RETINAL NERVE FIBER LAYER THICKNESS IN GLAUCOMA AND ALZHEIMER'S DISEASE BY SPECTRAL DOMAIN OPTICAL COHERENCE TOMOGRAPHY: A PILOT STUDY

A thesis presented to the graduate faculty of
New England College of Optometry in partial fulfillment
of the requirements for the degree of Master of Science

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April, 2014

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New England College of Optometry, 2014

Abstract

Objective: Alzheimer's disease (AD) is a neurodegenerative disease which has visual symptoms, and shares a common neurodegenerative process found in glaucoma. Histological and imaging studies have shown retinal nerve fiber layer (RNFL) thinning in AD, particularly in the superior quadrant surrounding the optic nerve head. Optical coherence tomography (OCT) is a widely accepted *in vivo* imaging technique that quickly and non-invasively measures the RNFL. Previous models of OCT have quantified RNFL loss in AD and correlated it with cognitive impairment. Spectral domain OCT (SD-OCT), a newer technology with superior resolution, can accurately detect RNFL thinning from glaucoma. The purpose of this pilot study was to use SD-OCT to characterize the RNFL in patients with POAG or AD, and to evaluate correlations between the RNFL thickness and cognitive function in individuals with AD. We hypothesized that RNFL thickness was less in AD participants and would be correlated with cognitive function.

Research Design and Methods: In this cross-sectional pilot study, participants recruited from the Jamaica Plain Veterans Affairs (JPVA) Neurology, Geriatric Research Education and Clinical Center (GRECC), and optometry clinics were assigned to one of three groups matched for age, gender, and race. The test groups consisted of an AD group with mild to moderate AD (5 total participants), a POAG group composed of individuals without

dementia of any type, and were actively treated for POAG (19 total participants), and a control group composed of individuals without dementia or glaucoma of any type (17 total participants). Following informed consent, visual acuity (Snellen), St. Louis University Mental Status (SLUMS) Exam, the Delis-Kaplan Executive Function System (D-KEFS) including only the Trail Making Test (TMT), and the Color Word Interference Test (CWIT), SD-OCT scans (Spectralis, Heidelberg Engineering) including average RNFL thickness in four quadrants, six sectors, and globally, and cup-to-disc ratio in both eyes were acquired. Analyses of Variance (ANOVA) were used to examine differences in both the RNFL thickness and cognitive function tests between groups, and Pearson correlation coefficients were used to determine association between them.

Results: There was no difference in the demographics of our three groups based on age, refractive error, or visual acuity. RNFL was thicker in the AD group vs. the control group in the inferior temporal and temporal areas, and it was thinner, approaching significance in the superior nasal and nasal areas. There was no optic nerve head cupping in the AD group which differs from the glaucoma group when compared to the control group, and the pattern of RNFL loss was overall loss in all areas within the glaucoma group. The SLUMS exam had a positive correlation to global RNFL thickness. The TMT, and the CWIT were negatively correlated to global RNFL thickness. This shows that as cognitive function decreases, global RNFL thickness decreases in AD patients.

Conclusion: Further characterization of the RNFL in glaucoma and AD will improve understanding of early retinal changes, setting the foundation for larger studies aimed at earlier recognition and intervention.

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Table of Contents

Abstract……iii-iv

Acknowledgments……v

Table of Contents……vi

List of Tables .....……...vii

List of Figures.....viii

Chapter One: Introduction……..... 1-43
Alzheimer's Disease…… 1-14
Glaucoma……... 15-24
Alzheimer's Disease and Glaucoma…….. 24-27
Alzheimer's Disease, Glaucoma and the Visual System……....27-31
Measuring Retinal Ganglion Cell Degeneration in Alzheimer's Disease……..32-35
Optical Coherence Tomography..... 35-43

Chapter Two: Materials and Methods 44-51
Study Design.....44-46
Optical Coherence Tomography.....46-47
Cognitive Testing.....47-49
Statistical Analysis.....50-51

Chapter Three: Results.....…….....52-57
Study Population and Demographics.....52-53
Optical Coherence Tomography…….....…....53-55
Cognitive Testing.....55-57

