7F	13.6 7-18	47.6 22-66	28.3 25-30	48.2 26-61
AD	69.9 60.8-75.4	7M 7F	13.8 8-18	52.1 26-66	17.2# 2-28	

Data presented as mean and range.

Age represents age at MRI scan. Education presented in years of schooling. SES = socioeconomic status; MMSE = Mini-Mental State Examination; †WAIS-R Vocabulary data was not available for AD subjects.

*Significantly differs from YHE and AD

#Significantly differs from YHE and OHE

Table 2. Region of Interest Volumes.

Group	Intracranial Volume	Total Prefrontal Volume+	Prefrontal White Matter Volume+	Prefrontal Gray Matter Volume+	Gray/White Matter Ratio+
YHE	Mean 1199.2 SEM 24.6	Mean 26.5* SEM 0.83	Mean 10.1* SEM 0.39	Mean 16.5 SEM 0.50	Mean 1.7 SEM 0.05
OHE	Mean 1151.5 SEM 28.3	Mean 22.3 SEM 0.88	Mean 7.1 SEM 0.51	Mean 15.2 SEM 0.63	Mean 2.3# SEM 0.17
AD	Mean 1214.1 SEM 35.5	Mean 22.5 SEM 1.20	Mean 8.1 SEM 0.67	Mean 14.5 SEM 0.69	Mean 1.9 SEM 0.15

Data presented as mean volume and standard error of the mean; SEM.

+Regional volumes are presented as percent of intracranial volume

*YHE significantly differs from both OHE and AD

#OHE significantly differs from YHE only

4.6 Figure Legends

Figure 1. Volumetric method used to calculate prefrontal region of interest volumes. Total prefrontal volume and prefrontal white matter volume for all subjects were determined by outlining the structures with a cursor directly on a computer display. A. Total prefrontal volume was defined by tracing all gyri and sulci around the cortical ribbon of each hemisphere in the T_2-weighted image. B. Prefrontal white matter volume was traced in the proton-density weighted image. Prefrontal gray matter volume was calculated by subtracting prefrontal white matter volume from total prefrontal volume. Images are presented as displayed on the screen by REGION.

Figure 2. Comparison of region of interest volumes between old healthy elderly (OHE), Alzheimer's disease (AD), and young healthy elderly (YHE) groups. Circles within each group represent individual subjects. Bars represent group means. Volumes presented as percentage of total intracranial volume to correct for head size. A, total prefrontal volume; B, prefrontal white matter volume; C, prefrontal gray matter volume; and D, prefrontal gray/white matter volume ratio. Fisher's least significant differences test was used to determine group differences (*=P <0.05 compared to OHE and AD; #=P <0.05 compared to YHE).

Figure 3. Regression of subject volume on age in all healthy elderly (OHE and

YHE combined). A, total prefrontal volume; B, prefrontal white matter volume; C, prefrontal gray matter volume; D, prefrontal gray/white matter volume ratio. There were significant negative correlations between age and total prefrontal volume ($r = -0.467$, $p = 0.01$; A) and age and prefrontal white matter volume ($r = -0.541$, $p = 0.002$; B). Prefrontal gray to white matter volume ratio showed a significant positive correlation with age in YHE and OHE groups combined ($r = 0.469$, $p = 0.01$; D). No significant correlations with age were found for any ROI measure in the AD group or the YHE and OHE examined separately.

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Figure 1. Method for Obtaining Volumetric Data

A.

B.

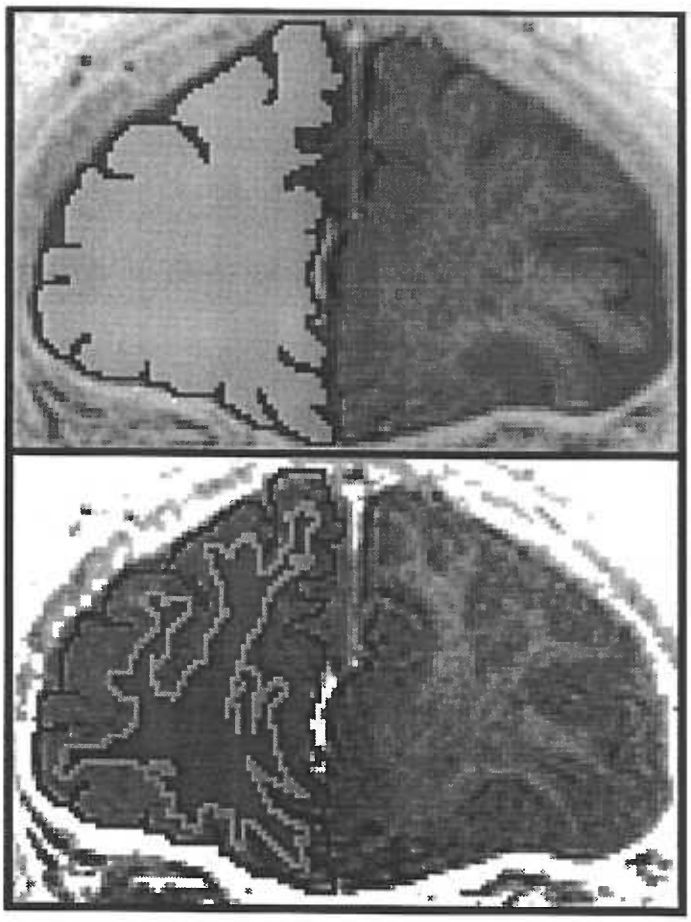


Figure 2. Region of Interest Volumes

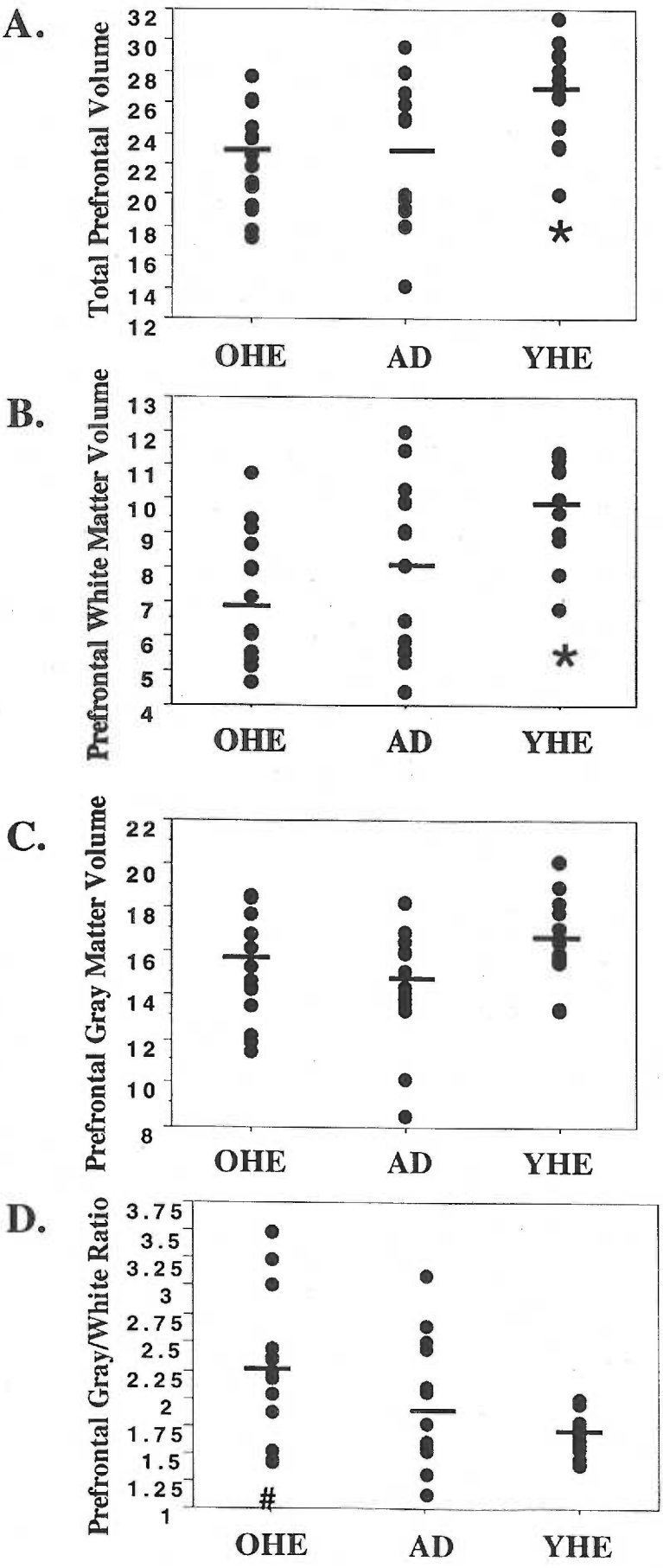
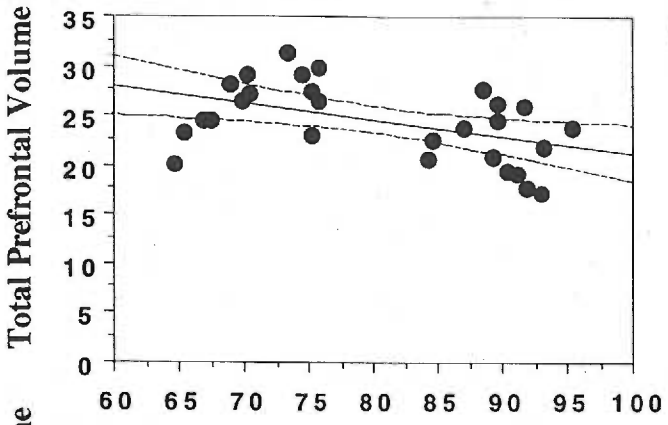


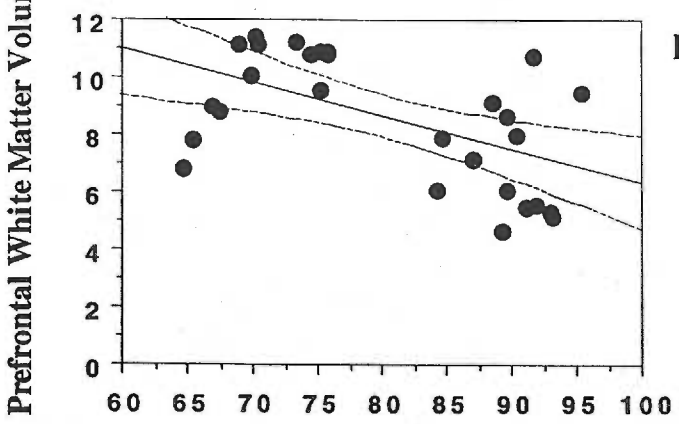
Figure 3. Regression of Region of Interest Volumes on Age

A.



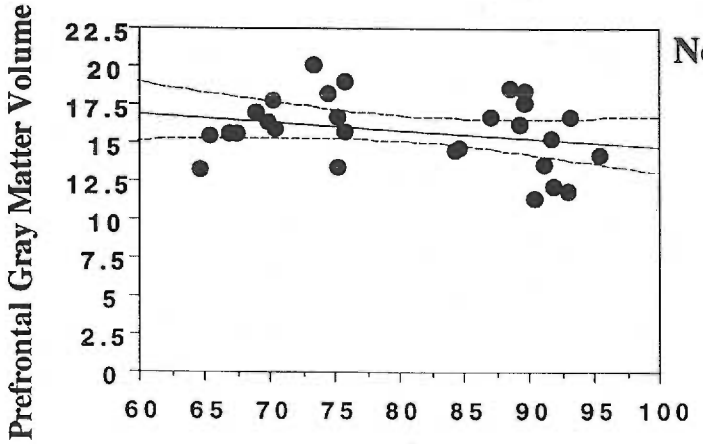
p = 0.01

B.



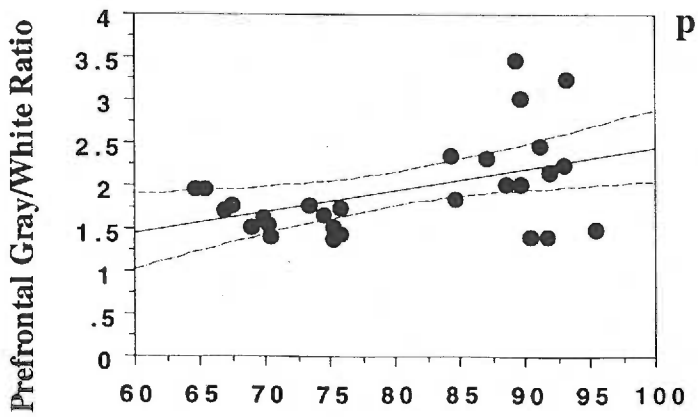
p = 0.002

C.



Not Significant

D.



p = 0.01

5. EXPERIMENT #2

Selective regional preservation and degeneration within the prefrontal cortex in healthy aging and Alzheimer's disease

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Running Title: Prefrontal degeneration in AD
Key Words: MRI, inferior frontal, gray matter, white matter, human

5.1 Abstract

The prefrontal cortex (PFC) is a heterogeneous cortical structure that supports higher cognitive functions including working memory and verbal abilities. This brain region is vulnerable to neurodegeneration with healthy aging and Alzheimer's disease (AD). We used volumetric magnetic resonance imaging to determine if any subregion within the PFC is more vulnerable than other PFC subregions to deterioration with late aging or AD. Study 1 showed that healthy oldest old subjects (n = 22, 11 men/11 women; mean age 88.9) had less PFC white matter than younger elderly subjects (n = 26, 14 men/12 women; mean age 71.7). The orbital subregion was selectively preserved relative to other PFC regions in the older subjects. Study 2 showed that subjects with AD (n = 22, 12 men/10 women; mean age 69.8) had less total cortical gray matter than age-matched healthy subjects when volumes were corrected for intracranial volume (n = 26, 12 men/14 women; mean age 71.4). AD subjects had significantly less volume in the inferior subregion only. These results suggest that orbital PFC is selectively preserved in healthy oldest old subjects. In contrast, degeneration within the PFC with AD is most prominent in the inferior PFC subregion. Thus, the PFC is regionally susceptible to both preservation and degeneration with aging and AD, respectively.

5.2 Introduction

Alzheimer's disease (AD) is an age-related neurodegenerative disorder that has a profound impact on memory, attention, and language functions (Locascio et al., 1995; Albert, 1996; Keilp et al., 1999; Perry and Hodges, 1999). Primary areas of degeneration have been determined through both post-mortem histopathological and in vivo neuroimaging techniques and include medial temporal and temporal-parietal cortex (Hyman et al., 1984; Double et al., 1996; Gomez-Isla et al., 1996; Detoledo-Morrell et al., 1997; Giannakopoulos et al., 1997; Kaye et al., 1997; Geula, 1998). Thus, many studies of AD pathophysiology focus on these regions of the brain. Much less attention has been placed on degeneration of the prefrontal cortex (PFC), although it is clear that the frontal lobe deteriorates in moderate to severe stages of the disease (Rusinek et al., 1991; Laakso et al., 1995; Brown et al., 1996; Double et al., 1996; Pantel et al., 1997a; Salat et al., 1999). The frontal lobe also degenerates with healthy aging (DeCarli et al., 1994; Raz et al., 1997; Salat et al., 1999), and shows greater age-related decline in volume than other areas of the brain (DeCarli et al., 1994; Raz et al., 1997). Regional blood flow within the PFC selectively predicts cognitive performance in both healthy young subjects (Wagner et al., 1998) and in subjects with AD (Eberling et al., 1992; Eberling et al., 1993). Thus, it is important to understand the patterns of degeneration in this region and to determine how PFC degeneration relates to cognitive decline. It is currently unknown if PFC degeneration is selective

(degeneration of a single region and not other regions) or preferential (greater degeneration in one region among other degenerating regions) or if degeneration is a general process affecting all regions of the PFC.

The PFC is a heterogeneous area of the brain that supports a variety of higher cognitive processes in human and in nonhuman primates. This region is a distinct area of the frontal lobe anterior to primary and secondary motor cortex (separated by the precentral sulcus). In fact, the PFC differs greatly from motor areas of the frontal lobe in anatomy, function, and development and has been suggested to be distinct from these motor regions (Brun, 1999). PFC regions critical for working memory (dorsolateral and inferior prefrontal) (Goldman-Rakic, 1990; Petrides et al., 1993; Goldman-Rakic, 1995; Petrides, 1995; Petrides, 1995; Braver et al., 1997; Cohen et al., 1997), attentional set-shifting (dorsolateral and orbital) (Dias et al., 1996; Robbins, 1996; Dias et al., 1997; Pantelis et al., 1999), and verbal abilities (inferior frontal) (Thompson-Schill et al., 1997; Gabrieli et al., 1998; Thompson-Schill et al., 1998; Poldrack et al., 1999) have all been described via lesion and functional neuroimaging studies.

Selective degeneration with AD might be expected in the PFC because of the cytoarchitectonic, connective, and functional architecture of this lobe (Pandya and Yeterian, 1990). Approximately 20% of Brodmann's original 52 defined cytoarchitectonic regions are located within the PFC (in contrast to about 6% in primary occipital cortex) (Brodmann, 1909) and cytoarchitectonic

regions could be differentially sensitive to degenerative processes (Coleman and Flood, 1987). Cytoarchitectonic regions are further subdivided by differential connectivity to posterior cortical structures. Decline in PFC cognition could be associated with degeneration of those PFC regions that have critical connectivity with posterior cortical regions (Morrison et al., 1986; Fuster, 1997). It is possible that degeneration in the PFC is more prominent in cytoarchitectonic regions that are connected with those posterior structures that degenerate in AD.

A number of studies, primarily performed in non-human primates, have examined prefrontal connectivity and suggest that certain areas within the PFC could be more susceptible to degeneration with AD than others. Many regions of the PFC are connected with temporal lobe and limbic regions (Petrides and Pandya, 1988; Carmichael and Price, 1995) that degenerate in the early (Hyman et al., 1984; Double et al., 1996; Gomez-Isla et al., 1996; Detoledo-Morrell et al., 1997; Giannakopoulos et al., 1997; Geula, 1998) and preclinical (Kaye et al., 1997) stages of AD. These PFC regions could be susceptible to anterograde or retrograde degenerative processes with progression of the disease.

In general, projections from the PFC to medial temporal lobe structures tend to originate in basal regions of the PFC including the medial, orbital, and ventrolateral PFC. For example, medial (Brodmann 32/24, 14, and 25) and orbital (Brodmann 11, 12, and 13) PFC send projections to the hippocampal

formation (Barbas and Blatt, 1995). Orbital PFC (Brodmann 13 and less in 11 and 12) and inferior lateral PFC (Brodmann 45) send projections to perirhinal cortex (Brodmann 35 and 36; Suzuki and Amaral, 1994). The parahippocampal cortex receives projections from lateral PFC regions (Brodmann 46), ventral to the principal sulcus (Suzuki and Amaral, 1994). Reciprocal connections between hippocampal and entorhinal regions and more dorsolateral PFC regions exist (Goldman-Rakic et al., 1984), but are sparse in comparison to the orbital and medial PFC connections (Insausti et al., 1987). Dorsolateral PFC (Brodmann 9 and 9/46) communicates with the medial temporal lobe more indirectly via the cingulum bundle terminating first in retrosplenial regions (Brodmann 30) (Morris et al., 1999). Similar to projections from the PFC, fibers that project from medial temporal lobe structures to the PFC tend to terminate in basal regions of the PFC. For example, medial and orbital PFC regions receive strong input from the hippocampal formation (Barbas and Blatt, 1995). Ventrolateral PFC regions receive sparse projections from the hippocampal formation and dorsolateral regions receive even fewer projections (Barbas and Blatt, 1995). The presubiculum projects to PFC regions in an opposite manner with the densest projections terminating in lateral PFC regions and the sparsest projections to medial regions (Barbas and Blatt, 1995). The entorhinal cortex sends projections to medial (Brodmann 24 and 25) and orbital (Brodmann 13 and 14) PFC regions as well (Insausti et al., 1987). In contrast, more dorsal and lateral regions (Brodmann 9, 6, and 8) are not

significantly connected with perirhinal regions (Suzuki and Amaral, 1994). Superior lateral and caudal regions such as Brodmann area 8, and 9 and 46 superior to the principal sulcus, receive very little input from medial temporal lobe regions (Barbas and Mesulam, 1981; Barbas, 1988; Barbas and Blatt, 1995).The temporal lobe regions described above are those that likely to degrade in early to moderate stages of the AD disease process as shown by plaque and tangle deposition (Braak and Braak, 1991) and neuron loss (Hyman and Gomez-Isla, 1994; Price and Morris, 1999). In sum, vulnerable temporal lobe and limbic regions communicate to a greater extent with basal PFC regions compared to dorsal and dorsolateral regions. Thus, orbital, medial, and inferior lateral PFC regions could be more susceptible to degeneration due to anterograde or retrograde degenerative processes originating in temporal lobe structures affected in AD. In contrast, more dorsal and lateral regions such as Brodmann areas 8, 9, and 46 could be less affected. Although dorsolateral regions do communicate with parahippocampal regions directly (Goldman-Rakic et al., 1984), the majority of medial temporal connections communicate with more basal prefrontal regions (Pandya and Yeterian, 1990; Barbas, 1995).

In contrast to Alzheimer's disease, PFC gray matter could be less vulnerable to age-related decline in volume. Although previous studies have demonstrated a decline in PFC volume with healthy aging (DeCarli et al., 1994; Raz et al., 1997; Salat et al., 1999), this volume loss could be due primarily to

loss of white matter and not gray matter of the region (Peters et al., 1996a; Peters, 1996b; Peters et al., 1998; Salat et al., 1999). Still, others have demonstrated an age-related decline in volume of both dorsolateral and orbital PFC gray matter (Raz et al., 1997). Studies showing gray matter loss could be due to the examination of volumes across a broad age-span with few subjects in the later decades (Raz et al., 1997) whereas white matter loss could be more prominent in those later decades (Salat et al., 1999).