Chapter Four: Discussion…….....……58-64

Tables.....65-69

Figures.....70-71

Bibliography……...72-82

List of Tables

Table 1: Summary of Various Studies on RNFL Thickness and AD 65

Table 2: Table of Participant Demographics 66

Table 3: Table of Cup to Disc Ratios and Global RNFL Thickness 66

Table 4: Table of RNFL Thickness by Quadrant and Sector 67

Table 5: Table of *Ad hoc* Analysis of RNFL Thickness 67

Table 6: Table of Cognitive Test Data 68

Table 7: Table of Correlation Coefficient of Cognitive Tests in Relation to Global RNFL Thickness.....69

Table 8: Table of Correlation Coefficient of Cognitive Tests Outcomes Compared to Global RNFL Thickness Across all Groups.....69

List of Figures

Figure 1: Scatterplot B/A Ratio vs. Global RNFL Thickness.....70

Figure 2: Scatterplot SLUMS Exam Scores vs. Global RNFL Thickness.....70

Figure 3: Scatterplot Stroop Interval vs. Global RNFL Thickness..... 71

Chapter One: Introduction

Alzheimer's Disease

Alzheimer's disease (AD) was first described by Dr. Alois Alzheimer in 1901. Dr. Alzheimer described a case of a 51 year old female patient, Auguste Deter, who presented with dementia with a rapid decline in cognitive function over a period of just a few years. At her autopsy, Dr. Alzheimer described the presence of what became known as the hallmark features of AD, the beta-amyloid plaques and neurofibrillary tangles within the brain. The disease was first coined as Alzheimer's disease by Emil Kraepelin, a prestigious psychologist, a several years later.

The prevalence of Alzheimer's disease within the United States is over five million people, most of whom are over age 65 (Herbert LE, Weuve J, Scherr PA, and Evans DA, 2013). Over three million of these individuals are females, which may be attributed to longer average life spans in females compared to males (Herbert LE, et al., 2013). African Americans are twice as likely to be diagnosed with AD compared to Caucasians and Hispanics are one and a half times more likely to be diagnosed, although it is unclear at this time as to what causes this difference (Gurland BJ, Wilder DE, Lantigua R, Stern Y, Chen J, Killeffer EH, and Mayeux R, 1999). In the absence of a medical breakthrough, it is estimated that by 2025 there will be over seven million Americans, aged 65 years and older, with AD (Alzheimer's Association, 2013). Increasing age is the largest risk factor in the development of AD. Other risk factors include smoking, cardiovascular disease (including obesity, diabetes, dyslipidemia, and hypertension) particularly in middle age, traumatic brain

injury, and first degree relatives with AD diagnosis (Alzheimer's Association, 2013). The primary protective factor is education level which is believed to increase cognitive reserve in order to stave off the presentation of symptoms (Plassman BL, Langa KM, Fisher GG, Heeringa SG, Weir DR, Ofstedal MB, 2007). However some studies show that such individuals have a faster rate of decline over time after the initial protective effect (Wilson RS, Bennett DA, Gilley DW, Beckett LA, Barnes LL, Evans DA, 2000). Other protective factors include exercise, diets low in saturated fats, and social and cognitive engagement (Alzheimer's Association, 2013). Healthcare costs for treatment and diagnosis of AD in 2013 were estimated to be approximately 203 billion dollars, 142 billion dollars coming from Medicare and Medicaid with a projected cost in 2050 of over one trillion dollars (Alzheimer's Association, 2013). This causes a huge drain on our healthcare system along with a large debt to taxpayers.

The Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition), 1994, defines AD as a progressive neurodegenerative disease that presents with a loss of memory, typically short term, and the accompaniment of one or more symptoms such as aphasia (difficulty forming speech), apraxia (difficulty with planning voluntary muscle movements), agnosia (difficulty identifying objects), or loss of executive functions (difficulty with planning and making decisions). These deficits also interfere with the ability to perform daily tasks, such as working and maintaining family and social relationships. More advanced stages of the disease present with loss of language function, loss of visual-motor sense, and incontinence, typically continuing to a persistent vegetative state (Holroyd S, 2001). Death follows usually due to an intercurrent infection such as aspiration pneumonia (Ropper A and Samuels M,

2009). Diagnosis is probable, based on the aforementioned symptoms, MRI scans for volume, and possibly cerebral spinal fluid (CSF) biomarker analysis. Definitive diagnosis is made only at autopsy, which uncovers characteristic beta-amyloid (Aβ) plaque formations and neurofibrillary tangles (NFT) within the brain.

MCI is the area of cognitive deficit in between normal aging and AD. This makes a diagnosis of MCI difficult to make but is defined by The Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, 1994, as the presence of two or more cognitive deficits in memory impairment, executive function, attention or speed of information processing, perceptual-motor abilities, or impairments in language. The individual must also not meet criteria for AD or other dementia including delirium.

Types of AD

AD can be separated into two general categories based on the age and characteristics of the onset of the disease. Early onset, or familial AD (FAD), is diagnosed before the age of 65, with some cases as early as the late twenties, though this is rare (Bettens K, SleegersK, Van Broeckhoven C, 2010). Late onset, or sporadic AD (SAD), is diagnosed after age 65 (Bettens K, et al., 2010). FAD only makes up about five percent of all AD cases, but is much more aggressive than SAD (Armstrong RA, 2009). Mutations found in three genes are definitively linked to FAD development, amyloid precursor protein (APP), presenilin 1(PSEN1), and presenilin 2 (PSEN2) (Armstrong RA, 2009 and Bettens K, et al., 2010). Mutations to these genes are autosomal dominant with most cases spread throughout family units (Armstrong RA, 2009). APP currently has 34 identified pathogenic mutations, mostly

single base substitutions with some single base deletions and whole gene duplications (Alzheimer's disease mutation database, 2013). Down syndrome patients are at greater risk of developing AD due to the trisomy of chromosome 21, which is where the APP gene is located (Hardy JA and Higgins GA, 1992). PSEN1 currently has 193 identified pathogenic mutations, mostly single base substitutions with a few deletions (Alzheimer's disease mutation database, 2013). PSEN2 currently has 20 identified pathogenic mutations, entirely made up of single base substitutions (Alzheimer's disease mutation database, 2013). All three genes are involved in either the formation or removal of Aβ peptide, and under pathologic conditions contribute to Aβ senile plaque formation as per the amyloid cascade hypothesis (Hardy JA, et al. 1992). SAD has only one gene definitively linked to an increased risk of development, the apolipoprotein E gene (APOE), specifically the APOE ε 4 allele (Bettens K, et al., 2010). APOE is involved in several intracellular pathways, in particular cholesterol metabolism and beta-amyloid processing. APOE exists in three different isomer forms, APOE ε 2, 3, and 4. APOE ε 3 is the most common form of the gene and does not seem to affect the risk of AD. APOE ε 4 is the next most common form and increases the risk of AD and APOE ε 2 is the least common form, and is protective against the development of AD(Castellano JM, Kim J, Stewart FR, Jiang H, DeMattos RB, Patterson BW, Fagan AM, Morris JC, Mawuenyega KG, Cruchaga C, Goate AM, Bales KR, Paul SM, Bateman RJ, Holtzman DM, 2011). It is believed that both FAD and SAD have similar etiologies, with FAD having a more aggressive proliferation due to the genetic mutations and SAD requiring more time to accumulate enough neuronal damage to manifest symptoms (Armstrong RA, 2009).