The present study used volumetric magnetic resonance imaging (MRI) to determine if specific subregions within the PFC degenerate preferentially in late aging or AD compared to healthy control subjects. It was expected that regions of the PFC with greater anatomical connectivity to medial temporal lobe and limbic structures, such as orbital, medial, and ventrolateral PFC regions, would show greater degeneration with AD than other PFC structures. In contrast, it was expected that healthy older subjects would show degeneration in dorsolateral regions as previously demonstrated in a study of aging across a broad range of ages (Raz et al., 1997). Previous studies have described degeneration of the frontal lobes with healthy aging and AD (Rusinek et al., 1991; DeCarli et al., 1994; Raz et al., 1997; Pantel et al., 1997b; Salat et al., 1999), yet no studies have examined volumetric degeneration of specific subregions within the PFC in AD or healthy aging. The current examination describes changes in PFC subregions in healthy oldest old in Study 1 and describes changes in the same PFC subregions with AD in Study 2.

5.3 Materials and Methods Study 1

Subjects. Magnetic resonance imaging (MRI) scans of younger healthy elderly (YHE; n = 26, 14 men and 12 women; mean age 71.7) and older healthy elderly (OHE; n = 22, 11 men and 11 women; mean age 88.9) were examined for the present study. Subjects were chosen to maximize the number of subjects in the study while keeping the YHE and OHE groups matched for demographic and cognitive factors including Mini-Mental Status Examination score (MMSE; Folstein et al., 1975). Scans for YHE and OHE were collected as part of the Oregon Brain Aging Study, a longitudinal study of brain aging and cognition at Oregon Health Sciences University and the Veterans Affairs Medical Center in Portland, Oregon. YHE and OHE were matched for years of education, socioeconomic status, and general knowledge (WAIS-R vocabulary (Wechsler, 1981); See Table 1 for subject demographics). All subjects signed informed consent to participate in the Oregon Brain Aging Study.

Detailed descriptions of the recruitment procedures and subject criteria for subject recruitment as well as extensive medical and cognitive data on these subjects have been published elsewhere (Howieson et al., 1993; Kaye et al., 1994). Briefly, healthy subjects were free from significant medical disorders such as diabetes mellitus, hypertension (supine BP> 160/95), ischemic heart disease, cardiac arrhythmia, stroke, active cancer, psychiatric disorders, any neurologic disorder, had vision correctable to 20/70 OU or better, had hearing that did not interfere with speech perception, and did not take any medications

known to affect cognitive function. All subjects performed within age-group norms on a battery of measures of cognitive function and behavior and did not have signs of early dementia (Howieson et al., 1993; Kaye et al., 1994).

MRI Procedure. Scan Protocol. MRIs were performed using a GE 1.5 Tesla scanner. The brain was visualized with a multi-echo coronal sequence, TR = 3000 msec, TE = 30 or 80 msec, 4 mm slices with no skip. T1-weighted images in the midsagittal plane were used to orient the coronal plane. The coronal plane was determined as the plane perpendicular to a line drawn from the lowest point of the genu to the lowest point of the splenium of the corpus callosum on the midsagittal image.

Region of Interest Analysis. Tissue analysis of PFC MR images was computer assisted utilizing a program called REGION as previously described (Salat et al., 1999; Salat et al., 2000). Briefly, data were first collected from three tissue regions of interest (ROI; total prefrontal volume, prefrontal white matter volume, and prefrontal gray matter volume). Structures were outlined with a cursor directly on a computer display (Figure 1). Sulcal and gyral boundaries were determined by tracing edges around and deep into the cortex. The prefrontal cortex was defined in the coronal plane beginning with the first slice in which the superior frontal gyrus could be visualized (the tip of the frontal pole), and continued posteriorly but did not include the first slice in which the anterior tip of the corpus callosum was visualized. This process utilizes approximately eight slices per subject. Total prefrontal volume was defined by tracing all gyri and

sulci around the cortical ribbon of each hemisphere in the T2-weighted image (Figure 1A). Prefrontal white matter volume was traced in the proton density weighted image of the same slice after standardized image adjustment to maximize gray matter to white matter contrast and reduce the number of ambiguous pixels (Figure 1B). Prefrontal gray matter volume was calculated by subtracting prefrontal white matter volume from total prefrontal volume.

After defining gray matter/white matter boundaries, the PFC was further subdivided into five ROIs within each cerebral hemisphere; superior, middle, inferior, orbital, and anterior cingulate ROIs. Each area was hand traced with the cursor using an atlas-defined protocol and visual inspection of the image (Figure 1C). The regions were defined using a method modified from PFC areas described by Damasio (Damasio, 1991; Damasio, 1995) in which the gyral and sulcal patterns are used as regional landmarks in the T2-weighted image. The superior region encompassed superior medial and superior lateral portions of the PFC. The superior region was defined as beginning at the most ventral portion of the superior frontal sulcus and traced dorso-medial to the dorsal extent of the cortex and then ventral down the interhemispheric fissure to the most lateral portion of the anterior cingulate sulcus. This region was expected to contain the majority of Brodmann area 8 and a portion of area 9. The middle region encompassed mid-dorsolateral regions of the PFC. The middle region began at the most ventral portion of the superior frontal sulcus and was traced lateral and ventral down the middle frontal gyrus and continued

past the middle frontal sulcus to the most medial portion of the inferior frontal sulcus. This region was expected to contain the majority of Brodmann area 46 and a portion of area 9. The inferior region encompassed ventrolateral regions of the PFC. The inferior region began at the most medial portion of the inferior frontal sulcus and continued lateral, and then ventromedial to the most dorsal portion of the orbital sulcus. This region was expected to contain the majority of Brodmann areas 45 and 47 and a portion of area 44. The orbital region encompassed orbital and ventromedial regions of the PFC. The orbital region began at the most dorsal portion of the orbital sulcus and continued ventromedial and up the interhemispheric fissure until the most dorsal portion of the gyrus (within the interhemispheric fissure) was reached. This region was expected to contain the majority of Brodmann area 11 and 12. Data for ambiguous regions were obtained by tracing through the midpoint of the ambiguous region. Pixel areas were transformed to volumes by multiplying total pixel counts by a derived constant that transforms pixel size from REGION to cubic centimeters given the MR slice thickness of 4 mm [pixel area * .8789 (pixels to mm^2) *4 (mm^2 to mm^3 by multiplying by slice thickness in mm) * 0.001 (mm^3 to cm^3)].

[Figure 1 about here]

Data were first analyzed using absolute regional volumes. Data were then

normalized in two ways: (a) Regional volumes were divided by the subject's total intracranial volume (ICV). ICV is strongly related to premorbid absolute brain volume and does not change with age (for example, see Blatter et al., 1995; additionally, there was no relationship between age and ICV in all subjects in this study). Thus, when regional volumes are divided by ICV, the resulting measurement provides an index of atrophy of the region. (b) Regional volumes were divided by all other PFC subregions combined. This measurement provides a relative index of selectivity of preservation or degeneration of a particular region. Data are presented as a percentage of the subregion relative to the rest of the PFC. Thus, this proportion would provide larger ratios to regions selectively preserved relative to the rest of the PFC and smaller ratios to regions of selective degeneration relative to the rest of the PFC.

ICV was defined as all non-bone pixels beginning with the first slice in which the frontal poles were visible and ending at the occipital pole (Kaye et al., 1997). Recursive segmentation was completed automatically by successively applying a discriminant function to tissue type sample intensities and subtracting bone from the image, leaving only intracranial contents for analysis. At the base of the brain, brainstem and infratentorial structures including the cerebellum were excluded from supratentorial structures by manually tracing boundaries according to atlas-based rules as described below. The cerebellum and all structures inferior to the cerebellum were excluded by

tracing along the superior aspect of the structure, below the tectum and quadrigeminal plate. Infratentorial structures were excluded at the level of the pons by tracing a line from connecting the most dorsomedial aspects of the middle cerebellar peduncles and the cerebral peduncles on more anterior slices. This procedure continued by tracing out all noncortical structures ventral to the mammillary bodies on the most anterior slices containing infratentorial structures. Total ICV for each subject was determined as the sum of the supratentorial pixel area and transformed using the equation described for regional volumes above.

Data for all subjects were collected by one analyst. The examiner was blind to subject group status and sex. Five brains were analyzed five times each to generate reliability data. Reliability (intraclass correlations) on each subregion was >0.99 for total PFC, >0.97 for total gray matter, >0.94 for total white matter, >0.76 for superior, >0.84 for middle, >0.85 for inferior, >0.89 for orbital, and >0.62 for anterior cingulate. Because only moderate reliability for the anterior cingulate region was achieved and because of the small portion of this structure in the slices analyzed, we report results in this region as speculative.

Statistical Analysis. Demographic characteristics and subregional volumes were compared between YHE and OHE by separate unpaired t-tests. Differences were considered significant when two-tailed p values < 0.05.

5.4 Results Study 1

Subject Characteristics. Clinical and demographic characteristics of the subjects are described in Table 1. As per the study design, there was a significant difference in age between YHE and OHE subjects ($t(46) = 17.2$, $p < 0.01$). There were no group differences in years of education, socioeconomic status, MMSE, or WAIS-R vocabulary performance.

[Table 1 about here]

MRI Region of Interest Analysis. Absolute regional and intracranial volumes are presented in Table 2. OHE had significantly less absolute total PFC volume ($t(46) = 2.9$, $p < 0.01$) and absolute PFC white matter volume ($t(46) = 3.3$, $p < 0.01$). When corrected for ICV, OHE similarly had significantly less total PFC volume than YHE ($t(46) = 3.2$, $p < 0.01$; Figure 2). This difference was primarily due to a difference in white matter with OHE having significantly less than YHE ($t(46) = 3.3$, $p < 0.01$; Figure 2). There was a trend for a difference in PFC gray matter volume in OHE compared to YHE ($p = 0.06$; Figure 2). There were no differences in subregional PFC volumes corrected for ICV (all $ps > 0.2$; Figure 3A). To examine whether any region was particularly lost or preserved relative to the rest of the PFC, each region was analyzed as a ratio of all other regions combined. The proportion of PFC occupied by the orbital region was significantly greater in the OHE subjects compared to the YHE subjects ($t(46) =$

2.4, $p < 0.02$). No differences between YHE and OHE were found in the proportions of any other PFC subregion (Figure 3B and 3C).

[Table 2 and Figures 2 and 3 about here]

5.5 Discussion Study 1

Degeneration of the PFC is most prominent in white matter in healthy late aging. In contrast to white matter changes, gray matter in the orbital PFC subregion was preserved in healthy older subjects relative to other subregions within the PFC. Study 2 compared the same PFC subregions in healthy subjects and age matched subjects with AD to determine if degeneration in the PFC with dementia is associated with regions with strong medial temporal lobe connectivity and if this degeneration differs from degeneration in the PFC with healthy late aging.

5.6 Materials and Methods Study 2

Subjects. MRI scans of younger healthy elderly (YHE; n = 26, 12 men and 14 women; mean age 71.4) and age-matched subjects with Alzheimer's disease (AD; n = 22, 12 men and 10 women; mean age 69.8) were examined for the present study. The YHE subjects in Study 2 overlapped with the YHE subjects in Study 1 by 92%. Differences in YHE subjects used for the two studies were due to maximizing the number of subjects while keeping YHE and AD subjects matched for age in Study 2 ($p > 0.1$). Scans for YHE were collected as part of the Oregon Brain Aging Study and scans for AD were collected as part of the Oregon Alzheimer's Disease Center clinical protocol at Oregon Health Sciences University. YHE and AD were matched for age, education, and socioeconomic status (See Table 2 for subject demographics). All subjects or their responsible caregiver signed informed consent for Oregon Brain Aging and Oregon Alzheimer's Disease Center studies. YHE subject demographics and recruitment procedures were described in Study 1. AD patients met NINDS-ADRDA criteria for probable or possible AD (McKhann et al., 1984).

MRI Procedure and Data Analysis. MRI procedures including scan protocol and region of interest analyses as well as statistical analyses were performed as described in Study 1.

5.7 Results Study 2

Subject Characteristics. Clinical and demographic characteristics of the subjects are described in Table 3. As per the diagnostic criteria, AD subjects had significantly lower MMSE scores than YHE ($t(45) = 6.9$, $p < 0.01$). There were no group differences in age, years of education, or socioeconomic status (see Table 3 for subject demographics).

[Table 3 about here]

MRI Region of Interest Analysis. Absolute regional and intracranial volumes are presented in Table 4. YHE and AD did not differ in absolute regional volumes. Still, subjects with AD had slightly larger ICVs (yet not significantly so) and differences in these small regions could be masked by this difference. When corrected for ICV, AD had significantly less total PFC volume than YHE ($t(46) = 2.2$, $p = 0.03$). This difference was primarily due to a difference in gray matter with AD having significantly less than YHE ($t(46) = 2.4$, $p = 0.02$). There was no difference in PFC white matter volume in AD compared to YHE ($p = 0.13$, n.s.; Figure 4). There was a regional difference with AD having significantly less volume in inferior PFC ($t(46) = 2.1$, $p < 0.05$) but not in any other PFC region (all $ps > 0.18$; Figure 5A and 5B). There were no differences between YHE and AD subjects when each region was analyzed as a proportion of all other regions combined, although there was a trend for the proportion of

orbital region proportion to be greater in AD compared to YHE ($p = 0.07$). No other region proportion showed this trend (all $ps > 0.22$; Figure 5C).

[Table 4 and Figures 4 and 5 about here]

5.8 Discussion Study 2

Degeneration of the PFC is most prominent in gray matter in subjects with AD. Although this degeneration may be widespread in PFC gray matter, regional differences were significant in the inferior PFC only, suggesting greater degeneration of this region. Additionally, AD subjects showed a trend suggesting preservation of orbital region relative to the rest of the PFC. This finding was similar to the significant preservation with healthy aging demonstrated in Study 1. Thus, the orbital region may be relatively resistant to atrophy in AD as well as in healthy aging.

5.9 General Discussion

We used a regional volumetric analysis of the PFC to determine if degeneration is selective to particular regions within the PFC in healthy aging and AD. OHE had significantly less total PFC volume and PFC white matter volume than YHE as previously reported in a smaller sample of partially overlapping subjects (Salat et al., 1999). Also, OHE had a significantly greater proportion of orbital regional volume to all other regions suggesting that this region is resistant to atrophy with healthy aging relative to the rest of the PFC. In contrast, AD subjects had significantly less total PFC and PFC gray matter volume when corrected for ICV than YHE as previously reported (Salat et al., 1999). AD subjects had significantly less inferior PFC volume when corrected for ICV compared to YHE and thus this region is more susceptible to degeneration with AD than other regions of the PFC. Still, there was a significant loss of total PFC gray matter in AD compared to YHE, suggesting a more widespread degeneration of PFC regions with the disease.

Regional preservation of the Orbital Prefrontal Cortex with Healthy Aging.

The orbital region of the prefrontal cortex was expected to show the greatest degeneration with AD due to connectivity with temporal lobe structures that degenerate in the early stages of the disease process. However, our results suggest that the orbital region did not degenerate more than other PFC regions with AD. In contrast, it is very interesting that this region was preserved in very healthy aging as the proportion of the orbital region to all other regions

combined was significantly greater in OHE compared to YHE. Additionally, if the orbital region is truly resistant to degeneration, then there should be a similar preservation in the AD group compared to the YHE group. In fact, there was a trend for the AD group towards a greater mean ratio for this region only ($p = 0.07$; all other $ps > 0.22$), supporting the finding that the orbital region is preserved relative to other PFC regions. An alternate interpretation of this finding is that this measurement does not reflect preservation but that subjects destined to remain neurologically healthy into late aging have a greater orbital ratio throughout the lifespan and that this preservation declines with the development of AD. This theory is in accord with a prior histological study demonstrating that orbital region (Brodmann area 11) showed greater neural density with increasing age (Haug, 1985). The authors suggested that the increased neural density was due to preservation and not due to an actual increase in neurons in the region. A quantitative neuropathological study of these subjects would be useful to understand how cellular changes differ in orbital compared to other PFC regions.

A prior study (Raz et al., 1997) found loss of volume in orbital PFC yet the age range (18-77 years) and anatomical boundaries in that study differed from those in the current study. The orbital region in the previous study contained some of the inferior region described here. Thus, differences in findings are likely related to both subject selection (age) and the region measured. Alternatively, the current data could result from cohort effects with healthy oldest

old having relatively larger orbital PFC to begin with. Longitudinal studies examining this region of the PFC would be useful in further understanding the significance of atrophy versus preservation of PFC subregions.

Regional degeneration of the Inferior Prefrontal Cortex with Alzheimer's Disease. The present study found degeneration in the inferior PFC. This degeneration was only apparent when correcting for head size. Still, the absolute volume of the inferior PFC region showed the greatest mean difference compared to the other PFC regions between YHE and AD suggesting that there is truly a predilection for degeneration of this region. Alterations in the inferior PFC have been reported in prior studies of AD. Activation was reduced in AD subjects compared to healthy older subjects in the inferior PFC in a functional imaging study of divided attention (Johannsen et al., 1999). Neuronal counts per square millimeter were significantly lower in the inferior frontal gyrus in a group of demented patients compared to age-matched control subjects (Mountjoy et al., 1983). Although other regions showed decreased neural measurements in this study using other counts (e.g. neuronal counts in four cortical columns), only two regions out of nine examined (superior temporal and inferior frontal gyri) showed differences using neuronal counts per square millimeter as the measurement. The inferior prefrontal gyrus is often combined with more dorsal regions in other studies to form a 'dorsolateral' region. Thus changes selective to the more specific inferior region could have been masked in those studies. A portion of the

inferior region described in this study was previously combined with portions of middle and superior PFC to form a dorsolateral region in another study which showed greater volume loss of PFC gray matter than other brain regions with aging (Raz et al., 1997).

Orbital and inferior PFC atrophy was predicted with AD given the anatomical connectivity between PFC regions and temporal lobe structures (Petrides and Pandya, 1984; Barbas and Pandya, 1987; Seltzer and Pandya, 1989; Pandya and Yeterian, 1990; Barbas, 1993). Regions more strongly connected to sites of primary degeneration with AD were expected to show greater volumetric differences in AD compared to YHE due to anterograde or retrograde degenerative processes. In contrast to these expectations, the orbital region did not show significant degeneration with AD. Because this region has strong connections with temporal lobe structures that degenerate with AD, it is possible that degeneration of the PFC could be unrelated to connections with these temporal lobe structures. Still, cross-sectional studies such as the current study are limited in their ability to infer changes from discreet groups of subjects. A longitudinal study of alterations in orbital and inferior PFC across in the same subjects across time would be very informative in this respect.

It is unclear if the connections described for orbital PFC in the monkey overlap with the connections of the ventrolateral PFC described in the human. It has been noted that prior studies examining the anatomical homology of the prefrontal cortex between human and non-human primates have defined

regions based on differential cytoarchitectonic and anatomical criteria (Petrides and Pandya, 1999). The present study attempted only to describe degeneration based on probable distribution of anatomically significant (cytoarchitectonic and hodological) regions and it is possible that the inferior PFC region described in this study contains regions homologous with what has been referred to as orbital PFC in non-human primates. In fact, very little data exist on connectivity of specific prefrontal regions and temporal lobe structures in the human brain as the vast majority of cortical anatomical information has been derived from nonhuman primate studies (Pandya and Kuypers, 1969; Pandya et al., 1971; Van Hoesen et al., 1975; Arnsten and Goldman-Rakic, 1984; Goldman-Rakic et al., 1984; Petrides and Pandya, 1984; Barbas and Pandya, 1987; Seltzer and Pandya, 1989; Cavada and Goldman-Rakic, 1989; Pandya and Yeterian, 1990; Goldman-Rakic, 1995). Thus, it is unclear how human cytoarchitectonic regions of the inferior frontal gyrus that are proportionately smaller or absent in the macaque PFC (e.g. Brodmann 45 and 47) are connected with medial temporal lobe structures. Future studies examining the relationship between PFC and temporal lobe degeneration would clarify this issue.

Contrasting Prefrontal Morphology in Healthy Aging and Alzheimer's Disease

The majority of studies of neural degeneration with AD focus on the temporal lobe and medial temporal lobe structures such as the hippocampus (Ball, 1987; de Leon et al., 1995; de Leon et al., 1996; de Leon et al., 1997;

Kaye et al., 1997) and entorhinal cortex (Gomez-Isla et al., 1996; Juottonen et al., 1998; Frisoni et al., 1999; Juottonen et al., 1999). Although it is clear that these structures degenerate in the primary and even preclinical stages of AD (Kaye et al., 1997; Price and Morris, 1999), it is important to contrast healthy aging with AD in other areas of the brain for a better understanding of the neurobiological bases of AD degeneration and cognitive decline. AD is currently only conclusively diagnosed histopathologically. Thus, understanding how alterations in the brain differ between healthy older people and people with AD will be useful for potential in vivo diagnostic and intervention applications. The PFC is particularly important to contrast between healthy aging and AD as this region of the brain degenerates preferentially in healthy aging compared to other structures of the brain (Raz et al., 1997) and regional differences between healthy old and subjects with AD could reflect particularly accelerated regions of degeneration.

It is of note that PFC measurements were not related to disease severity in the AD subjects (MMSE score; data not shown). Thus, it is unclear how prefrontal degeneration is related to cognitive decline in AD. Still, the MMSE is a global scale of disease severity and thus is not likely to contribute to understanding more subtle cognitive changes related to prefrontal function. Studies are currently underway in our laboratory examining the relationship between regional volumes and cognitive performance on tasks demonstrated to critically depend on different PFC regions in healthy oldest old subjects.

ACKNOWLEDGEMENTS:

This research was supported by NIA grants AG12611 and AG08017, NIMH grant MH11855, and a VA Merit Review Grant.

The authors thank Milar Moore, Tamara Karnos, and Dave Kerr for technical assistance with this study.

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TABLE 1. Study 1: Subject Demographic Characteristics.[H]

Group	*Age (Years)*	*Sex*	*Education (Years)*	*SES*	*MMSE*
YHE	**71.7 (64.6-76.8)**	**14M/12W**	**14.8 (9-20)**	**50.1 (25-66)**	**28.7 (27-30)**
OHE	**88.9* (84.3-95.4)**	**11M/11W**	**15.0 (7-20)**	**49.8 (22-66)**	**28.2 (25-30)**

Data presented as mean and range.

[H] Abbreviations referred to:

YHE = younger healthy elderly; OHE = older healthy elderly; SES = socioeconomic status; MMSE = Mini-Mental State Examination (Folstein et al., 1975).

* Significantly greater than YHE ($p < 0.01$).