Pathophysiology of AD

The amyloid cascade hypothesis outlined by James Hardy and Gerald Higgins in 1992 explains the initial formation of senile plaques through the aggregation of Aβ. APP is a transmembrane protein that resides in the cytoplasmic membrane of neurons. When this protein is metabolized, it can be cleaved by either α, or β and γ-secretases. Under normal circumstances APP is cleaved by α secretase and Aβ is not formed. When a mutation is present in APP or when normal cleavage is disturbed, β and γ-secretases aberrantly cleave the protein into 38 to 42 amino acid long peptides, known as Aβ. PSEN1 and PSEN2 genes produce proteins that assist in the cleavage of APP by γ-secretase. Mutations in PSEN1 and 2 increase the formation of Aβ. Aβ is an insoluble peptide that aggregates within the brain forming the basis for the senile plaque which is characteristically found in AD. Senile plaques are complex structures that also contain a host of other components such as APOE, antichymotrypsin, silicon, aluminum, macroglobulins, interleukin-6, clusterin, vibronectin, cell adhesion molecule 1, and complement proteins C1q, C3, and C4 (Armstrong RA, 2009). Their various component concentrations change over the course of the disease and allow for the staging of plaques into various types (Armstrong RA, 2009).

Along with senile plaque formation, neurofibrillary tangles (NFTs) are another hallmark sign of AD. NFTs are also made up of multiple components such as ubiquitin, APOE, synaptophysin, basic fibroblast growth factor, glial fibrillary acidic protein, and Aβ, with the bulk of it being made of hyperphosphorylated tau proteins (3R and 4R tau) (Armstrong RA, 2009). Tau is a normal microtubule associated protein involved in axoplasmic transport

within the cytoskeleton of the neuron. NFTs form from the presence of senile plaques in response to cellular damage (Armstrong RA, 2009). There are two types of NFTs, internal and external, named based on their location relative to the neurons. Each type varies in their component structure and external tangles tend to present in later stages of the disease (Armstrong RA, 2009). When NFTs form they cause damage to neurons by halting axoplasmic transport and by disrupting cellular processes. There are several other models that also contribute to cellular damage such as glutamate excitotoxicity, oxidative stress, chronic inflammation, and cholinergic deprivation within AD that will not be discussed in detail here.

Treatment for AD

Treatment for AD is currently limited to preventing loss of function and decreasing the onset of new symptoms. The most widely used treatment for AD is acetylcholinesterase inhibitors (Holroyd S, 2001). Decreased levels of acetylcholine within the brain were found in patients with AD in the 1970's (Jacobsen JS, Reinhart P, Pangalos MN, 2005). Acetylcholinesterase inhibitors inhibit the acetylcholinesterase enzyme responsible for breaking down the neurotransmitter within the synaptic cleft. This class of drug increases the amount of neurotransmitter available within the cleft, indirectly increasing acetylcholine levels. Tacrine was the first drug widely prescribed for the treatment of AD symptoms, but hepatotoxicity led to its discontinuation (Jacobsen JS, et al., 2005). Donepezil is often used now for treatment, though galantamine is quickly replacing it; these are less hepatotoxic than tacrine (Jacobsen JS, et al., 2005).

Memantine is a non-acetylcholinesterase inhibitor class of drug used to treat AD symptoms. It works based on the excitotoxic glutamate hypothesis that states that increased levels of glutamate in the presence of oxidative stress can lead to neurodegeneration from over stimulation (Jacobsen JS, et al., 2005). N-methyl D aspartate (NMDA) receptors stimulate production of glutamate, stimulating this neurodegeneration. Memantine functions as a non-competitive, NMDA receptor antagonist, lowering glutamate levels and decreasing neurodegeneration (Jacobsen JS, et al., 2005).

Strategies for developing future treatments for AD focus mainly on inhibiting the generation of senile plaques and NFTs by interrupting various stages of formation based on the β-amyloid hypothesis (Jacobsen JS, et al., 2005). Drugs are being developed that decrease Aβ formation through inhibiting β- and γ-secretase activity, decreasing Aβ aggregation, and breaking down Aβ directly through vaccines and antibodies, both actively and passively (Jacobsen JS, et al., 2005, and Rafii MS and Aisen PS, 2009). The hyperphosphorylated tau protein is also a promising drug target for AD. Methylene blue and a small peptide called NAP have shown some promise of inhibiting the hyperphosphorylation of tau (Rafii MS, et al., 2009). Other more basic neuroprotective strategies have also been considered in the treatment of AD such as the use of receptors for advanced glycosolated end products (RAGE) inhibitors to decrease neurodegeneration along with Vitamin E treatments to limit oxidative stress (Rafii MS, et al., 2009). To date none of these treatment modalities have yielded promising results.