TABLE 2. Study 1: Absolute regional volumes.[H]

Group	*ICV*	*Total PFC*	*PFC Gray*	*PFC White*	*SUP*	*MID*	*INF*	*ORB*	*CING*
YHE	1198.7	107.1	70.3	36.9	12.9	9.0	11.2	8.8	4.1
	(20.4)	(3.6)	(2.1)	(1.9)	(0.6)	(0.6)	(0.6)	(0.4)	(0.1)
OHE	1183.5	92.9*	64.4	28.4*	12.3	7.9	10.0	8.9	4.1
	(26.8)	(3.4)	(2.4)	(8.2)	(0.7)	(0.7)	(0.6)	(0.5)	(0.2)

[H] Regional volumes presented in cubic centimeters.

Data presented as mean and (standard error of the mean).

Abbreviations referred to: PFC = prefrontal cortex; SUP = superior region; MID = middle region; INF = inferior region; ORB = orbital region; CING = cingulate region.

YHE = younger healthy elderly; OHE = older healthy elderly;

* Significantly less than YHE ($p < 0.01$).

TABLE 3. Experiment 2: Subject Clinical and Demographic Characteristics.[H]

Group	*Age (Years)*	*Sex*	*Education (Years)*	*SES*	*MMSE*
YHE	71.4 (64.6-75.8)	12M/14W	14.7 (9-20)	49.4 (25-66)	28.8 (27-30)
AD	69.8 (62.3-75.4)	12M/10W	13.5 (8-20)	49.6 (22-66)	17.0* (0-28)

Data presented as mean and range.

[H] Abbreviations referred to:

YHE = healthy control; AD = Alzheimer's disease; SES = socioeconomic status; MMSE = Mini-Mental State Examination (Folstein et al., 1975).

* Significantly less than YHE ($p < 0.05$).

Data not available for 1 AD education, 2 AD SES, and 1 AD MMSE.

TABLE 4. Study 2: Absolute regional volumes.[H]

Group	*ICV*	*Total PFC*	*PFC Gray*	*PFC White*	*SUP*	*MID*	*INF*	*ORB*	*CING*
YHE	1200.2	107.5	69.9	37.6	12.9	8.7	11.1	8.7	4.1
	(20.3)	(3.6)	(2.1)	(1.8)	(0.6)	(0.6)	(0.6)	(0.4)	(0.1)
AD	1233.9	100.3	65.6	34.7	12.3	8.2	9.7	8.7	4.3
	(33.3)	(5.2)	(2.9)	(2.7)	(0.8)	(0.8)	(0.6)	(0.5)	(0.2)

[H] Regional volumes presented in cubic centimeters.

Data presented as mean and (standard error of the mean).

Abbreviations referred to: PFC = prefrontal cortex; SUP = superior region; MID = middle region; INF = inferior region; ORB = orbital region; CING = cingulate region.

YHE = younger healthy elderly; AD = Alzheimer's disease;

5.10 FIGURE LEGENDS

Figure 1. Cartoon representation of volumetric method used to calculate prefrontal region of interest (ROI) volumes. The images are the same as those used in data collection but regional demarcations have been smoothed for publication purposes. Total prefrontal volume, prefrontal white matter volume, and subregion volumes for all subjects were determined by edge tracing the cortical ribbon and gray/white boundary with a cursor directly on a computer display. A. The most posterior slice of the prefrontal ROI is shown. Total prefrontal volume was defined by tracing all gyri and sulci around the cortical ribbon of each hemisphere in the T_2-weighted image. Pixel areas were transformed to volumes as described in text. B. Prefrontal white matter volume was traced in the proton-density weighted image of the same exact slice as A. Prefrontal gray matter volume was calculated by subtracting prefrontal white matter volume from total prefrontal volume. C. Left panel shows the unaltered T_2-weighted image of four posterior coronal PFC slices (top = most posterior). Right panel shows regional delineation on the exact same slices for superior (yellow), middle (pink), inferior (orange), orbital (green), and anterior cingulate (blue) regions. Subregions were defined as described in the text.

Figure 2. Comparison of tissue region of interest volumes between Younger Healthy Elderly (YHE) and Older Healthy Elderly (OHE) subjects. Volumes presented as percentage of total intracranial volume (ICV) to correct for head size. OHE had less total prefrontal volume and prefrontal white matter volume

than YHE (*ps < 0.05). Data presented as mean and standard error of the mean.

Figure 3. Comparison of subregion volumes between Younger Healthy Elderly (YHE) and Older Healthy Elderly (OHE) groups. A. Subregional volumes did not differ between YHE and OHE when corrected for intracranial volume (ICV). Data presented as mean and standard error of the mean. B. YHE had significantly less orbital/all other region ratio than OHE ($p < 0.05$). Data presented as mean and standard error of the mean. C. Scattergram of orbital/all other region ratio. OHE had a significantly greater ratio compared to YHE. Circles represent individual subjects. Bars represent group means.

Figure 4. Comparison of tissue region of interest volumes between Younger Healthy Elderly (YHE) and subjects with Alzheimer's disease (AD). Volumes presented as percentage of total intracranial volume (ICV) to correct for head size. AD had less total prefrontal volume and prefrontal gray matter volume than YHE (*ps < 0.05). Data presented as mean and standard error of the mean.

Figure 5. Comparison of subregion volumes between Younger Healthy Elderly (YHE) and subjects with Alzheimer's disease (AD). A. AD had significantly less volume in the inferior PFC subregion and no other subregion compared to YHE (*$p < 0.05$). Data presented as mean and standard error of the mean. B. Scattergram of inferior PFC region corrected for ICV. AD had a significantly less inferior PFC volume than YHE. Circles represent individual subjects. Bars represent group means. C. Subregional volumes did not differ between YHE

and AD when examined as a ratio to all other regions combined. Data presented as mean and standard error of the mean.

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Figure 1.

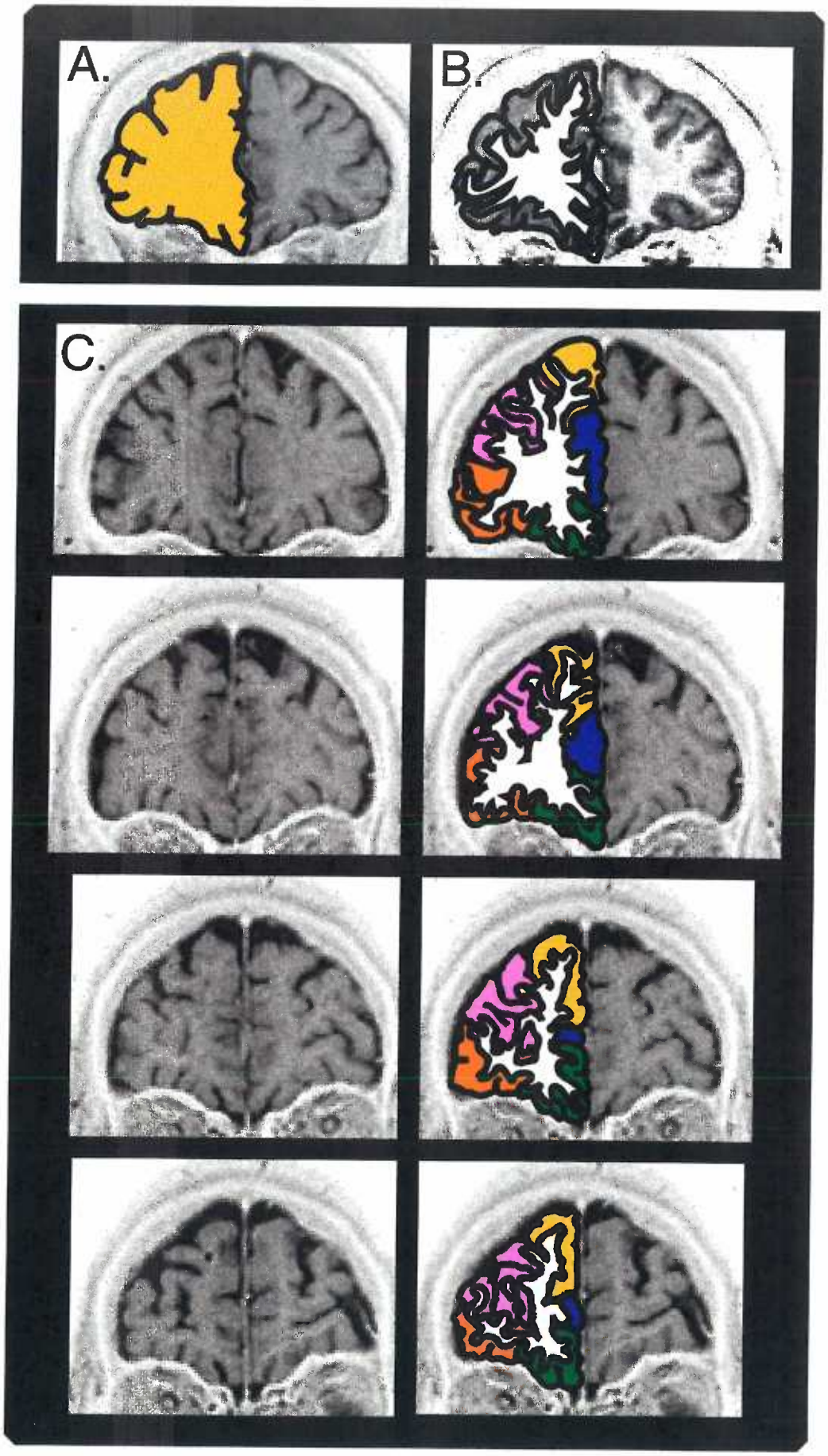

Figure 2.

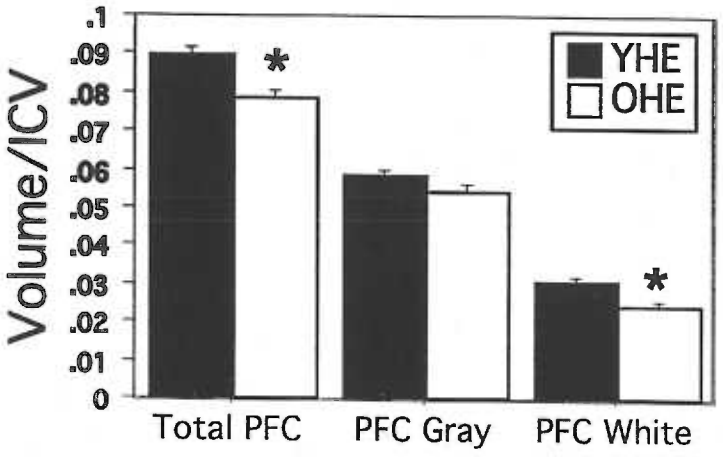
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Figure 3.

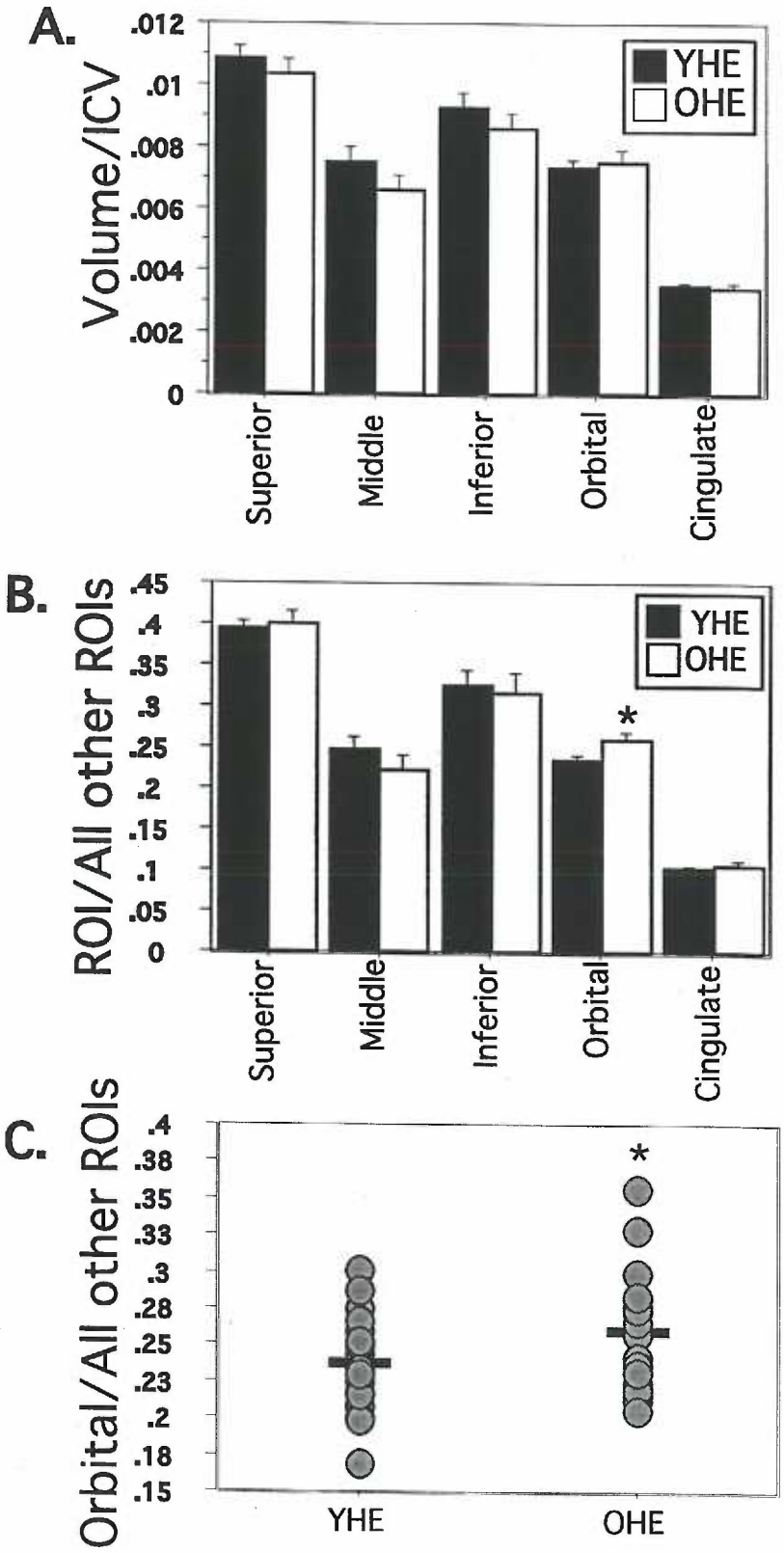
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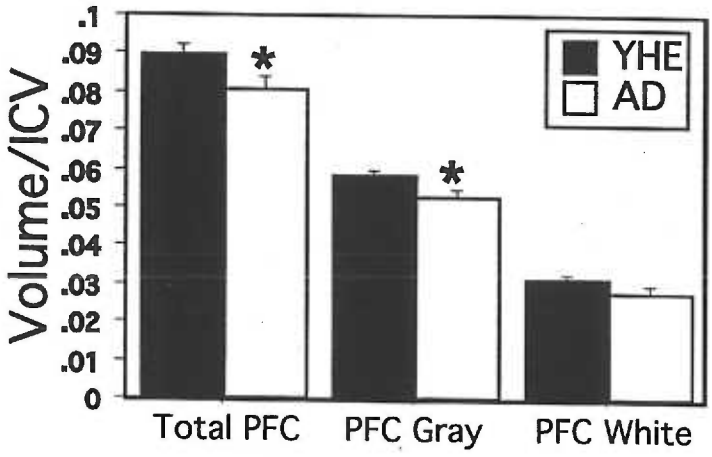
Figure 4.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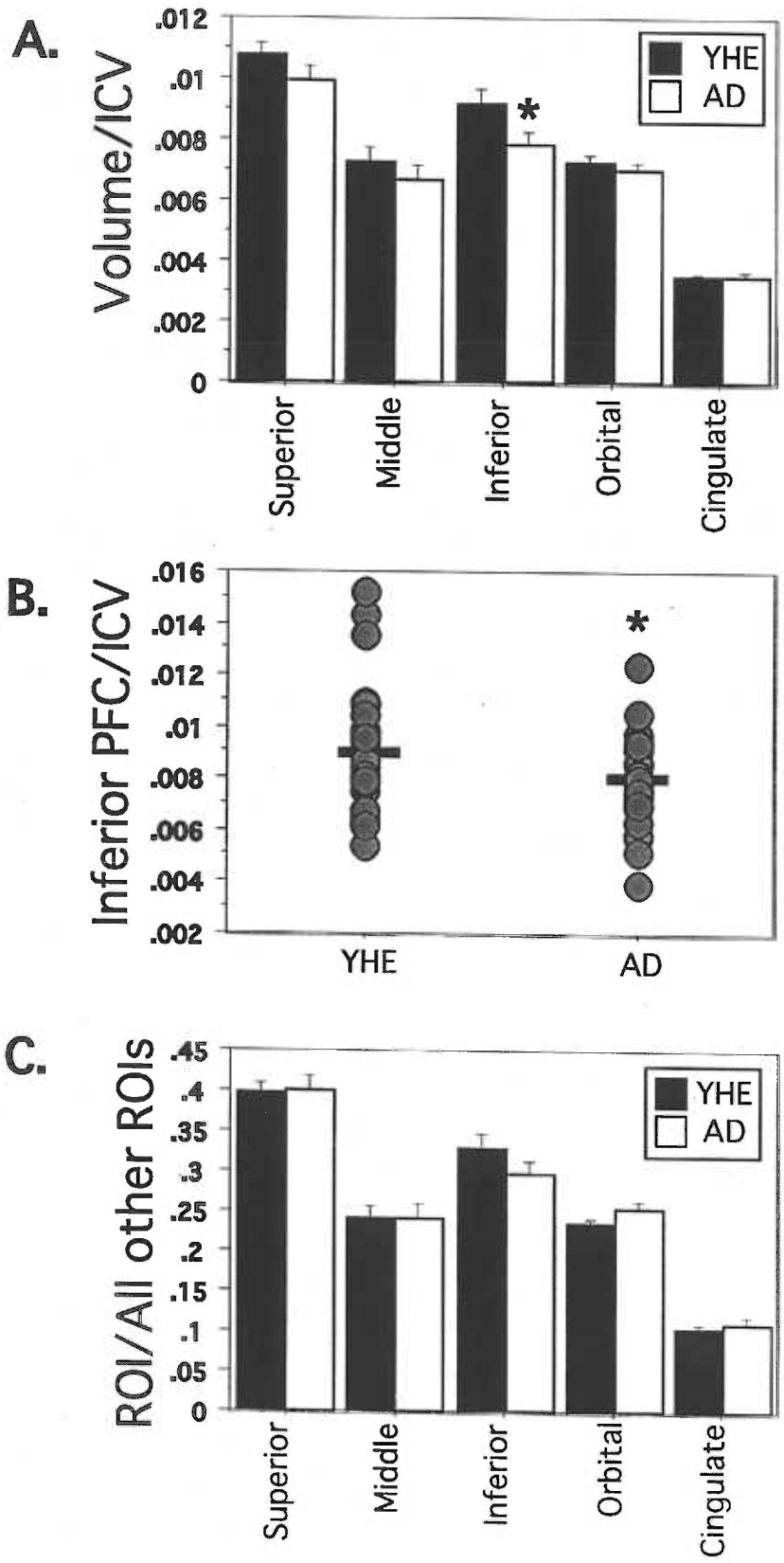
PFC White

Figure 5.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6. Experiment 3

Age-related alteration of orbital prefrontal cortex selectively predicts decline in working memory performance

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Abbreviated Title: Prefrontal degeneration in aging

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Acknowledgments: This research was supported by NIA grant AG12611, NIMH grant MH11855, and a VA Merit Review Grant. The authors thank Milar Moore, Dara Wasserman, Alison Dame and Suzanne Lehman for technical assistance and subject coordination for this study and Drs. Barry Oken and Gary Sexton for statistical consultation.

6.1 ABSTRACT:

Studies of neural dysfunction with aging show that degeneration of the prefrontal cortex (PFC) contributes to age-related cognitive decline in older adults. We examined the specificity of PFC degeneration, and the relationship between age-related degeneration and cognitive performance. Study 1 tested older and younger subjects using cognitive tasks that depend critically on different subregions of the PFC. Selective decline in cognitive function would, in turn, suggest selective regional degeneration. Study 2 used volumetric magnetic resonance imaging to determine if subregional volumes related to performance on regionally supported tasks. Differences in performance on tasks of working memory were greatest between old and young subjects and these measures were selectively correlated with age in both groups. Working memory ability accounted for most of the variance in performance of all prefrontal tasks. The volume of different PFC regions selectively predicted performance on tasks supported by those regions. Most notably, larger volume of orbital PFC selectively predicted worse working memory performance. The relationship between orbital PFC volume and working memory was also apparent when controlling for age suggesting that there is an age-independent relationship between performance and regional volume. Superior PFC predicted performance on other cognitive measures. Results from both studies demonstrate that working memory is a sensitive measure of cognitive aging and that regional morphology is associated with age-related changes in

cognitive performance. Alterations in orbital PFC volume with age could contribute to working memory decline through attenuated inhibitory mechanisms.

Key Words: prefrontal, aging, working memory, MRI, volume, cognitive neuroscience, orbitofrontal, dorsolateral

6.2 Introduction

Age-related neurodegeneration and subsequent cognitive decline are important to study from both a public health and a cognitive neuroscience perspective. Recent census estimates project that 70 million seniors, representing 20% of the population, will exist in the United States by the year 2030 (Administration on Aging, 1999). Age-related decline in cognition prevents functional independence in older adults. Thus, understanding the basis for cognitive decline that accompanies aging is a significant health-care interest. Aging also provides a type of lesion model in which selective regional degeneration may permit an understanding of how specific brain structures contribute to different cognitive processes.

The present study employed tasks of PFC cognition that have been used in a number of prior studies. This battery included the Conditional Association Task (testing the ability to form associations between two arbitrary stimuli; Petrides, 1985), the Self-Ordered Pointing (Petrides and Milner, 1982) and the N-Back Tasks (Cohen et al., 1997); two widely studied tasks of working memory (WM; the ability to store and manipulate information 'online' to perform a task), and the Object Alternation Task (testing the ability to shift cognitive or behavioral set; Freedman et al., 1998). Conditional Association performance is more impaired in subjects with lesions more superior in the PFC as opposed to more ventrolateral lesions (Petrides, 1985) and activates a superior and posterior PFC region (Brodmann area 8) in functional imaging studies

(Petrides et al., 1993). Human lesion (Petrides and Milner, 1982), monkey lesion (Petrides, 1995), electrophysiological (Pelosi and Blumhardt, 1999), transcranial magnetic stimulation (Jahanshahi et al., 1998), and functional neuroimaging (Petrides et al., 1993; Cohen et al., 1997; Braver et al., 1997) studies have all demonstrated a role for dorsolateral and inferior PFC regions in the performance of tasks of WM. Orbital and ventromedial PFC damage results in an increase in perseverative responses on Object Alternation and similar tasks in monkeys (Meunier et al., 1997) and humans (Freedman et al., 1998). Thus, impairments in Conditional Association learning could be related to superior PFC dysfunction, WM impairments could reflect dorsolateral and/or inferior PFC dysfunction, and deficits in Object Alternation performance could be an index of orbital PFC dysfunction. Accordingly, selective degeneration of PFC subregions with aging could result in selective cognitive deficits in one or more of the tasks described.

There are a number of degenerative changes that occur in the brain with healthy aging. Both degeneration of white matter (Jernigan et al., 1991; Dickson et al., 1992; Peters et al., 1994; Raz et al., 1997; Salat et al., 1999) and gray matter (Jernigan et al., 1991; Peters et al., 1994; Raz et al., 1997; Mueller et al., 1998) have been reported. White matter changes with aging include myelin 'ballooning' which causes a splitting of the myelin sheath (Feldman and Peters, 1998). Gray matter degeneration is due more to loss of synapses and recession of dendrites than actual cell death (Peters et al., 1998; Morrison and

Hoff, 1997).

We performed a detailed analysis of age-related alterations in PFC function and morphology. In Study 1, performance on the PFC cognitive battery was compared between older and younger adults. It was expected that age-related changes in WM would be most significant and most closely related to age compared to other tasks. We further examined which cognitive processes best account for age-related change by performing effect size calculations for each task. Finally, a discriminant function analysis was performed to determine how well the PFC task battery could classify older and younger subjects and which tasks were most important in that classification. In Study 2, task performance was related to volumetric measurements of PFC subregions. Dorsolateral PFC degenerates preferentially (i.e. this region degenerates more than other areas of the brain) with aging (Raz et al., 1997) and it was hypothesized that this degeneration could underlie the decline in WM performance in the aged. It was expected that age-related deterioration would be most prominent in the middle and inferior dorsolateral PFC regions and that WM would be most closely related to the volumes of these regions. Thus, we examined how regional volumes change with age and how regional volumes related to cognitive performance. We further examined the relationship between regional volumes and cognitive performance by performing a set of partial correlations examining the relationship between regional volumes and cognitive performance while controlling for the variance in the volume of the other PFC regions and the

variance in performance on the other PFC tasks. Finally, a factor analysis was performed to determine how performance on each PFC task relates to performance on other PFC tasks. Independent factor adjusted scores were then correlated with regional volumes to determine how regional alterations related to cognitive performance.

6.3 STUDY 1:

MATERIALS AND METHODS:

Subjects. Cognitive data were collected on a group of young subjects (n = 20, 10 men and 10 women; mean age 29.9) and a group of older subjects (n = 31, 15 men and 16 women; mean age 84.0). Older and younger subjects were matched for WAIS-R vocabulary score, a measure of general intellectual functioning, and there was a similar proportion of men to women in each group (50/50%, young and 48/52% old). All subjects were right handed except for two left handed young subjects. Young subjects were recruited through flyers displayed at local universities and were screened for a variety of factors related to health and general cognitive function. Young subjects were not taking prescription or nonprescription medications known to affect cognitive function, had no prior neurological problems including seizures, had good vision, and no health concerns. Older subjects were recruited through the Oregon Brain Aging Study, a longitudinal study of cognitive, neurological and other aspects of aging. Detailed descriptions of the recruitment procedures and subject criteria for the Oregon Brain Aging Study as well as extensive medical and cognitive data on the older subjects has been published elsewhere (Howieson et al., 1993; Kaye et al., 1994). Healthy older subjects entered into the Oregon Brain Aging Study were functionally independent, had English as their principal language, had not sought evaluation for cognitive impairment, and scored well on a variety of tests including the Instrumental Activities of Daily Living

(Fillenbaum, 1985), the Mini-Mental State Examination (Folstein et al., 1975), the Cornell Depression Scale (Yesavage, 1983), the Geriatric Depression Scale (Yesavage et al., 1982), and the Clinical Dementia Rating Scale (Hughes, 1982). They did not have significant medical disorders [e.g. diabetes mellitus, hypertension (supine BP > 160/95), ischemic heart disease, cardiac arrhythmia, stroke, active cancer, psychiatric disorders, any neurologic disorder], had vision correctable to 20/70 OU or better, had hearing that did not interfere with speech perception and did not use medicines that affect cognitive function. No subjects had cerebral infarctions. Only minor degrees of periventricular white matter hyperintensity were present in these subjects' images. These subjects were examined biannually for signs of dementia or changes in their medical status and had annual neurologic, neuropsychological (Weschler Adult Intelligence Scale-Revised (Weschler, 1981), Weschler Memory Scale (Weschler, 1981), and Verbal Fluency) and MRI examinations. Thus, these subjects were exceptionally healthy when entered into the Oregon Brain Aging Study. A portion of the older subjects (13 of the 31 subjects) still met these extreme health criteria for entry described above when the cognitive tests for this study were administered (tasks were administered a mean of 4.7 years after entry in these subjects). The remaining older subjects (18 of the 31 subjects) were deficient in one of the above criteria at the time of this study (tasks were administered a mean of 7.6 years after entry in these subjects). All subjects were free from any signs of possible dementia and no

subjects had experienced a stroke at the time of cognitive testing. Health concerns in the older subjects were mostly due to heart disease or blood pressure (8 subjects). The other health concerns were cancers outside of the nervous system (3 subjects), angina (2 subjects), depression with medication (2 subjects), and rheumatoid arthritis, trigeminal neuralgia, and the presence of an old lacunar infarct (each in one subject). Subjects with health concerns did not differ from subjects still meeting Oregon Brain Aging Study entry criteria in cognitive performance on PFC tasks (see results).

Cognitive Testing. All subjects were tested with a battery of cognitive tasks supported by PFC function as shown in previous studies using functional imaging or lesion models. Tasks were administered either exactly the same or in a similar manner to tasks described in the literature. The battery included the Conditional Association Task (Petrides, 1985), the Self-Ordered Pointing Task (Petrides and Milner, 1982), the N-Back Task (Cohen et al., 1997; Braver et al., 1997), and the Object Alternation Task (Freedman et al., 1998). All subjects were trained on computer tasks and response procedures prior to task performance to assure that younger and older subjects did not differ in competency of computer usage.

Conditional Association Task. The Conditional Association Task examined the subject's ability to learn associations between two arbitrary visual stimuli. Performance on this task is supported by superior and posterior dorsolateral PFC regions (Petrides, 1985; Petrides et al., 1993). Subjects were presented

with six abstract designs positioned in two rows of three designs in each row on a computer monitor (Figure 1a). The designs were obtained as part of a larger set from Dr. Michael Petrides (see Self-Ordered Pointing Task description below) but digitized for computer display. These were the same stimuli as used in prior studies of PFC function (Petrides, 1985; Petrides et al., 1993). The stimuli were created such that they would be easy to distinguish from one another but were are abstract so they are difficult to code verbally. One of six different colored lines appeared above the six designs on the monitor (Figure 1a). Subjects were told that each colored line was arbitrarily matched (associated) with one and only one abstract design. The goal of the task was to learn, through trial and error and feedback from the computer (correct and incorrect beeps), which colored line was associated with each specific abstract design. Subjects chose designs by using designated keys on the computer keyboard that matched the spatial location of the design chosen. If an incorrect response was made, the computer would beep indicating an incorrect response and the subject was to choose again. When the correct design was chosen, the six designs would clear from the screen and reappear 1000 ms later in a new spatial arrangement with a new colored line at the top of the screen. Subjects would then have to learn the association between the new colored line and a different abstract design. Once an association was learned, subjects were to respond by choosing that same design whenever the associated colored line appeared at the top of the monitor. Thus, subjects were

to chose a particular design conditional upon the color of the line at the top of the screen, ignoring the spatial position of the design on the screen. Stimuli were presented pseudorandomly such that any colored line could appear on any trial with any one of six spatial arrangements of abstract designs. All six lines and all six spatial arrangements were presented before repeating any one. Trials continued until subjects reached a 12 consecutive correct response criterion or until 180 trials had been administered. Data for the Conditional Association Task were analyzed as the average number of errors per trial (total number of errors divided by the total number of trials).

Self-Ordered Pointing Task. The Self-Ordered Pointing Task is a self-paced task of nonverbal working memory (keeping nonword items in mind). Performance on this task is dependent on mid-dorsolateral PFC regions (Petrides and Milner, 1982). Subjects were presented with a book of 8.5 X 11 inch pages. Each page displayed a set of abstract designs, with the designs in a different randomly assigned spatial arrangement for each page (Figure 1b). The stimuli were the same stimuli as used in prior studies of PFC function (Petrides and Milner, 1982). Subjects were told that the goal of the task was to choose one design on each page in the series without choosing the same design more than once. The task was self-ordered in that designs could be chosen in any order by the subject, but choosing from the same spatial location repeatedly was discouraged. Thus, the subject had to keep the designs previously chosen in working memory and update memory with each choice in

order to not choose the same design on subsequent pages. Four working memory load conditions (conditions differing in the amount of working memory used to perform the task) were presented to all subjects. Load conditions consisted of six, eight, ten, and twelve design load conditions (See examples Figure 1b). Each load condition contained a new group of designs and no design was repeated across load conditions. Each load condition was administered three times for a total of twelve blocks (three runs of each of four load conditions). Errors (choosing the same design more than once in a run) were summed for each run of each load condition and examined as percent correct for each load condition.

N-Back Task. The N-Back Task is a timed task of verbal working memory (keeping letters in mind) and performance of this task causes activation of mid-dorsolateral and inferior lateral PFC regions as well as other nonPFC regions (Cohen et al., 1997; Braver et al., 1997). Subjects were presented with a string of random letters, one at a time, at the center of a computer display. Subjects were told to respond by pressing one of two buttons on a button box for each and every letter, dependent on whether the letter was a 'target' letter or a 'nontarget' letter (described below). The task contained four different working memory load conditions. The zero-back condition was the control condition of the task and contained all stimuli and response components as the other conditions with no working memory requirements. Subjects were instructed to press the target button every time they saw a specific letter, whether it was in

uppercase or lowercase, (e.g. 'Respond with the target button every time you see the letter 'q') and to press the nontarget button to all other letters (Figure 1c). In the one-back condition (low working memory load condition), subjects were told to respond with the target button to any letter that was repeated one letter back (e.g. 'a-a'), whether it was in upper or lower case, and respond with the nontarget button to all other letters. In the two and three-back conditions (moderate and high working memory load conditions, respectively), subjects were to respond with the target button to any letter repeated two and three letters back, respectively (e.g. a repeated letter separated by one letter such as 'a-q-a' in the two-back condition, a repeated letter separated by two letters such as 'a-q-m-a' in the three back condition). Thus, subjects had to keep letters in working memory and update their working memory with each stimulus (Figure 1c). Letters were presented for 500 ms with a 2500 ms inter-stimulus interval between letters. Targets were presented on approximately 20% of the trials in each condition. Each condition of the task was completed three times in random order for a total of 12 blocks (three runs of each of the four conditions). Data were analyzed using a sensitivity measurement that takes into account the number of targets correctly identified (responding with the target button to a target letter) and the number of nontargets incorrectly rejected (responding with the target button to a nontarget letter). Specifically, data were analyzed as the Z score of all subjects for %correct target minus the Z score of all subjects for %incorrect nontarget.

Object Alternation Task. The Object Alternation Task examines the subjects ability to shift responses and cognitive set and is dependant on orbital PFC function (Freedman et al., 1998). Errors on this task reflect perseveration to a specific incorrect response. Subjects were presented with two shapes on the computer monitor, a red circle and a blue square. Subjects were told that there was a star 'hidden' behind one of the shapes (the circle or the square) and that their task was to find the star on each and every trial. The correct strategy to find the star on every trial was to alternate back and forth between the circle and the square regardless of their spatial position. If the subject chose the incorrect object they would be informed by a beep (incorrect feedback). After a correct response, a correct beep sounded and a star appeared in the object's place for 500 ms (correct feedback). The stimuli then cleared from the screen and a circle and square returned five seconds later, with either shape randomly in the left or right position (Figure 1c). Subjects performed the task until reaching the criterion of ten consecutive correct responses or until a total of 50 trials were reached. Data for the Object Alternation Task were analyzed as the number of errors per trial for each subject (total number of trials divided by the total number of errors).

[Figure 1 about here]

Statistical Analyses. Demographic data and data for the Conditional

Association and Object Alternation tasks and the 0-Back condition (control condition) of the N-Back task were compared between young and old subjects by separate unpaired t-tests. Data for the Self-Ordered Pointing and N-Back tasks were analyzed as 2 factor (group X working memory load) repeated measures ANOVAs with working memory load as the repeated measure. Repeated measures ANOVAs were used to determine if there is a greater difference between younger and older subjects at the higher load conditions. An interaction between group and load would suggest that the number of items to be kept in mind is a critical factor in age-related decrement in performance of the working memory task. Effect size calculations (Eta^2) were made to determine the magnitude of differences between groups on all tasks. The relationship between age and cognitive performance was examined using Pearson's correlations in the older and younger subjects separately. A conservative significance value of $p < 0.01$ was used to correct for the number of correlations performed. Significance values of $p < 0.05$ were considered suggestive.

Most subjects performed all cognitive tasks with a few exceptions. Two older subjects were unwilling to complete the Conditional Association Task due to difficulty with task performance. One older and one younger subject were unable to complete the Object Alternation Task because of time constraints. The data reported maximizes the number of subjects for each analysis (all subjects available for each cognitive task).

6.4 RESULTS:

Subject Characteristics. We divided older subjects into 'criteria' and 'noncritera' groups, based on the health criteria for entry into the Oregon Brain Aging Study, to determine if there were any difference in cognitive performance due to medical comorbidities as a preliminary analysis. There were no differences in age or PFC cognitive performance between subjects meeting the Oregon Brain Aging entry criteria at testing and those not meeting this criteria at testing (all ps > 0.25). Thus all subjects were used in the analyses presented. Data for the two subjects with depression have been highlighted in figures as it has been suggested that prefrontal function may be altered with depression (Drevets, 1999).

Clinical and demographic characteristics of the young and older subjects are described in Table 1. As per the study design, there was a significant difference in age between young and older subjects ($t(49) = -32.6$, $p < 0.001$). There were no differences in WAIS-R vocabulary performance and all older subjects performed within the normal range on the Mini-Mental State Examination (MMSE; Folstein et al., 1975). Although there was a small difference in years of education between groups (mean difference 1.5 years; $t(49) = 2.01$, $p = 0.049$), education was unrelated to all cognitive variables examined in older and younger subjects although there was a marginal relationship between performance on the working memory composite and education in the young subjects ($p = 0.056$).

[Table 1 about here]

Cognitive Analyses. Older subjects made significantly more errors than younger subjects on all tasks. Older subjects made a greater number of errors than younger subjects on the Conditional Association Task ($t = 3.3$, $p < 0.001$; Figure 2a). The repeated measures ANOVA for the Self-Ordered Pointing Task revealed that Older subjects made significantly more errors compared to younger subjects ($F(1,49) = 32.4$, $p < 0.001$; Figure 2b). There was a significant effect of working memory load with errors increasing with increasing working memory load in all subjects ($F(3, 49) = 105.2$; $p < 0.001$; Figure 2b) and there was a significant group by working memory load interaction ($F(3, 49) = 11.1$; $p < 0.001$; Figure 2b) with greater differences between groups at the higher working memory load conditions. Older subjects performed worse than young subjects on the 0-Back condition of the N-Back Task ($t(49) = 2.5$, $p = 0.02$). The repeated measures ANOVA for the N-Back Task revealed that older subjects performed significantly worse than younger subjects ($F(1,49) = 66.4$, $p < 0.001$; Figure 2c). There was no effect of working memory load or interaction, yet this type of effect would be difficult to obtain given the use of standardized sensitivity scores normalized for all subjects at each working memory load. Thus, we performed a similar repeated measures ANOVA on percent target correct scores (percent of trials in which a target was correctly

identified as a target). In this analysis, there was a significant effect of group ($F(1, 49) = 25.2$, $p < 0.001$; Figure 2d) and WM load ($F(2, 49) = 61.5$, $p < 0.001$; Figure 2d) with performance declining with increasing working memory load in all subjects and no interaction between group and load. Older subjects made more errors per trial than younger subjects on the Object Alternation Task ($ts(47) = 3.3$, $p < 0.001$; Figure 2a).

[Figure 2 about here]

Estimates of effect size (Eta^2) between younger and older subjects were calculated to further characterize the magnitude of the cognitive differences. The main effect for the 10 picture condition of the Self-Ordered Pointing Task and the 1-Back condition of the N-Back task showed the greatest effect of aging ($Eta^2 = 0.44$) followed by the 2-Back condition of the N-Back task ($Eta^2 = 0.41$), and the 3-Back condition of the N-Back Task ($Eta^2 = 0.35$). The 0-Back condition of the N-Back task showed the least effect of age ($Eta^2 = 0.11$) supporting the contention that tasks of sustained attention without a working memory load are minimally affected by age (Albert, 1996).

A discriminant function analysis was performed to determine how well performance on the PFC battery classified older and younger subjects and to determine which cognitive processes were most important in that classification. Data were reduced for this analysis by using a composite of the

two working memory tasks (the sum of normalized %correct for all sets of the Self-Ordered Pointing Task and normalized performance score for all working memory conditions of the N-Back task divided by two to obtain a mean normalized score). This analysis showed that the PFC battery correctly classified 95% of the younger subjects and 93% of the older subjects. Performance on the working memory composite was most important for this classification as this measure correlated greatest with the standardized canonical discriminant function ($r = 0.96$, all other $rs < 0.51$). The working memory composite alone correctly classified 85% of the young subjects and 94% of the older subjects.

All correlations between cognitive performance and age for younger and older subjects are presented in Table 2. In the younger subjects, there was a suggestive relationship between age and performance on the 2-Back condition of the N-Back Task ($r = -0.46$, $0 < 0.04$; Figure 3c) and significant relationships between age and performance on the 3-Back condition of the N-Back task ($r = -0.638$, $p < 0.002$; Figure 3a), and total performance of the WM conditions of the N-Back task ($r = -0.596$, $p = 0.005$; Figure 3b). Performance on these working memory measures declined with increasing age. Performance was not related to age on any other task.

[Table 2 about here]

[Figure 3 about here]

In the older subjects, there was a significant relationship between Self-Ordered Pointing percent correct in the six design load condition and age (r =-0.509, p = 0.003), a suggestive relationship between the twelve design load condition and age (r = -0.41, p = 0.02), and a significant relationship between all sets combined of the Self-Ordered Pointing Task (r = -0.46, p < 0.01; Figure 4a) and age with the percent correct declining with increasing age. There was a significant relationship between performance of the 2-Back condition of the N-Back Task and age (r = -0.45, p = 0.01; Figure 4b). There was a suggestive relationship between age and total performance of all WM load conditions of the N-Back task combined (r = -0.40, p = 0.03; Figure 4c). Performance was not related to age on any other task.

[Figure 4 about here]

6.5 DISCUSSION STUDY 1:

There was an age-related decline in the performance of all of the PFC tasks. Measures of effect size and the discriminant analysis showed that the greatest decrements are seen in the performance on tasks of WM. The strong age-related decline in WM performance was supported by the number of relationships between WM performance (two different tasks each at a number of working memory loads) and age in both young and older subjects. Similar

relationships were not apparent for performance on the other PFC tasks.

Both the N-Back and Self-Ordered Pointing Tasks are considered tasks of working memory yet the pattern of results were different for the two tasks. For example, there was a group by load interaction for the Self-Ordered Pointing Task, with greater differences between groups at the higher working memory loads. This effect was not present for the N-Back task. There are a number of differences between these tasks that could account for this dissimilarity. First, the Self-Ordered Pointing Task is a task of nonverbal working memory that uses abstract designs as stimuli whereas the N-Back task is a task of verbal working memory that uses letters as stimuli. Thus, older subjects could have greater difficulty keeping novel stimuli (abstract designs) online compared to familiar, language-related stimuli (letters). Another important difference between tasks is that subjects choose from multiple stimuli in the Self-Ordered Pointing Task whereas stimuli are presented one at a time during the N-Back task. Thus, a 'decision making' component of responding on the Self-Ordered Pointing Task (deciding which of multiple stimuli to choose) could be altered with aging.

Performance on the three back condition of the N-Back task correlated with age in the younger subjects and performance on the two back condition correlated with age in the older subjects. These relationships could reflect a staging of working memory load capabilities from younger (3-Back) to older (2-Back) adults and demonstrates the sensitivity of this task as a measure of age-

related cognitive decline. Performance on non-working memory PFC tasks did not correlate with age in either group, yet these measures were significantly affected by aging. Conditional association learning and response alternating processes could decline rapidly in the age-range separating the younger and older groups of this study but not show a significant decline after this initial loss. This theory is supported by a trend towards a relationship between age and performance in the younger subjects on the Conditional Association Task ($r = 0.39$, $p < 0.09$) which is completely absent in the older subjects ($r = 0.09$, $p = 0.65$) . An examination of subjects approximately 45-75 years of age would be useful in further staging age-related decline in these cognitive functions.

Study 2 examined the neural correlates of the age-related cognitive decline demonstrated in Study 1. Volumetric magnetic resonance imaging was used to relate morphological measurements of specific PFC subregions to performance on cognitive tasks supported by those regions. It was expected that the age-related decline in working memory tasks would be related to the volume of specific structures within the PFC, most specifically the volume of the middle (dorsolateral) PFC region, and that this region would show significant age-related degeneration.

6.6 STUDY 2:

Subjects.

Magnetic resonance imaging (MRI) scans from 30 of the 31 older subjects from Study 1 (n = 30, 14 men and 16 women; mean age 84.2) were examined for Study 2. Volumetric data were collected on an additional subject who was unable to attend the cognitive test session (M/75 years). This subject's data were used in the analyses of the relationship between age and PFC volumes only. Scans for older subjects were collected as part of the Oregon Brain Aging Study, a longitudinal study of brain aging and cognition at Oregon Health Sciences University and the Veterans Affairs Medical Center in Portland, Oregon (See Table 1 for demographics of all subjects tested on the PFC cognitive battery). All subjects signed informed consent to participate in the Oregon Brain Aging Study.

MRI Procedure. Scan Protocol. MRIs were performed using a GE 1.5 Tesla scanner. The brain was visualized with a multi-echo coronal sequence, TR = 3000 msec, TE = 30 or 80 msec, 4 mm slices with no skip. T1-weighted images in the midsagittal plane were used to orient the coronal plane. The coronal plane was determined as the plane perpendicular to a line drawn from the lowest point of the genu to the lowest point of the splenium of the corpus callosum on the midsagittal image.

Region of Interest Analysis. Tissue analysis of PFC MR images was computer assisted utilizing a program called REGION as previously described (Salat et

al., 1999; Salat et al., 2000). Briefly, data were first collected from three tissue regions of interest (ROI; total PFC volume, PFC white matter volume, and PFC gray matter volume). Structures were outlined with a cursor directly on a computer display (Figure 5). Sulcal and gyral boundaries were determined by tracing edges around and deep into the cortex. The prefrontal cortex was defined in the coronal plane beginning with the first slice in which the superior frontal gyrus could be visualized (the tip of the frontal pole), and continued posteriorly but did not include the first slice in which the anterior tip of the corpus callosum was visualized. This process utilizes approximately eight slices per subject. Total PFC volume was defined by tracing all gyri and sulci around the cortical ribbon of each hemisphere in the T2-weighted image (Figure 5a). PFC white matter volume was traced in the proton density weighted image of the same slice after standardized image adjustment to maximize gray matter to white matter contrast and reduce the number of ambiguous pixels (Figure 5b). PFC gray matter volume was calculated by subtracting PFC white matter volume from total PFC volume.

After defining gray matter/white matter boundaries, the PFC was further subdivided into five ROIs within each cerebral hemisphere; superior, middle, inferior, orbital, and anterior cingulate ROIs. Each area was hand traced with the cursor using visual inspection of the image and an atlas-defined protocol as described below (Figure 5c). The regions were defined using a method modified from regions described by Damasio (Damasio, 1991; Damasio,

1995) in which the gyral and sulcal patterns were used as regional landmarks in the T2-weighted image. The superior region was defined as beginning at the most ventral portion of the superior frontal sulcus and traced dorso-medial to the dorsal extent of the cortex and then ventral down the interhemispheric fissure to the most lateral portion of the anterior cingulate sulcus. The middle region began at the most ventral portion of the superior frontal sulcus and was traced lateral and ventral down the middle frontal gyrus and continued past the middle frontal sulcus to the most medial portion of the inferior frontal sulcus. The inferior region began at the most medial portion of the inferior frontal sulcus and continued lateral, and then ventro-medial to the most dorsal portion of the orbital sulcus. The orbital region began at the most dorsal portion of the orbital sulcus and continued ventro-medial and up the interhemispheric fissure until the most dorsal portion of the gyrus (within the interhemispheric fissure) was reached. Data for ambiguous regions were obtained by tracing through the midpoint of the ambiguous region.

[Figure 5 about here]

Regional pixel areas were first transformed to volumes by multiplying total pixel counts by a derived constant that transforms pixel size from REGION to cubic centimeters given the MR slice thickness of 4 mm [pixel area * .8789 (pixels to mm^2) *4 (mm^2 to mm^3 by multiplying by slice thickness in mm) *

0.001 (mm^3 to cm^3)]. Volumes were then adjusted by the subject's total intracranial volume (ICV) to correct for head size and provide a measure of atrophy as in prior studies (Kaye et al., 1997; Salat et al., 1999). ICV was defined as all non-bone pixels beginning with the first slice in which the frontal poles were visible and ending at the occipital pole (Kaye et al., 1997). Recursive segmentation was completed automatically by successively applying a discriminant function to tissue type sample intensities and subtracting bone from the image, leaving only intracranial contents for analysis. At the base of the brain, brainstem and infratentorial structures including the cerebellum were excluded from supratentorial structures by manually tracing boundaries according to atlas-based rules as described below. The cerebellum and all structures inferior to the cerebellum were excluded by tracing along the superior aspect of the structure, below the tectum and quadrigeminal plate. Infratentorial structures were excluded at the level of the pons by tracing a line from connecting the most dorsomedial aspects of the middle cerebellar peduncles and the cerebral peduncles on more anterior slices. This procedure continued by tracing out all noncortical structures ventral to the mammillary bodies on the most anterior slices containing infratentorial structures. Total ICV for each subject was determined as the sum of the supratentorial pixel area and transformed as described for regional volumes. The examiner was blind to subject age and sex. Five brains were analyzed five times each to generate reliability data. Reliability (intraclass correlations) on

each subregion was >0.99 for total PFC, >0.97 for total gray matter, >0.94 for total white matter, >0.76 for superior, >0.84 for middle, >0.85 for inferior, >0.89 for orbital, and >0.62 for anterior cingulate. Because only moderate reliability for the anterior cingulate region was achieved and because of the small portion of this structure in the slices analyzed, results are not discussed for this region.

Cognitive Data. Cognitive data collected in Study 1 were related to the regional volumes collected in Study 2.

Statistical Analysis.

The relationship between cognitive performance and regional volume was first analyzed by Pearson's correlations. These relationships were further explored in two ways to support the interpretation of the data. First, a set of partial correlations was performed to determine the specificity of relationships between regional volumes and PFC task performance when controlling for the shared variance of other volumes/tasks. Second, factor analyses were used to determine how performance of the different tasks was interrelated. The significant factor adjusted scores were then related to regional volumes. These analyses are described in greater detail below.

Bivariate Correlations:

Pearson's correlations were used to examine the relationship between the volume of each PFC subregion and age and the volume of each PFC subregion and performance on each PFC task.

Partial Correlations:

Partial correlations were performed relating performance on each cognitive task with each volumetric region controlling for the volume of all other regions. A second set of analyses was performed controlling for performance on all other cognitive tasks. The first set of analyses provides information about variance in task performance that is specifically related to each region of interest and the second set provides information about variance in regional volume that is related specifically to performance on each cognitive task. Data were reduced for these analyses by using the working memory composite described for Study 1. Hypothesized relationships were first examined followed by exploratory analyses of other task/region relationships.

Cognitive Factor Analyses:

Cognitive data were reduced by factor analysis (principal components analysis) and related to PFC volumes. This factor analysis serves two purposes. First, reduction in the number of variables reduces the likelihood of making type 1 statistical errors. Second, factor analysis creates a set of uncorrelated (independent) factors. Thus, the confound of multicoliniarity in the data (i.e. highly intercorrelated data makes the interpretation of the specificity of effects difficult) is greatly reduced. An additional theoretical benefit is derived from observing which cognitive domains load together on each factor. This information is useful in understanding how certain cognitive tasks could be similar or differ in their underlying cognitive mechanisms. The factor analysis was performed on the following parameters: Errors per trial on the Conditional

Association Task, percent correct on each of the four Self-Ordered Pointing Task working memory loads, performance on each of the three N-Back working memory loads, and errors per trial on the Object Alternation Task. Additional factor analyses was performed with fewer working memory variables to examine if the use multiple working memory variables weighted the results towards significant findings for working memory. Thus, one analysis was performed using only two working memory loads from each task, and one analysis was performed using the working memory composite in addition to performance on the Conditional Association and Object Alternation tasks. Factor analyses were performed in both the younger and older subjects to compare factor loadings between the two groups. A significance value of $p < 0.01$ was used to correct for the number of correlations performed. Significance values of $p < 0.05$ were considered suggestive.

6.7 RESULTS:

Bivariate Correlations Between Regional Volumes and Age and Regional Volumes and Task Performance.

Correlations between age and regional volumes corrected for ICV are presented in Table 3. There was a suggestive increase in orbital PFC volume with increasing age ($r = 0.04$, $p = 0.03$; Figure 6). No other regional volume was related to age.

[Table 3 about here]

[Figure 6 about here]

Correlations between PFC task performance and regional volumes are presented in Table 4. There was a significant relationship between performance on the Conditional Association Task and the volume of the superior PFC region ($r = 0.47$, $p = 0.01$; Figure 7a). Performance on the Conditional Association Task did not correlate with any other regional volume. There was a relationship between the Working Memory Composite and the volume of the orbital PFC region ($r = -0.46$, $p = 0.01$; Figure 7b). Performance on the Working Memory Composite did not correlate with any other regional volume. Because age was related to both orbital PFC volume and performance on the Working Memory Composite, we performed an additional partial correlation between orbital PFC volume and performance on the Working

Memory Composite controlling for age to determine if there was an age-independent contribution of orbital volume to working memory performance. This analysis reduced the relationship between the two factors ($r = -0.33$, $p = 0.08$).

[Table 4 about here]

[Figure 7 about here]

Partial Correlations Between Cognitive Performance and Regional Volumes Controlling for all Other Subregions. Partial correlations between cognitive performance and regional volumes controlling for all other subregions are presented in Table 5. There was a significant correlation between errors per trial on the Conditional Association Task and volume of the superior region ($r = 0.53$, $p < 0.01$; Figure 8a) and performance on the Working Memory Composite and the volume of the orbital region ($r = -0.59$, $p < 0.01$; Figure 8b). Larger regions were related to worse task performance.

[Table 5 about here]

[Figure 8 about here]

Partial Correlations Between Regional Volumes and Cognitive Performance Controlling for all Other Cognitive Tasks. Partial correlations between regional

volumes and cognitive performance controlling for all other cognitive tasks are presented in Table 6. There was a significant relationship between performance on the Working Memory Composite and volume of the orbital PFC region ($r = -0.57$, $p = 0.003$; Figure 9). No other task/region relationship was significant in these analyses.

[Table 6 about here]

[Figure 9 about here]

Cognitive Factor Analyses. The factor analyses generated three factors with eigenvalues > 1.0 that differed in their cognitive contributions (Table 7) in both the young and older subjects. Note that greater positive loadings refer to worse performance on the Conditional Association Task and Object Alternation Task (i.e. a greater number of errors per trial) whereas positive loadings on the Self-Ordered Pointing Task and N-Back Task refer to better performance (greater percent correct and greater sensitivity, respectively). Strong loading for each factor was determined using a loading criterion of > 0.6. Strongly loaded factors were then used to determine the theoretical cognitive domain of each factor.

Factor Analyses in Older Subjects. The Factor analysis of the older subjects produced three significant factors with eigenvalues greater than 1.0 that accounted for 66.9% of the variance all cognitive performance. Factor 1 explained 34.7% of the variance in performance among older subjects. This

factor was strongly loaded by performance on the 2-Back condition of the N-Back working memory task and all conditions of the Self-Ordered Pointing Task. Thus, this factor was termed 'Working Memory Factor'. Factor 2 explained 20.1% of the variance in PFC task performance. This factor was strongly loaded by errors per trial on the Object Alternation Task, errors per trial on the Conditional Association Task, and performance on the 3-Back condition of the N-Back task. The cognitive root of this factor was somewhat ambiguous yet loadings from the Self-Ordered Pointing Task were minimal. This factor was termed 'General PFC Cognition' because multiple cognitive tasks loaded strongly on it and this factor was used to examine regional correlations on a factor with less working memory requirements (self-ordered working memory in particular). Factor three explained 12.1% of the variance in PFC task performance. No tasks loaded strongly on this factor although the 1-Back condition of the N-Back task loaded moderately strongly (0.59). This factor could represent cognitive processes essential for working memory yet less emphasized in the other load conditions of the two working memory tasks. In particular, the 1-Back condition of the N-Back task does not require the storage and manipulation of more than one item online to perform the task. Thus, this factor was termed 'Single Item Working Memory'.

[Table 7 about here]

Factor Analyses in Younger compared to Older Subjects.

The factor analysis of the PFC cognitive variables was performed on the younger subjects to determine if cognitive tasks loaded similarly in younger and older subjects (Table 7). In the young subjects, three significant factors with eigenvalues greater than 1.0 that explained 73.2% of the cognitive variance were obtained. Factor 1 explained 37.1% of the variance in PFC cognitive performance and was strongly loaded by the four working memory load conditions of the Self-Ordered Pointing Task. Factor 2 in the young subjects was strongly loaded by performance on the 3-Back condition of the N-Back Task, errors per trial on the Conditional Association Task and errors per trial on the Object Alternation Task. Factor three was loaded most by performance on the 1-Back condition of the N-Back task and errors per trial on the Object Alternation Task. Notably, the young subjects differed from the older subjects in the direction of loading of Conditional Association Performance in Factor 2. Whereas young subjects that performed well on the 3-Back and Object Alternation Task also performed well on the Conditional Association Task, this relationship was opposite in the older subjects (better performance on the Conditional Association Task was related with worse performance on the other tasks).

Correlations of Cognitive Factors with Regional Volumes. Correlations of cognitive factors with regional volumes are presented in Table 8. Cognitive factors obtained in the factor analysis were related to regional volumes in the

older subjects. There was a significant negative correlation between the superior PFC region and General PFC Cognition Factor ($r = -0.48$; $p = 0.01$; Figure 10a). Subjects with larger superior PFC regions performed better on the 3-Back working memory load condition of the N-Back task, made more errors per trial on the Conditional Association Task, and fewer errors per trial on the Object Alternation Task. There was a significant negative correlation between the volume of the orbital PFC region and performance on the Working Memory Factor ($r = -0.50$, $p < 0.01$; Figure 10b). Subjects with smaller orbital PFC volumes had a greater percent correct in the different working memory load conditions of the Self-Ordered Pointing Task and performed better on the two back working memory load condition of the N-Back Task. A partial correlation was performed between the Working Memory Factor and orbital PFC volume controlling for age to determine the if the relationship between the cognitive and volume measurements was age dependent. This correlation remained suggestive after controlling for age ($r = -0.41$, $p = 0.04$) suggesting that there is a relationship between working memory ability and orbital PFC volume that is independent of age.

[Table 8 about here]

[Figure 10 about here]

Additional factor analyses. Two additional factor analyses were performed to determine if the number of working memory variables in the analysis were

weighting the findings towards significance for working memory. The first analysis used the 6 and 10 picture condition of the Self-Ordered Pointing Task and the 1 and 2 back conditions of the N-Back task in addition to errors per trial on the Conditional Association and Object Alternation Tasks. This analysis showed that working memory still accounted most for the variance in performance of the older subjects as the 2-Back, 10 and 6 picture conditions loaded most strongly on Factor 1 (loadings of 0.78, 0.66, and 0.63 respectively). The Object Alternation Task loaded marginally strongly on Factor 1 (loading of-0.60). Similarly, a factor analysis using only the working memory composite score in addition to errors per trial on the Conditional Association and Object Alternation Tasks showed that although all three variables loaded strongly onto one factor, working memory performance loaded substantially more strongly (0.90) than Conditional Association or Object Alternation performance (-0.63 for both tasks) suggesting that the number of working memory load conditions did not greatly influence the original factor analysis.

6.8 DISCUSSION STUDY 2:

The partial correlations demonstrated that there is a relationship between the volume of specific PFC subregions and performance of tasks supported by those regions. The analyses supported a role for superior PFC in conditional association learning and orbital PFC in working memory performance. Relationships existed when controlling for shared variance in volume among

regions and controlling for shared variance in task performance. Larger structures were associated with worse task performance suggesting that degenerative processes could result in increased or preserved volume of the region. This interpretation is supported by the suggestive relationship between increasing age and increasing volume of the orbital PFC region. Task performance loaded onto three factors with differential cognitive contributions in both the older and younger subjects. In the older subjects, Factor 1 (Working Memory Factor) explained a large amount (34.7%) of the variance in PFC task performance and was loaded strongly by performance of all working memory load conditions of the Self-Ordered pointing task and the 2-Back condition of the N-Back task. Factor 2 (General PFC Cognition Factor) was strongly loaded by performance on the 3-Back working memory load condition of the N-Back task and the errors per trial on the Conditional Association Task and the Object Alternation Task. Volume of the orbital PFC ROI was related to the Working Memory Factor suggesting that age-related alterations in the orbital region contribute to a decline in working memory task performance. There was also an age-independent contribution of volume of the orbital PFC region to working memory demonstrated by partialing out age from the correlation between orbital PFC volume and the Working Memory Factor. Thus, the morphology of the superior and orbital PFC regions selectively predict measures of PFC cognition with aging.

6.9 GENERAL DISCUSSION:

The present study demonstrated that a specific cognitive function, working-memory, is a highly sensitive measure of cognitive aging and that alterations in orbital PFC function may underlie age-related cognitive decline. These data contribute to a growing body of evidence implicating PFC dysfunction as major cause of cognitive decline in older and even middle-aged adults.

There were relationships between cognitive performance and superior and orbital PFC volumes yet only the volume of the orbital region showed a relationship with age. Interestingly, the orbital region showed an increase in volume with increasing age. Alteration of the region was associated with worse performance on working memory parameters and the relationship between orbital volume and the Working Memory Factor remained suggestive even when controlling for age. This suggests that there is a relationship between working memory performance and orbital PFC volume that is independent of age. Larger PFC subregions were associated with worse performance on other tasks as well. These relationships were region and task selective supporting the speculation that region specific dysfunction would lead to task specific attenuation in performance.

There is no *a priori* reason to expect that performance would be adversely affected only by loss of tissue of a structure and thus differential change (or preservation of volume) of specific regions of the brain should be considered in interpretations of cognitive decline. Prior studies have found a relationship

between greater regional volumes and poorer cognitive performance. For example, a study of seventy healthy subjects showed that larger hippocampal volumes are associated with worse explicit memory performance (Chantome et al., 1999). Although the mechanism of such potential alteration of neural regions is unclear, prior studies have suggested a proliferation of astroglia and gliosis that is preferential to the frontal lobe with age (Amenta et al., 1998). This proliferation could be a maladaptive compensatory response to a disproportionate loss of white matter (Peters, 1996; Salat et al., 1999) as astrocytes protect oligodendrocytes from certain types of damage including oxidative stress (Noble et al., 1994). Also, age-related hypertrophy of other neural regions including the hypothalamus (Rance et al., 1993) has been reported and neuronal hypertrophy has been suggested to be an early compensatory mechanism with the development of Alzheimer's disease (de Lacalle et al., 1993). These results and interpretations should be considered with caution as a longitudinal replication of these findings in a larger sample of subjects will be necessary to rule out cohort or other effects that might contribute to these relationships. Additionally, similar studies in aged nonhuman primates would be very important in understanding the mechanism of regional alterations with age.

The critical component root of cognitive aging has been the topic of many prior investigations (Salthouse, 1995; Hasher et al., 1991). The current study suggests that if there is a single dominant mechanism it would have to be a

component of all prefrontal tasks administered as older subjects were attenuated in performance of all tasks. The neuropsychology literature has suggested a number of different cognitive mechanisms that could be responsible for general cognitive decline. Working memory attenuation (Salthouse et al., 1989; Salthouse, 1992; Salthouse and Meinz, 1995), disinhibition (Hasher et al., 1991; Hasher et al., 1999), and decline in cognitive processing speed (Babcock and Salthouse, 1990; Hasher et al., 1991; Salthouse, 1992; Hasher et al., 1999) have all been examined as potential mechanisms. It is possible that some interaction of these factors results in the greatest cognitive decline. Working memory task requirements typically include inhibition of prepotent responses and the ability to shift responses. The original hypotheses predicted that working memory performance would be related to mid-dorsolateral PFC regions as function of this region has been associated with WM performance. Performance of any given cognitive task utilizes a variety of cognitive subprocesses mediated by a number of neural regions. The decline in performance of WM tasks could be related to attenuated orbitofrontally mediated inhibitory processes. Orbital PFC cortex has been implicated in a variety of cognitive processes including attentional set-shifting (Dias et al., 1996), behavioral inhibition (Dias et al., 1997), guessing (Elliott et al., 1999) and advantageous decision making (Bechara et al., 1994; Bechara et al., 1997). Findings of involvement of orbital PFC cortex in working memory performance have been limited. One study dissociated the cognitive processes

of working memory from decision making and found that lesions of dorsolateral PFC disrupted working memory performance but not decision making whereas ventromedial PFC lesions disrupted decision making performance (Bechara et al., 1998). Still, subjects with ventromedial lesions did have working memory deficits if the lesion was more posterior in the PFC. The ventromedial region described in this prior study included some of the orbital region described in the current study and thus suggests a role of the orbital PFC in working memory performance.

There are reasons to think that the errors on working memory in aging are caused by processes supported by orbital PFC regions such as perseveration and disinhibition. For example, older subjects make more perseverative errors on the Self-Ordered Pointing Task than younger subjects (West et al., 1998). Data on errors from this task are being examined to determine if there is a similar finding in the current study. Data from the N-Back task lends support to this finding. Older subjects had faster reaction times with incorrect responses compared to correct responses (data not shown). The younger subjects do not show such a reaction time difference suggesting that disinhibition or a perseveration to the non-target button could contribute to errors on the N-Back in older subjects. Inhibitory and cognitive set-shifting abilities are mediated by orbital and ventromedial PFC (Lineberry and Siegel, 1971; Dias et al., 1996; Dias et al., 1997) Accordingly, age-related changes in orbital PFC could result in attenuated performance on working memory tasks via an attenuated

inhibitory or impaired response-shifting mechanisms. Additional studies examining performance on working memory tasks with different inhibitory requirements could be illuminating in this respect.

Another interesting finding was that working memory performance is a sensitive measure of cognitive aging in the young group as well as in the older group. There were correlations between age and working memory in the younger and older groups, yet age correlated with performance in the higher working memory load condition in the younger subjects and the lower working memory load in the older subjects. This pattern of results suggests that working memory load could be used for staging age-related cognitive decline. Although it was expected that there would be an attenuation of working memory with age, it was not expected that there would be a relationship with performance and age in the younger subjects. Studies attempting to prevent this decline could use working memory as a sensitive measure of change with time, and different load conditions to compare expected outcomes in older and younger subject groups. For example, a pharmacological agent might be found to affect working memory similarly in younger older subjects only when the younger subjects are at a higher working memory load.

A recent study in our laboratory found a selective preservation of orbital PFC volume in relation to the other subregions in older subjects and a trend towards preservation of the same region in a group of subjects with Alzheimer's disease (Salat et al., in preparation). This prior finding is

compelling given the relationship between increasing orbital PFC volume with advancing age in this independent sample of scans (there was very little overlap in the subjects studied in this and the prior study and there was no overlap in scans analyzed between the two studies). These data suggest that there is either a preservation, growth, cohort effect, or some combination of these factors contributing to the increase in volume of orbital PFC with advancing age. An attractive speculation is that the volume of this region is larger in those likely to advance to healthy, late aging. Though these interpretations should be considered with discretion, converging evidence from this study and our prior study of aging and Alzheimer's disease recommend further study of this region.

TABLE 1. Study 1: Subject Demographic Characteristics.†∞

Group	*Age (Years)*	*Sex*	*Education (Years)*	*WAIS-R Vocab*	*MMSE*
Young	29.9 (21-43)	10M/10W	16.4 (12-22)	55.4 (34-66)	¥
Old	84.0* (72-94)	15M/16W	14.9 (12-24)	53.4 (34-66)	28.5 (24-30)

Data presented as mean and range.

† Abbreviations referred to:

SES = socioeconomic status; MMSE = Mini-Mental State Examination (Folstein et al., 1975).

* Significantly greater than younger subjects ($p < 0.01$)

¥ Data were not collected for young subjects on MMSE.

∞ Older subjects in Study 2 overlapped with the older subjects in Study 1 by 93% and demographics for Study 2 were similar to those reported in Study 1.

TABLE 2. Correlation Table of Relationships Between Age and Cognitive Performance in Younger and Older Subjects¥

Task	*Load*	*Young*	*Old*
CAT	**N/A**	**0.39**	**0.09**
SOP	**6-Design**	**-0.23**	**-0.51***
	8-Design	**-0.17**	**-0.35**
	10-Design	**-0.41**	**-0.08**
	12-Design	**-0.17**	**-0.41***
	Total SOP	**-0.27**	**-0.46***
N-Back	**0-Back**	**-0.17**	**-0.24**
	1-Back	**-0.06**	**-0.26**
	2-Back	**-0.46***	**-0.45***
	3-Back	**-0.64***	**-0.15**
	Total N-Back	**-0.60***	**-0.40***
OAT	**N/A**	**0.25**	**0.10**

¥ Pearson's correlation r values presented.

* $p < 0.05$

Abbreviations referred to: CAT = Conditional Association Task; SOP = Self-Ordered Pointing Task; N-Back = N-Back task; OAT = Object Alternation Task.

TABLE 3. Correlation Table of Relationships Between Age and Regional Volumes in Older Subjects.¥

	Superior	*Middle*	*Inferior*	*Orbital*
Age	**0.03**	**0.01**	**-0.05**	**0.39***

¥ Pearson's correlation r values presented.

* $p < 0.05$

TABLE 4. Correlation Table of Relationships between Cognitive Task Performance and Regional Volumes.¥

	Superior	*Middle*	*Inferior*	*Orbital*
CAT	**0.47***	**0.27**	**-0.14**	**0.05**
WM COMP	**-0.18**	**0.04**	**0.13**	**-0.46***
OAT	**-0.20**	**-0.20**	**-0.26**	**0.11**

¥ Pearson's correlation r values presented.

* $p < 0.05$

Abbreviations referred to: CAT = Conditional Association Task; WM COMP = Working Memory Composite; OAT = Object Alternation Task.

TABLE 5. Partial Correlation Table of Relationships between Cognitive Task Performance and Regional Volumes Controlling for the Volumes of All other PFC Subregions.¥

	Superior	*Middle*	*Inferior*	*Orbital*
CAT	**0.53***	**0.35**	**-0.31**	**-0.34**
WM COMP	**-0.13**	**0.34**	**0.25**	**-0.59***
OAT	**-0.18**	**-0.16**	**-0.21**	**0.33**

¥ Pearson's correlation r values presented.

* $p < 0.05$

Abbreviations referred to: CAT = Conditional Association Task; WM COMP = Working Memory Composite; OAT = Object Alternation Task.

TABLE 6. Partial Correlation Table of Relationships between Regional Volumes and Cognitive Task Performance Controlling for performance on all other PFC Tasks.¥

	Superior	*Middle*	*Inferior*	*Orbital*
CAT	**0.23**	**0.21**	**-0.26**	**-0.30**
WM COMP	**-0.35**	**-0.09**	**-0.25**	**-0.57***
OAT	**-0.39**	**-0.25**	**-0.38**	**-0.22**

¥ Pearson's correlation r values presented.

* $p < 0.05$

Abbreviations referred to: CAT = Conditional Association Task; WM COMP = Working Memory Composite; OAT = Object Alternation Task.

TABLE 7. Cognitive Factor Analysis Component Matrix¥

Young	*%Var*	*CAT*	6 Pic	8 Pic	10 Pic	12 Pic	1-Back	2-Back	3-Back	OAT
Factor 1: WM	37.1	-0.17	{0.78}	{0.91}	{0.95}	{0.85}	-0.21	0.35	0.28	-0.03
Factor 2: GENPFC	20.2	{0.70}	0.20	0.29	-0.03	0.10	-0.06	-0.41	{-0.75}	{0.68}
Factor 3: 1WM	16.0	-0.21	-0.33	0.12	0.19	0.01	{0.88}	0.32	0.07	{0.60}
Old										
Factor 1: WM	34.7	-0.51	{0.60}	{0.68}	{0.67}	{0.66}	0.39	{0.75}	0.37	-0.56
Factor 2: GENPFC	20.1	{-0.65}	-0.12	0.48	0.07	-0.3	0.49	-0.24	{-0.61}	{0.68}
Factor 3: 1WM	12.1	0.05	0.22	0.06	-0.51	-0.47	{0.59}	0.25	0.38	0.09

¥All factors with eigenvalues > 1.0 are reported; Components with significant loading on factor (>0.6) are enclosed in parentheses; Abbreviations referred to: %Var = percent of total cognitive variance explained by each factor; CAT = Conditional Association Task; Pic = design load condition of the Self-Ordered Pointing Task; OAT = Object Alternation Task. WM = Working Memory Factor; GENPFC = General PFC Factor; 1WM = Single Item Working Memory Factor

TABLE 8. Correlation Table of Relationships between Cognitive Task Factors and Regional Volumes.¥

	Superior	*Middle*	*Inferior*	*Orbital*
WM	**-0.34**	**-0.12**	**-0.08**	**-0.50***
General PFC	**-0.48***	**-0.34**	**-0.07**	**-0.01**
Single Item	**-0.01**	**-0.14**	**-0.36**	**-0.16**

¥ Pearson's correlation r values presented.

* $p < 0.05$

Abbreviations referred to: WM = Working memory factor; General PFC = General PFC cognitive factor; Single Item = Single item working memory factor.

6.10 FIGURE LEGENDS:

Figure 1. Cognitive tasks differentially supported by regions within the PFC. See text for full descriptions of prefrontal tasks. A. The Conditional Association Task. Subjects were to choose a particular line conditional upon the color of the line at the top of the monitor. For this example, the top center design is highlighted to demonstrate the choice of this design every time the associated colored line (the purple line) appeared above the designs. B. The Self-Ordered Pointing Task. Examples of the 8-design working memory load condition (top panel) and 12-design working memory load condition (bottom panel). In each condition, subjects were to choose a design on each and every page in the set without choosing the same design more than once. C. The N-Back Task. The example shows the 2-Back working memory load and 0-Back (control) conditions of the task. Subjects viewed a string of letters presented sequentially on the screen, one at a time. In the 2-Back condition, subjects were to respond as targets to any letter that repeated itself two letters back in the sequence (i.e. Any letter that repeated itself separated by one letter in the sequence). In this example, the letter 'a' is repeated two letters back, separated by one letter ('q'). In the 0-Back condition, subjects were to respond every time they saw a specific letter, and this letter did not have to be repeated. In this example, the subject responds as a target to every 'q' in the sequence. D. The Object Alternation Task. Subjects had to learn that the best strategy for performance of the task was to alternate between choosing the circle and

square on each trial, regardless of the objects spatial position (to the left or right of the other object). Thus, subjects would choose the circle on trials 1, 3, 5, 7, 9, etc. And the square on trials 2, 4, 6, 8, 10, etc. For optimal performance.

Figure 2. Cognitive performance on PFC cognitive battery in young and older subjects. A. Older subjects made more errors per trial on both the Conditional Association Task and the Object Alternation Task (*ps < 0.01). B. Older subjects performed worse than younger subjects on all working memory loads of the Self-Ordered Pointing Task ($p < 0.001$). Both young and older subjects performed worse with increasing working memory load ($p < 0.001$). There was a significant interaction with greater differences between groups at the higher working memory loads ($p < 0.001$). C. Older subjects performed worse than younger subjects on all working memory loads of the N-Back task ($p < 0.001$). D. Younger subjects had a significantly greater number of targets correctly identified in all working memory loads of the N-Back task ($p < 0.001$). Both younger and older subjects decreased the number of correct responses with increasing working memory loads ($p < 0.001$).

Figure 3. Scattergrams of performance on PFC cognitive battery and age in younger subjects. There was a relationship between age and performance on A. 3-Back condition, B. all N-Back conditions combined, C. and the 2-Back condition of the N-Back task in the younger subjects.

Figure 4. Scattergrams of performance on PFC cognitive battery and age in older subjects. There was a relationship between age and percent correct of A. all

load conditions of the Self-Ordered Pointing Task combined, B. the 2-Back condition of the N-Back task, and C. total performance on all conditions of the N-Back task. Other relationships existed but are not shown in the figure.

Figure 5. Cartoon representation of volumetric method used to calculate prefrontal region of interest (ROI) volumes. The images are the same as those used in data collection but regional demarcations have been smoothed for publication purposes. Total prefrontal volume, prefrontal white matter volume, and subregion parcellation for all subjects were determined by edge tracing the cortical ribbon and gray/white boundary with a cursor directly on a computer display. A. The most posterior slice of the prefrontal ROI is shown. Total prefrontal volume was defined by tracing all gyri and sulci around the cortical ribbon of each hemisphere in the T_2-weighted image. Pixel areas were transformed to volumes as described in text. B. Prefrontal white matter volume was traced in the proton-density weighted image of the same exact slice as A. Prefrontal gray matter volume was calculated by subtracting prefrontal white matter volume from total prefrontal volume. C. Left panel shows the unaltered T_2-weighted image of four posterior coronal PFC slices (top = most posterior). Right panel shows regional delineations on the exact same slices for superior (yellow), middle (pink), inferior (orange), orbital (green), and anterior cingulate (blue) regions. Subregions were defined as described in the text.

Figure 6. Scattergram of correlation between age and orbital PFC volume. Older subjects had larger orbital PFC volumes.

Figure 7. Scattergrams of correlations between cognitive task performance and regional volumes. A. Greater superior PFC volumes were associated with more errors per trial on the Conditional Association Task, B. Greater orbital PFC volumes were associated with worse performance on the working memory composite.

Figure 8. Scattergrams of partial correlations between cognitive task performance and regional volumes when controlling for the shared variance in volume among regions. A. Greater superior PFC volumes were associated with more errors per trial on the Conditional Association Task, B. Greater middle PFC volumes were associated with better performance on the working memory composite, C. Greater orbital PFC volumes were associated with more errors per trial on the Object Alternation Task, and D. Greater orbital PFC volumes were associated with worse performance on the working memory composite.

Figure 9. Scattergram of partial correlations between cognitive task performance and regional volume when controlling for the shared variance in performance among tasks. Greater orbital PFC volumes was associated with worse performance on the working memory composite.

Figure 10. Scattergrams of correlations between cognitive factors obtained in the principal components analysis and regional volumes. A. Greater superior PFC volumes were associated with worse performance on the Conditional Association Task and better performance on the 3-Back condition of the N-Back task and less errors per trial on the Object Alternation Task (loadings on the

general PFC factor), B. Greater orbital PFC volumes were associated with worse performance on the Working Memory Factor.

6.11 Reference List

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Figure 1.

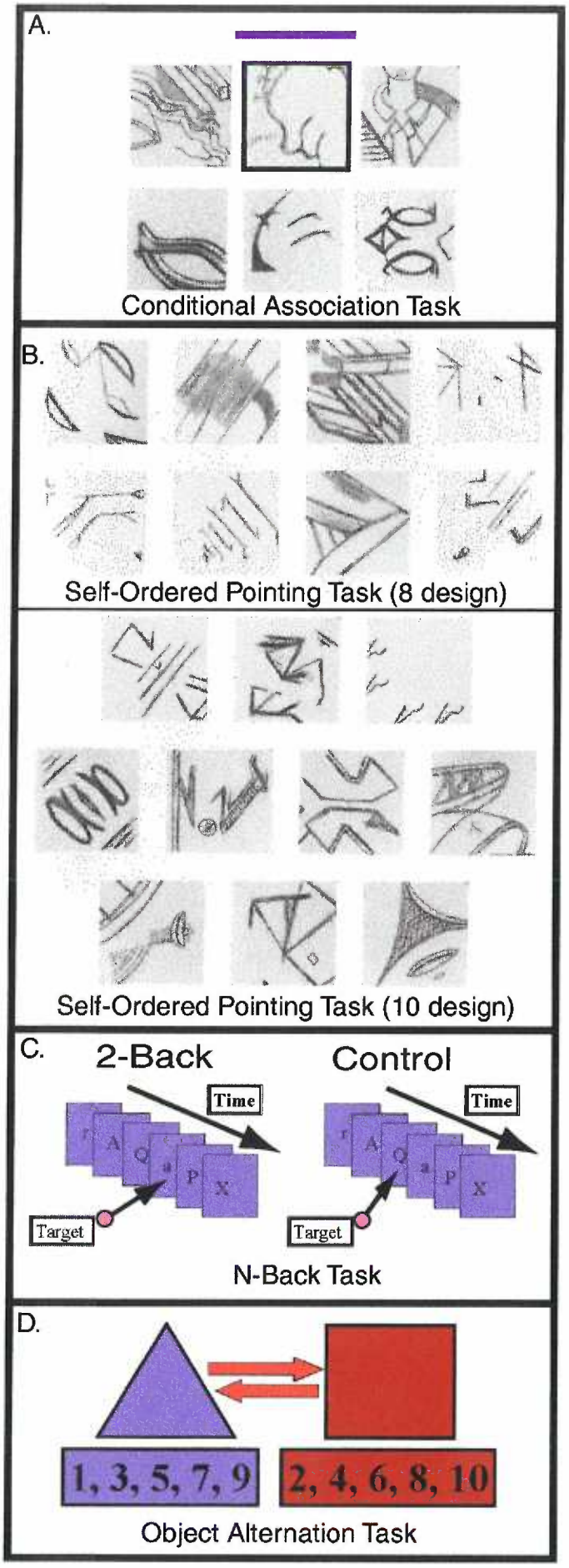

A.
Conditional Association Task
B.
Self-Ordered Pointing Task (8 design)
Self-Ordered Pointing Task (10 design)
C.
2-Back
Control
Time
Time
Target
Target
N-Back Task
D.
1, 3, 5, 7, 9
2, 4, 6, 8, 10
Object Alternation Task

Figure 2.

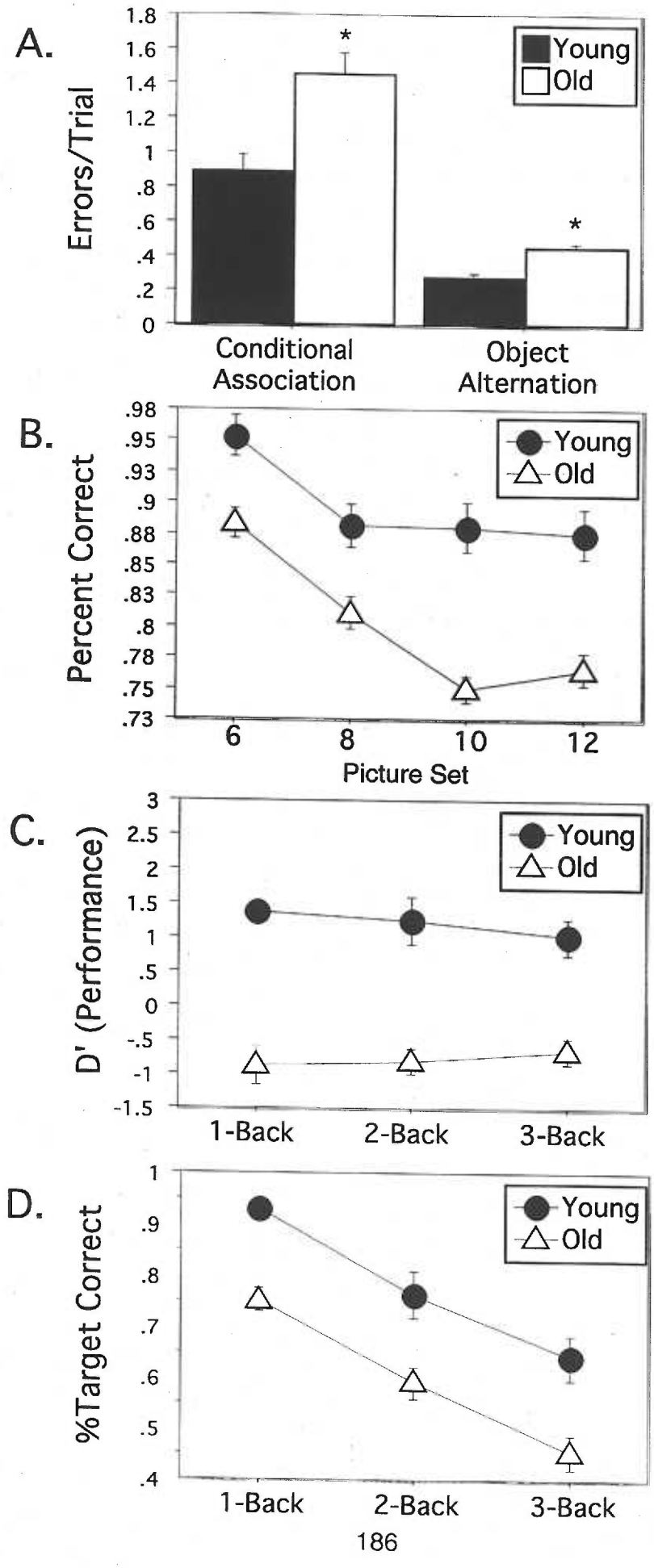

A.
Errors/Trial
1.8
1.6
1.4
1.2
1
.8
.6
.4
.2
0
*
*
Young
Old
Conditional Association
Object Alternation
B.
Percent Correct
.98
.95
.93
.9
.88
.85
.83
.8
.78
.75
.73
Young
Old
6
8
10
12
Picture Set
C.
D' (Performance)
3
2.5
2
1.5
1
.5
0
-.5
-1
-1.5
Young
Old
1-Back
2-Back
3-Back
D.
%Target Correct
1
.9
.8
.7
.6
.5
.4
Young
Old
1-Back
2-Back
3-Back

Figure 3.

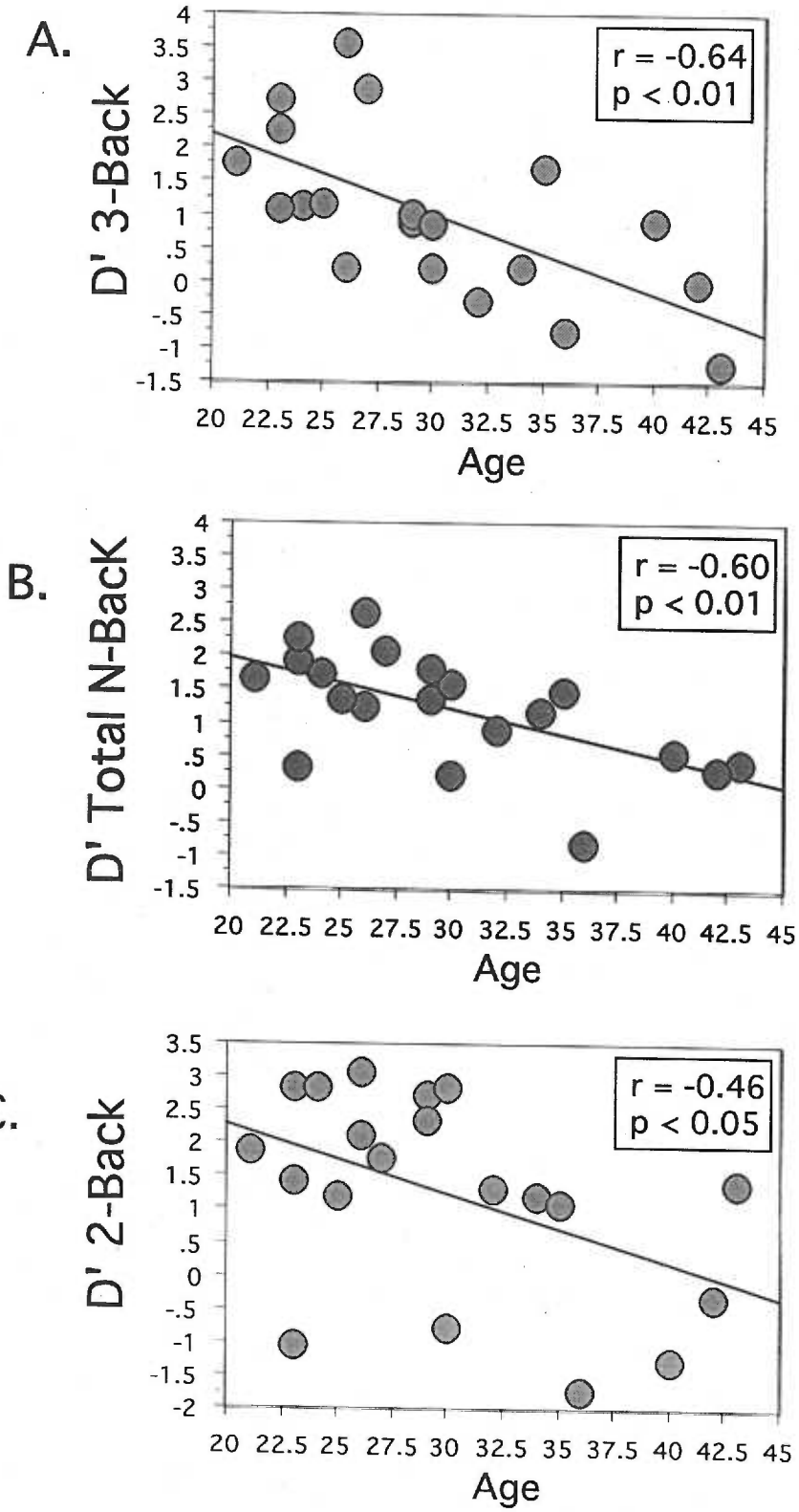
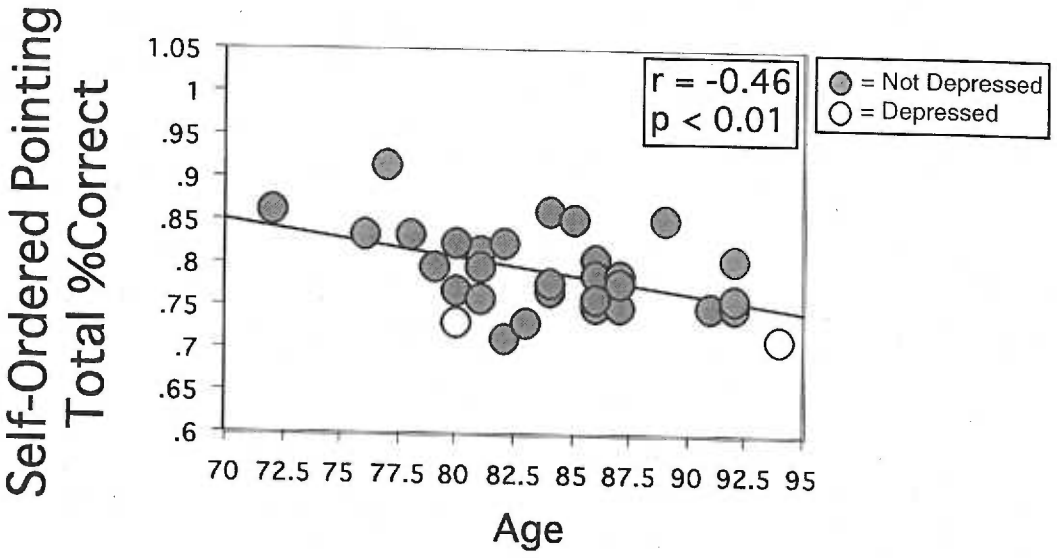

A.
D' 3-Back
r = -0.64
p < 0.01
Age
B.
D' Total N-BacK
r = -0.60
p < 0.01
Age
C.
D' 2-Back
r = -0.46
p < 0.05
Age

Figure 4.

A.


Self-Ordered Pointing
Total %Correct
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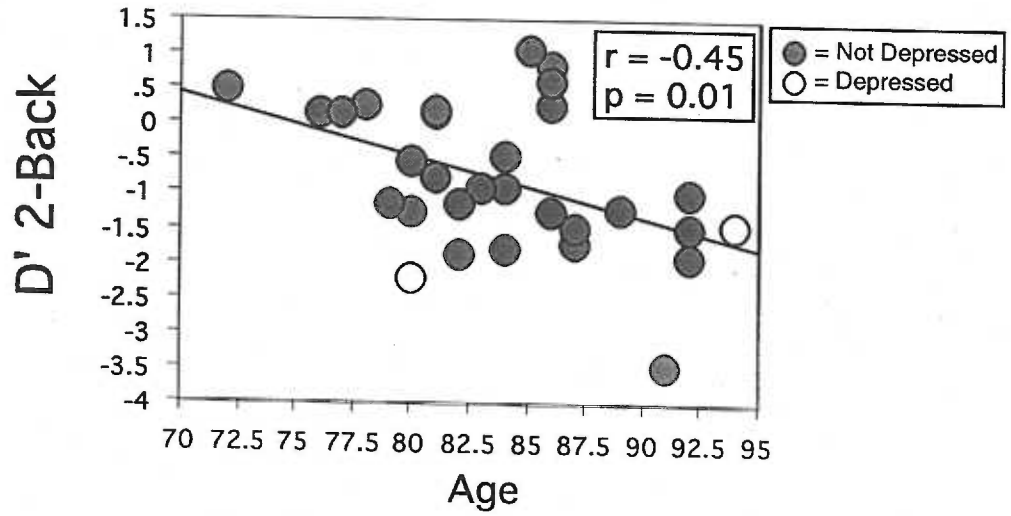
1
.95
.9
.85
.8
.75
.7
.65
.6
r = -0.46
p < 0.01
= Not Depressed
= Depressed
70 72.5 75 77.5 80 82.5 85 87.5 90 92.5 95
Age

B.


D' 2-Back
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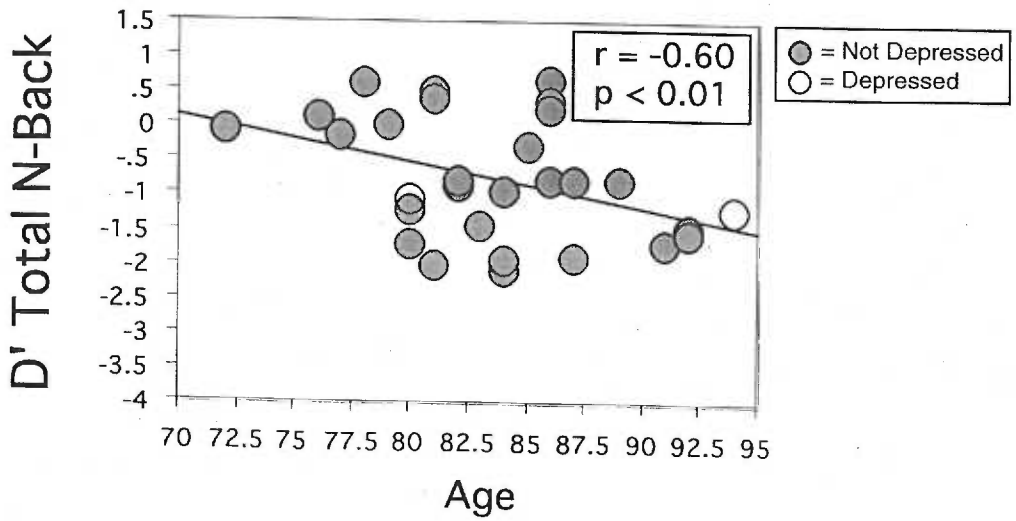
1
.5
0
-.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
r = -0.45
p = 0.01
= Not Depressed
= Depressed
70 72.5 75 77.5 80 82.5 85 87.5 90 92.5 95
Age

C.


D' Total N-Back
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.5
0
-.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
r = -0.60
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= Not Depressed
= Depressed
70 72.5 75 77.5 80 82.5 85 87.5 90 92.5 95
Age

Figure 5.

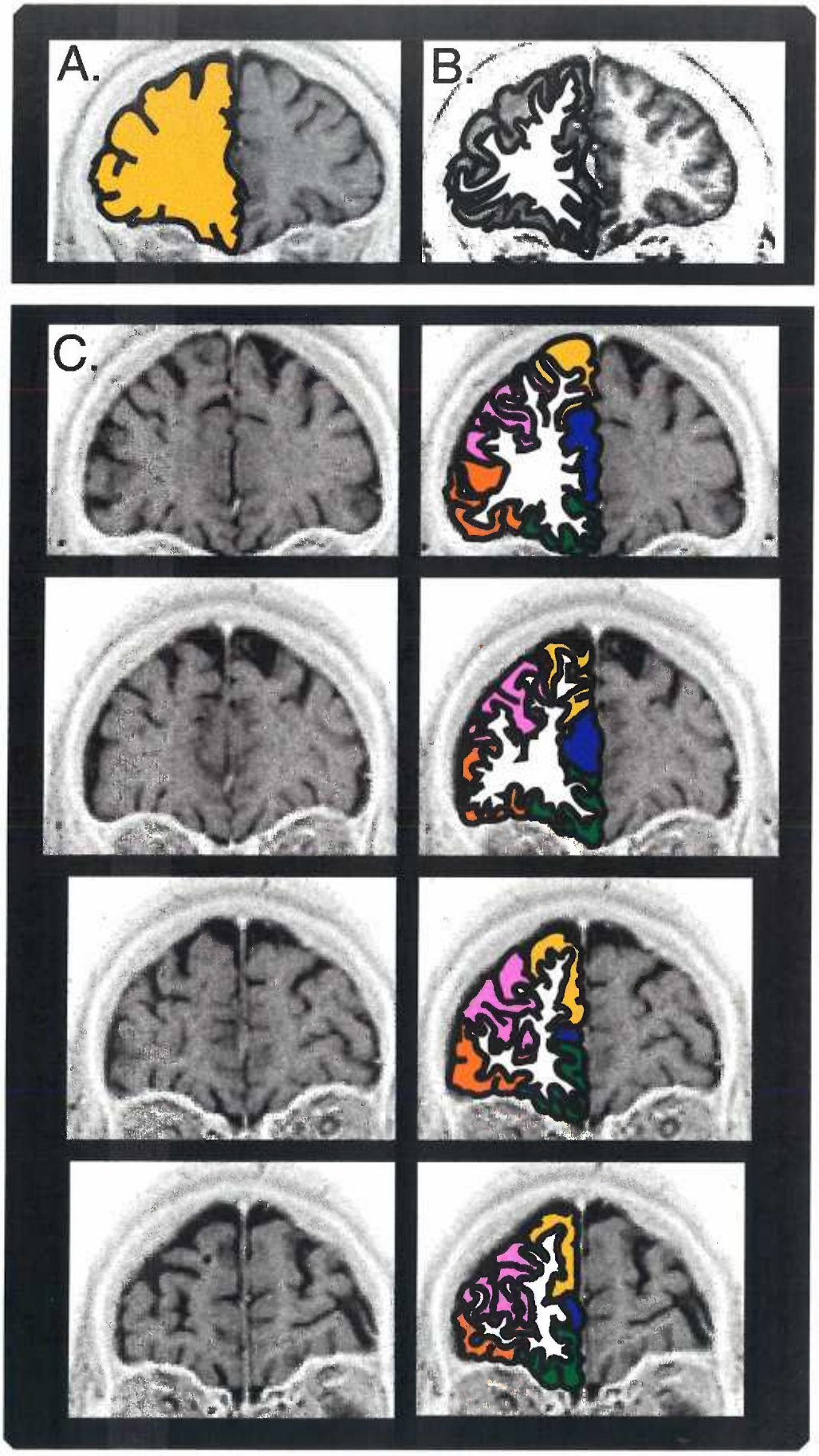


Figure 6.

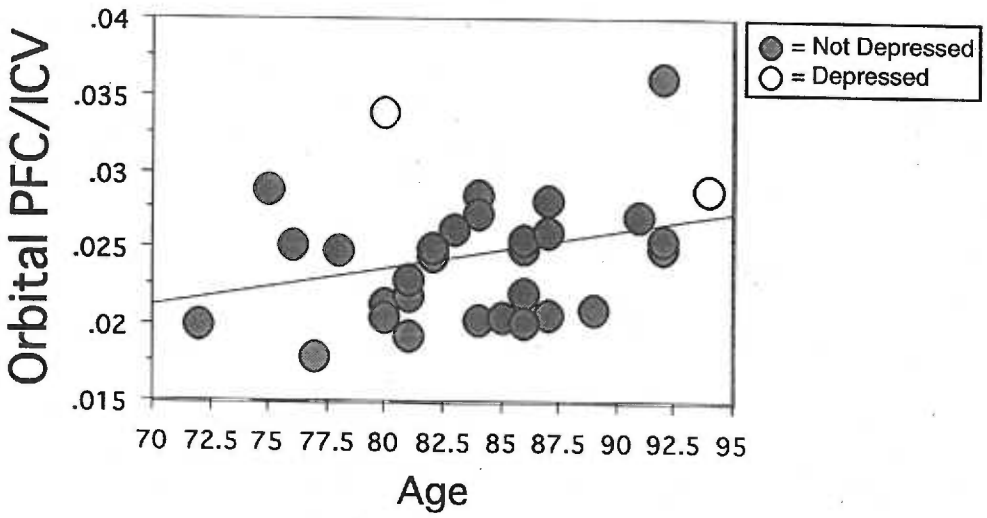
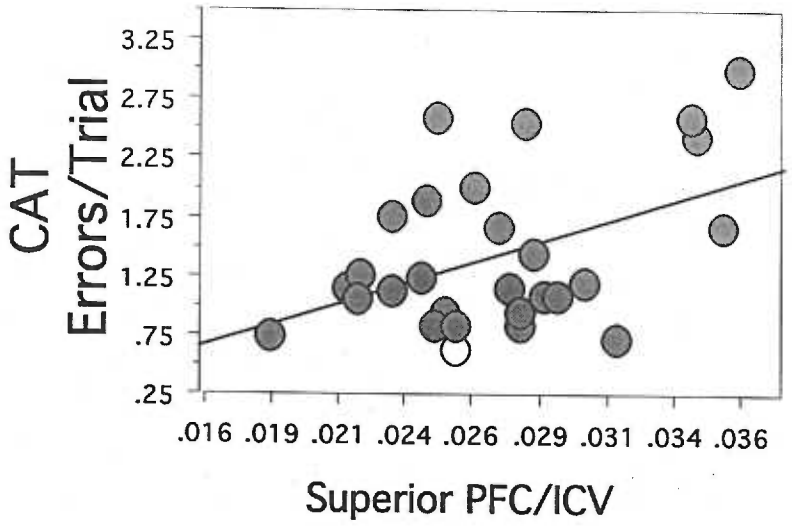

Orbital PFC/ICV
.04
.035
.03
.025
.02
.015
70 72.5 75 77.5 80 82.5 85 87.5 90 92.5 95
Age
= Not Depressed
= Depressed

Figure 7.

A.


CAT Errors/Trial
3.25
2.75
2.25
1.75
1.25
.75
.25
.016 .019 .021 .024 .026 .029 .031 .034 .036
Superior PFC/ICV

B.

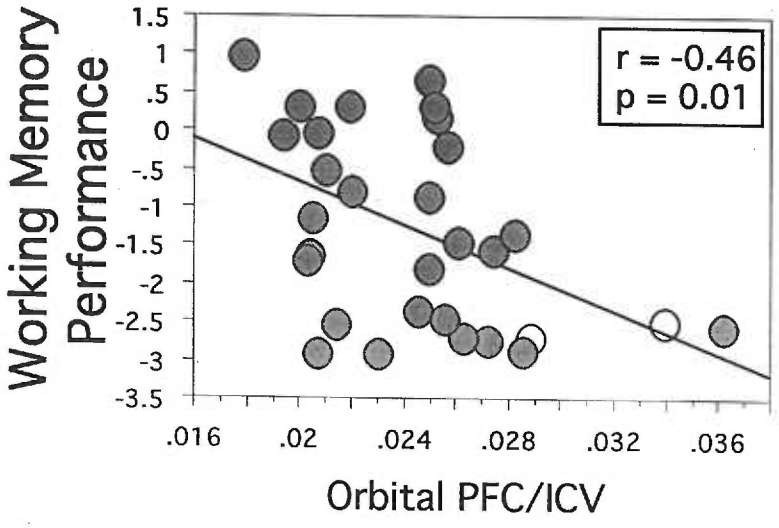
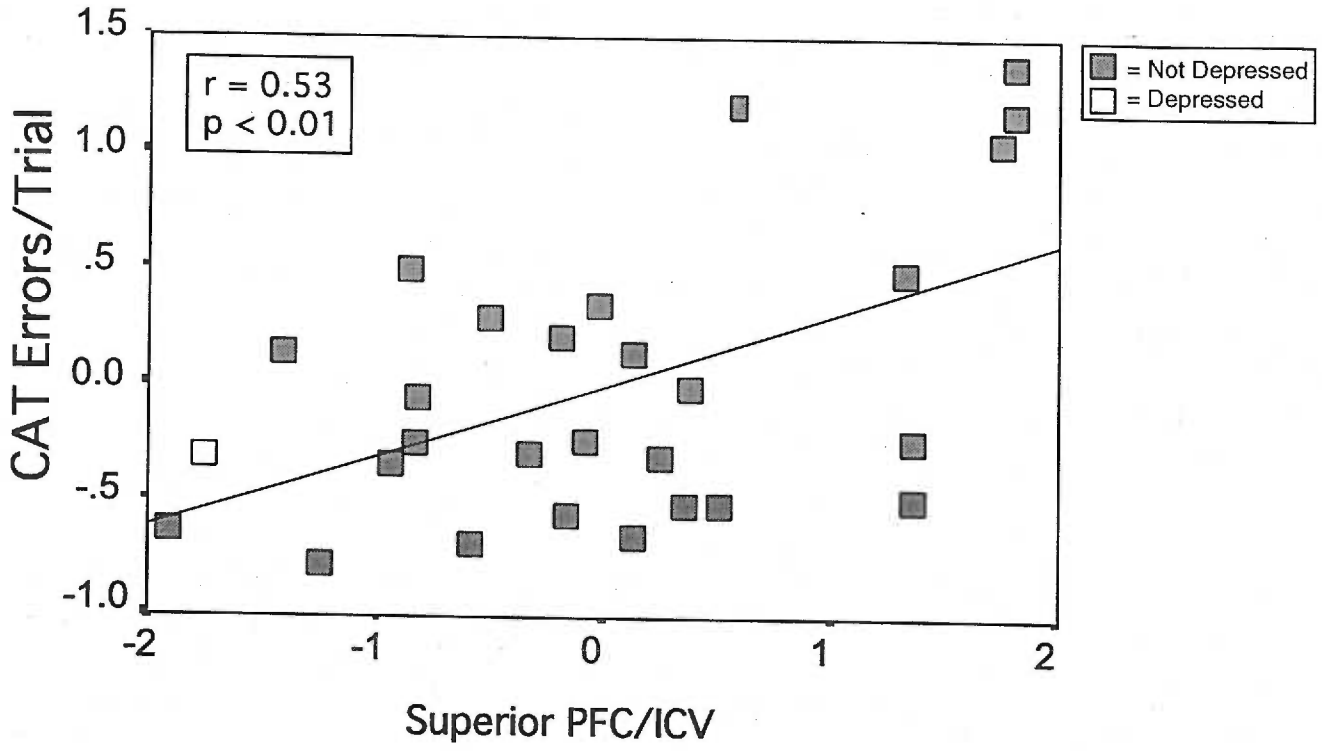

Working Memory Performance
1.5
1
.5
0
-.5
-1
-1.5
-2
-2.5
-3
-3.5
r = -0.46
p = 0.01
.016 .02 .024 .028 .032 .036
Orbital PFC/ICV

Figure 8.

A.


r = 0.53
p < 0.01
= Not Depressed
= Depressed
CAT Errors/Trial
1.5
1.0
.5
0.0
-.5
-1.0
-2
-1
0
1
2
Superior PFC/ICV

B.

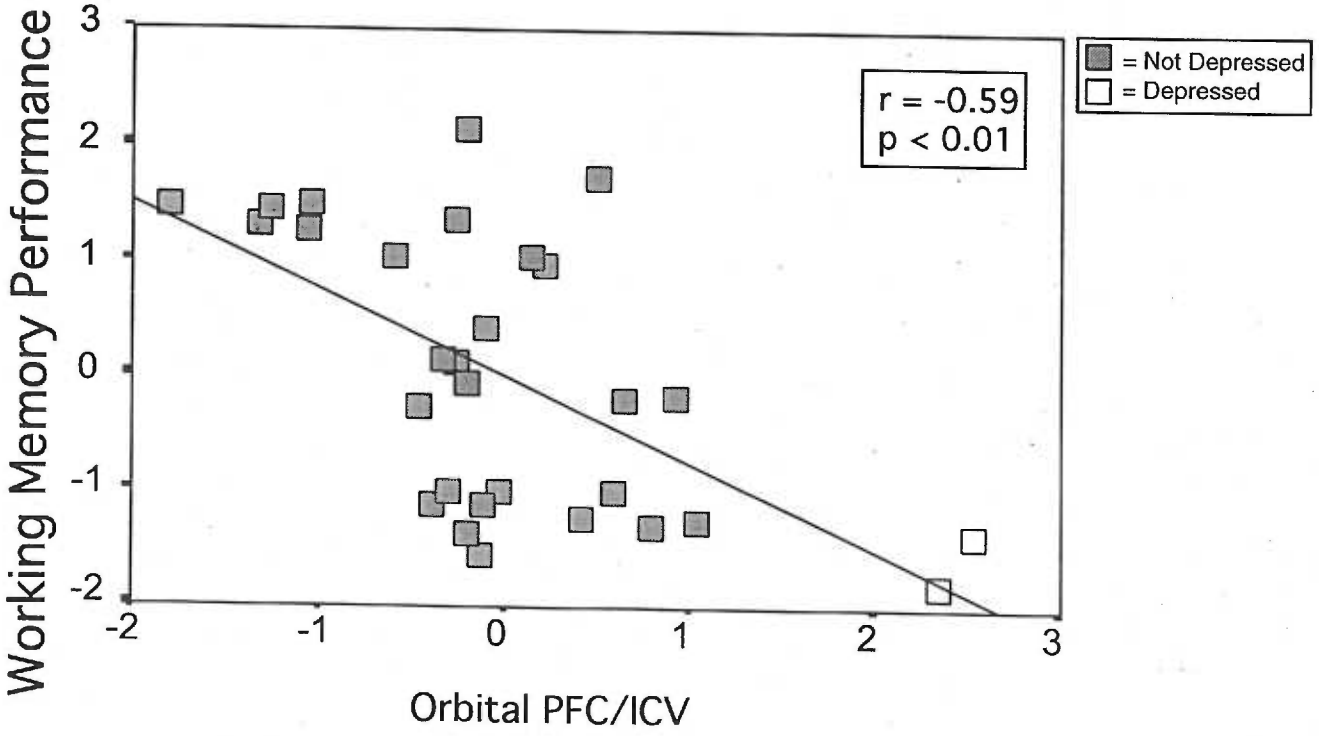
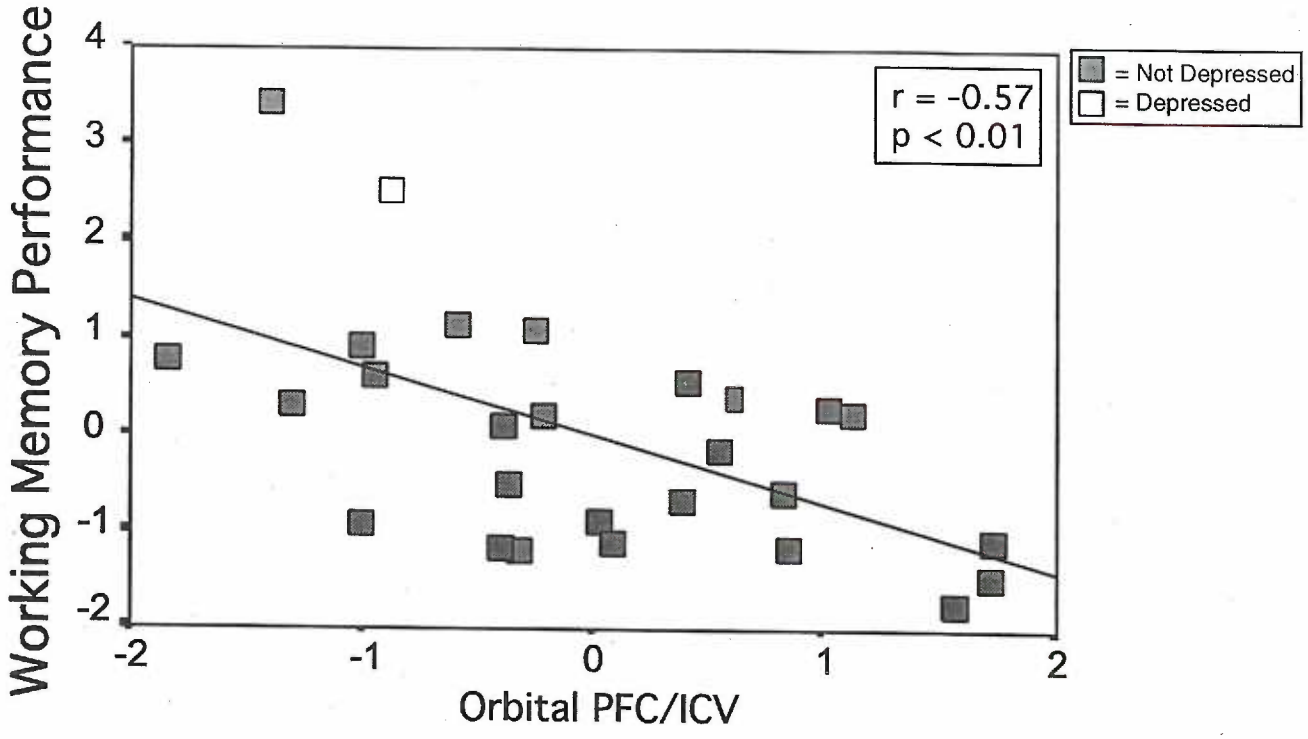

r = -0.59
p < 0.01
= Not Depressed
= Depressed
Working Memory Performance
3
2
1
0
-1
-2
-2
-1
0
1
2
3
Orbital PFC/ICV

Figure 9.

A.


Working Memory Performance
4
3
2
1
0
-1
-2
-2
-1
0
1
2
Orbital PFC/ICV
r = -0.57
p < 0.01
= Not Depressed
= Depressed

7. GENERAL DISCUSSION

7.1 Prefrontal structure and function are both altered with healthy aging. There are a number of changes that occur in the PFC of both healthy older people and people with AD. Healthy oldest old lose a greater amount of white matter compared to gray matter. It is possible that the reverse is true with AD. Although there was a significant loss of white matter in Experiment 1, changes in gray matter were more profound in AD, particularly in the larger sample of subjects examined in Experiment 2. Experiment 2 showed there is a preservation of the volume of orbital PFC with healthy aging and greater degeneration in the inferior PFC with AD compared to other prefrontal subregions. Experiment 3 showed that older subjects perform worse than younger subjects on a battery of tasks of prefrontal function. Older subjects showed the greatest deficits on tasks of working memory, and working memory abilities explained the majority of the variance in prefrontal cognitive performance. Experiment 3 also showed that the seeming preservation of orbital PFC volume with healthy aging could actually be related to pathologic changes as larger orbital PFC volumes predicted worse working memory performance. Greater volume in the superior prefrontal region also predicted worse performance on prefrontal cognitive parameters.

Degeneration of gray matter is not uniform but differs according to regional divisions. Although absolute regional gray matter measurements did not differ in younger and older subjects, there was a marginal decline in total gray matter with healthy aging (Experiment 2, Study 1, $p = 0.06$) and it is

apparent that some regions may degenerate more than others (e.g. the middle and inferior PFC regions; see Experiment 2 Figure 3). Because regional volumes were related to performance on tasks supported by those regions, it is possible that this marginal loss of gray matter with healthy aging contributes regionally to selective cognitive decline in the older population. These studies suggest the need for a closer, evaluation of changes in white matter, orbital PFC, and performance on tasks of working memory with healthy aging and inferior frontal degeneration in AD. Longitudinal approaches to brain aging (Kaye et al., 1997) would be appropriate for this task.

7.2 Contrasting aging and AD. There were both differences and similarities in the patterns of degeneration with healthy aging and AD. The greater loss of white matter than gray matter with healthy aging was absent in subjects with AD suggesting that pathological processes target white matter to a greater degree with healthy aging. In contrast, the regional analyses of Experiment 2 did not point to qualitative as much as quantitative differences between healthy aging and AD except in the inferior PFC region (e.g. compare Experiment 2, Figures 3a and 5a). Comparisons of age and AD related changes relative to the younger healthy group suggest a similar pattern of gray matter degeneration in healthy aging and AD. Still, AD subjects had a significant decrease in volume in the inferior PFC region, supporting theories that secondary cortical degeneration occurs in structures with temporal lobe and limbic connections with AD (Braak and Braak, 1991). Thus, degeneration of white matter could be

selective to healthy aging whereas degeneration in inferior PFC could be accelerated in AD.

7.3 Mechanisms of degeneration in healthy aging and AD. The finding of white matter degeneration with healthy aging is consistent with the post-mortem literature in both humans (Kemper, 1994) and monkeys (Peters et al., 1991; Peters, 1996). Mechanisms of white matter degradation are currently unknown. The myelin balloon is an abnormal swelling of the myelin found with aging that causes splitting of the myelin sheath (Feldman and Peters, 1998). Also, age-related inclusions are apparent in the cell bodies of oligodendroglia (Peters, 1996) that could be toxic to the cell. One theory asserts that excessive free radical damage due to the highly peroxidizeable phospholipids in myelin leads to white matter breakdown (Weber, 1990). Oligodendroglia also have low levels of glutathione and high iron content and are more dependent on oxidative phosphorylation than other glial cells such as astrocytes. These are all factors contributing to potential damage by reactive oxygen species (Juurlink, 1997; Juurlink and Sweeney, 1997). Both plasma antioxidant levels (Schmidt et al., 1998) and myelin integrity (Peters et al., 1996) have been related to cognitive function. Thus, it is possible that oxidative damage to white matter could affect cognition in the healthy older subjects of this study. White matter volume was related to only one measure in Experiment 3 (the Conditional Association Task, data not shown) and the lack of correlation with working memory measures suggests that alterations in gray matter are more strongly

causal in working memory cognitive decline. Other studies have shown that performance on a delayed memory task correlate with the thinning of layer 1 in area 9/46 of the rhesus monkey (Peters et al., 1998), supporting the importance of gray matter in working memory. A longitudinal examination of the relationship between plasma antioxidant levels, prefrontal white matter volume, and performance on tasks of prefrontal function in older subjects with antioxidant supplementation would be of interest in determining if oxidative stress contributes to age-related cognitive decline.

The trend towards gray matter loss in healthy aging (Experiment 2 Study 1), could be a secondary result of a demyelination of axons. This theory would be in accord with more recent studies suggesting that earlier post-mortem reports of neuronal death are more artefactual than real and are likely greatly exaggerated (Haug et al., 1984). In contrast to healthy aging, white matter changes in AD (Experiment 1) could be independent or a secondary result of the disease process as suggested by Englund and Brun (1990). Multiple mechanisms of neuronal degeneration have been proposed for AD (Mattson, 1994; Dickson, 1997; Vickers, 1997; Kanfer et al., 1998; Lin et al., 1999). One theory suggests that plaque deposition creates channels in neurons that allow toxic levels of calcium into the cell resulting in cell death (Lin et al., 1999). Other studies have examined the role of beta amyloid in free radical damage with the disease (Keller et al., 1999). It is likely that multiple mechanisms contribute to final degenerative stages. Histological studies examining damage of white matter due to reactive oxygen species in healthy aging and unbiased

stereological (Geinisman et al., 1992) neuronal and glial counts in the orbital and inferior PFC regions with healthy aging and AD would be useful in understanding the pathology underlying degeneration of the PFC.

7.4 Orbital PFC Alteration With Healthy Aging. Experiment 3 confirmed that there are indeed differential age-related alterations in subregions of the PFC. In contrast to expectations, there was an *increase* in the volume of the orbital PFC with increasing age. This result is supported by the fact that the OHE subjects had a greater (but not significantly so) absolute mean volume in the orbital PFC region compared to YHE in Experiment 2 (See Experiment 2 Figure 3). These findings suggest that the seeming 'preservation' of the orbital PFC region could actually result in pathological function in the region. The volume of specific regions predicted performance on specific cognitive tasks supported by those subregions, though the relationships typically showed that larger structures were associated with worse performance. These findings support the theory that larger volumes could represent very early pathological change in previously healthy older subjects.

Volumetric studies are limited as alterations in regional volumes measured by imaging procedures could be related to a number of factors. For example, there are a number of potential reasons why the volume of a region might be preserved or increased even though degenerative processes are occurring in the region. First, an increase of up to 44% of microglia has been found in area 17 of older compared to younger monkeys (Peters et al., 1991).

Astroglia also proliferate in the frontal cortex of subjects over 70 years (Hansen et al., 1987) and oligodendroglia swell with aging (Feldman and Peters, 1998). Neuronal hypertrophy has been found in subjects with dementia (Vogels et al., 1990; Iraizoz et al., 1991) suggesting that volumetrically preserved structures could be a precursor to subsequent degeneration and cognitive decline. Moreover, cell hypertrophy in the medial septal area was associated with behavioral impairment in a study of aged monkeys (Stroessner-Johnson et al., 1992). Thus, the seeming 'preservation' of this area could actually represent pathological changes in the region. The finding of less volume loss in this region compared to other regions with aging (Experiment 2 Study 1), a similar trend in AD (Experiment 2 Study 2), an increase in the volume of this region with age (Experiment 3 Study 2), and the negative relationship between the volume of this region and cognitive performance (Experiment 3 Study 2) all argue that changes in orbital PFC might signal pathology in older subjects. It is unlikely that these results are simply due to a cohort effect as a correlation between orbital PFC volume and age was found in the fairly limited age-range of Experiment 3. Still, these results would have to be interpreted in the context of findings that there is significant volume loss detectable in the temporal lobes of very early preclinical stages of AD (Kaye et al., 1997). Also, these discrepancies demonstrate the limitations of volumetric studies as volume loss could indicate different processes in different neural regions and different health conditions. Longitudinal studies examining the volumetric progression of orbital PFC from preclinical to clinically apparent AD could be useful in determining if

tissue hypertrophy precedes dementia or subsequent volume loss.

7.5 Cognitive decline with healthy aging. Studies are still equivocal as to whether cognitive decline is a necessary result of physiologic aging (Rowe and Kahn, 1987; Rapp and Amaral, 1992). Some deterioration is clearly the norm. Older subjects perform worse than younger subjects on a number of cognitive tasks, including all tasks in the prefrontal battery of Experiment 3. Performance on tasks of working memory, and particularly self-ordered working memory, explained a substantial portion of the variance in all prefrontal task performance and was most affected by aging (as demonstrated by the effect size comparisons in Experiment 3 Study 2). Working memory or a subprocess of working memory tasks could therefore be of interest in the study of therapeutic interventions for age-related cognitive decline.

It is important to understand the true nature of decline in performance on working memory tasks in order to develop more specific therapeutic strategies. Prior studies have examined types of errors and suggest that deficits in working memory might be due to perseverative or defective inhibitory processes. For example, older subjects make more perseverative responses on the Self-Ordered Pointing Task than younger subjects (West et al., 1998). Also, a younger cohort of older subjects who used a strategy performed as well as younger subjects on the same task (Daigneault and Braun, 1993). Use of a strategy (especially a successful one) could potentially be a method to overcome degraded inhibitory mechanisms. Data from Experiment 3

corroborate the theory that disinhibitory and perseverative mechanisms play a role in performance of the working memory tasks examined. Older subjects make a large number of perseverative errors (choosing the same design sequentially on 2 or more cards) on the Self-Ordered Pointing Task (personal observation, data not shown) as previously reported (West et al., 1998). On the N-Back task, older subjects have quicker reaction times for incorrect target responses compared to correct target responses. Younger subjects do not show this reaction time difference, suggesting that older subjects could have been disinhibited in their incorrect responses leading to quicker reaction times (data not shown). Also, older subjects tend to 'correct' an incorrect response on the N-Back more often than younger subjects (pressing the target button after incorrectly pressing nontarget button; personal observation). Although subjects do not get credit for this response correction, this phenomenon suggests that older subjects do not necessarily have a storage or updating working memory deficit but in fact are disinhibited and make the prepotent response (i.e. responding 'nontarget'). These theories would be consistent with the relationship between performance and alterations in orbital PFC, a region involved in inhibitory and response shifting processes. Alternatively, alterations in working memory performance could be a result of dysfunctional connections between medial temporal lobe structures and the PFC. Specifically, orbital PFC has strong connections with medial temporal lobe structures (Barbas, 1995). Although the PFC seems to be more critical for the performance of WM tasks, it is clear that there is some involvement of the medial temporal lobes (Petrides,

1982). Thus, disruption in the function of the greatest medial temporal input region of the PFC could be responsible for decline in WM performance. Studies examining the contribution of inhibitory cognitive set shifting processes to the performance of tasks of working memory and the relationship between these processes and orbital PFC function will be useful in understanding neural and cognitive alterations that result in working memory deficits with aging.

Other cognitive processes, such as conditional association learning, could decline earlier and more rapidly than working memory with aging. For example, there was a trend towards a relationship between age and Conditional Association Task performance in the younger subjects that was absent in older subjects, suggesting that age-related decline in performance of this task is at a maximal level before the older age-range studied. Moreover, the factor loading of performance on this task changed in the young subjects compared to the older subjects suggesting that a profound alteration in performance of this task relative to the other PFC tasks occurs from the younger to older age-range. These results suggest that conditional association learning could be selectively and greatly altered in middle age compared to other PFC cognitive functions. Also, performance on the Conditional Association Task was related to superior PFC volume suggesting further study of this cognitive process with aging.

White matter loss with aging could potentially affect cognition in at least two ways. First, because myelin is instrumental in rapid neuronal signaling, white matter loss could simply slow cognitive processing as suggested in

neuropsychological studies (Salthouse, 1992). This slowing could be manifested in the disinhibited responses described above. For example, older subjects could be delayed in identifying a target letter in the N-Back task, and subsequently respond correctly when the signal is received. Alternatively, differential regional degeneration of white matter could disrupt critical timing of neuronal messages causing a dys-integration syndrome among neural regions. For example, white matter loss could be related to a lack of coordination or gait disruption leading to falling often reported with older people (Greenhouse A.J., 1999) similar to the disturbances of gait reported in even mild multiple sclerosis (Benedetti et al., 1999), a disease of myelin.

It has been questioned whether the cognitive decline with aging is a simple progression from a decline in the most difficult measures to the more simple tasks or if decline is related to the dysfunction of specific neural systems. An evaluation of the divergence in performance between young and older subjects shows the latter is more likely. The effect sizes showed that fewer errors per trial are made on the Object Alternation Task compared to the Conditional Association Task (Experiment 3 Study 2), even when correcting for the number of possible errors per trial on each task (data not shown). The larger number of errors suggests that the Conditional Association Task is more difficult than the Object Alternation Task. Still, there were greater differences between younger and older subjects on the Object Alternation Task than the Conditional Association Task. Similarly, in the N-Back Task there was a greater difference between older and younger subjects on easier working memory load conditions

(1-Back condition) than more difficult working memory load conditions (the 2 and 3-Back conditions) suggesting that increased difficulty has a greater effect on performance of younger compared to older subjects. This result is not likely due to a floor effect in the older subjects as this group correctly identified approximately 45% of the target letters correctly in the 3-Back condition. One potential explanation is that cognitive strategies used by younger subjects and not older subjects are less effective in the more difficult conditions of the task. Support for this assertion comes from prior studies demonstrating comparable performance between younger subjects and older subjects when older subjects use a strategy to perform the task (Daigneault and Braun, 1993).

7.6 Successful or normal agers? The normal subjects of all three studies were originally selected for their optimal health status in a variety of measured physiological domains. The study of 'successful aging' has suggested that physiological changes are not a product of chronological age, *per se*, and instead a product of age-related epiphenomena. It was possible, then, to expect that changes in this sample of older subjects would be minimal compared to the younger cohort. This theory was both true (minimal loss of gray matter) and not true (great loss of white matter). Also, a large cognitive decline was observed in these subjects. Still, it is unclear how the results of this study relate to changes in less healthy subjects. Also, there was a significant amount of variability in the neural changes and cognitive performance of these subjects suggesting that even 'successful aging' is a heterogeneous phenomenon. The data from the

studies presented could be useful for understanding the most likely changes to occur when multiple other physiologic processes are functioning well.

7.7 Limitations of the presented studies. Imaging studies provide a unique opportunity to study the living human brain, yet are limited for a number of reasons. First, the development of volumetric methods requires reliable reproduction of data collection and the validation of the methods using a phantom object or *post-mortem* tissue. The methods used in the studies presented were reliable, yet validation of the techniques in *post-mortem* tissue has not been achieved. Also, although differences in regional volumes can be detected among groups, these data provide little information as to the underlying neural change. Differences in volume could thus be due to loss of synapses, dendritic recession, neuronal death, change in neuronal size, or a number of other mechanisms. Also, changes in volume could mean different things in different regions and different conditions. While temporal lobe volumes could decline early in the AD process (Kaye et al., 1997), it is possible that other regions exhibit a 'hypertrophy' with early disease processes (See Experiment 3). Thus, volumetric studies provide a first step in the analysis of neural degeneration. Future studies using other imaging techniques (e.g. MR spectroscopy and MR microscopy) and histological procedures (e.g. stereology and numerous other histochemical techniques) would be useful in further analysis of volumetric change.

The presented studies are also limited for being cross-sectional. Thus,

differences or lack of differences among groups could be due to cohort some sort of cohort effect as opposed to being a reflection of regional change across time. Also, the subjects in the studies presented were selected to be optimally healthy. It is possible that optimally healthy oldest old could have particular neural features that differentiate them from their younger cohort, as all subjects in the younger cohort will not likely age as optimally as the oldest old studied. Thus, longitudinal imaging studies and *post mortem* histological studies of aged humans and nonhuman primates will be necessary to further explore the findings of the presented studies.

7.8 Potential physiological mechanisms of orbital PFC contributions to working memory performance. The orbital PFC is theorized to serve a number of functions in humans. Behavioral inhibition (Rolls et al., 1994; Damasio, 1996; Starkstein and Robinson, 1997; Murphy et al., 1999), decision making (Bechara et al., 1997; Bechara et al., 1998), guessing (Elliott et al., 1999), and social cognition (Adolphs, 1999) have all been related to this and similar ventromedial regions of the brain. Damasio's somatic marker hypothesis argues that the orbital PFC region is involved in controlling physiological processes (specifically, galvanic skin response) that are used by the subject for nonconscious biasing of decisions (Damasio, 1996) and studies have shown that orbital PFC damage is accompanied by defects in galvanic skin response and additionally by poor decision making compared to nonlesioned subjects (Bechara et al., 1997). A decrease in skin response to various stimuli has been

reported with aging (McDowd and Filion, 1992; Drory and Korczyn, 1993; Eisenstein et al., 1995). It is possible that orbital PFC dysfunction contributes to abnormal skin responses that are manifested as disinhibited or perseverative responses with aging. An examination of age-related changes in skin and other autonomic responses in relation to working memory task performance and functional and structural imaging measures of orbital PFC integrity could be useful for supporting or refuting this speculation.

7.9 Therapeutic implications of orbital PFC and working memory disorders in aging. Pharmacological manipulation of working memory performance has been demonstrated in a number of human and nonhuman primate studies examining dopaminergic (Williams and Goldman-Rakic, 1995) and noradrenergic compounds (Arnsten and Cai, 1993; Franowicz and Arnsten, 1999). Although the dopaminergic system of the PFC has been studied in greatest detail, the current studies suggest that a closer examination of noradrenergic and even serotonergic manipulations could be useful as these transmitter systems are more specifically localized to orbital PFC and ventromedial regions (Levitt et al., 1984). Alpha-2 adrenergic stimulation improved impaired working memory performance in aged monkeys (Arnsten and Contant, 1992; Arnsten and Cai, 1993) and in subjects with AD (Riekkinen et al., 1999) suggesting a role for the alpha-2 adrenergic receptor in age and disease-related working memory attenuation. Testosterone supplementation has been shown to influence working memory performance in older men. One

month of testosterone supplementation improved performance of older men on the same self-ordered task used in the current study (Janowsky et al., 2000). There are testosterone binding sites in both dorsolateral and orbital PFC (Clark et al., 1988) and there is a decline in testosterone levels with advancing age (Denti et al., 2000). Thus, declining testosterone levels could affect prefrontal function in aging. Moreover, orbital PFC lesions produce deficits on an object discrimination task in male but not female monkeys, yet female monkeys given androgen and orbital PFC lesions do show a deficit similar to male monkeys (Clark and Goldman-Rakic, 1989). Thus, testosterone levels could interact with orbital PFC function leading to cognitive decline. Future studies examining the pharmacologic and hormonal manipulation of PFC function will be of great interest in the amelioration of cognitive decline with healthy aging and AD.

7.10 Final thoughts. As the older population continues to expand in the Unites States, so do the fields of cognitive neuroscience and aging research. Wide-ranging studies of aging are currently underway in numerous laboratories around the world. The results of the experiments presented provide a basis for numerous potential future studies across broad disciplines to contribute to this research community. These studies could include a closer examination of prefrontal patterns of age-related neurodegeneration, the pathological mechanisms of such degeneration, the contribution of degeneration to age related cognitive decline, and the most attenuated cognitive functions with aging. The ultimate goal of such research will be in the amelioration of age-

related disease and cognitive decline. With increasing knowledge of the aging process, it is hopeful that this goal will be attainable.

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