Alzheimer's Disease Neuroimaging Initiative (ADNI)

In 2004, the National Institute on Aging began work on the ADNI to help better understand the ability of current detection methods to diagnose and monitor AD progression. Susan Molchan (project officer for ADNI) wrote in a US Neurology Review Business Briefing about the ADNI (2003). She stated that the study would help "…to identify the biomarkers of disease progression that are most promising for use as surrogate end-points in phase 2 and 3 clinical trials for the prevention and treatment of AD." The ADNI study took place over a five year period from 2005 to 2010 and enrolled 800 participants aged 55 to 90 years. Study participants underwent cognitive testing, magnetic resonance imaging (MRI) scans, positron emission tomography (PET) scans, and blood, cerebrospinal fluid (CSF), and urine biomarker analyses. The blood, CSF, and urine samples were tested for levels of isoprostanes, tau, Aβ, sulfatides, and homocysteine. Samples were maintained for future biomarker analysis. Participants were also screened for the presence of the APOE ε 4 gene allele. (Petersen R, Albert M, Weiner MW, Aisen PS, Toga A, Shaw LM, Jack C, and Jagust W, 2011a). Since the completion of the study, over 230 papers have been published (Petersen R., et al., 2011a). A follow up study began recruiting in 2010, the ADNI 2. The goals of this study build on the goals of the ADNI 1 study and further break down the continuum of participants into normal aged individuals, early mild cognitive impairment (MCI), late MCI, and early AD. The study is also extending participants from the ADNI 1 study into the ADNI 2 study to see the effects of conversion over a ten year period. Some cognitive tests were added, as well as F-AV-45 Amyloid Positron Emission Tomography (PET) studies. F-AV-45 Amyloid is a radioactive tracer compound incorporating a 18F

(Fluorine) molecule which binds to the Aβ plaques within the brain (Ono M, Saji H., 2012). Traces of the radioactive isotope are then picked up by the PET machine and locations and sizes of the plaques can be determined. The study will follow participants over a 54 month period, where after this time it may be extended with further protocols (Petersen R, Weiner MW, Albert M, Aisen PS, Morris J, Shaw LM, Jack C, Jagust W, Sayken A, and Toga A, 2011b).

One of the study aims of the ADNI 1 was to determine if there was a benefit to using CSF biomarkers in the detection and determination of treatment efficacy in AD. Due to the extremely invasive nature of lumbar puncture, it is valuable to know how much these biomarkers can tell us about the disease and its progression. Several earlier pilot studies determined which biomarkers were most effective in monitoring AD progression and at what levels they are found within the CSF of both healthy controls and AD patients. One such study used the ADNI 1 testing data to track changes in the levels of Aβ42, total-tau, and phosphorylated tau at baseline, one year, and two years (Fjell AM, Walhovd KB, Fennema-Notestine C, McEvoy LK, Hagler DJ, Holland D, Brewer JB, Dale AM, and the Alzheimer's Disease Neuroimaging Initiative, 2010). The levels of the CSF biomarkers were compared between the three groups and it was found that at baseline there was no significant difference between them. The levels of CSF biomarkers were also compared to the rate of brain atrophy, as measured by MRI. The rate of brain atrophy was higher in those individuals with a higher level of both total and phosphorylated tau and higher with a lower level of free Aβ42. Changes in biomarker levels were then compared to changes in cognitive testing over the two year period. There was no correlation between biomarker level change and cognitive

change. MRI was a better predictor of cognitive loss than CSF biomarker analysis (Fjell AM, et al., 2010). Another study analyzed the ADNI 1 to determine the effect of age on CSF biomarker levels in regards to determining cognitive loss in both controls and abnormal study participants (Schmand B, Eikelenboom P, van Gool WA, and the Alzheimer's Disease Neuroimaging Initiative, 2011). Both the MCI and the AD test groups were combined to create a cognitively abnormal group, which was compared to the normal control group. Over the two year period both MRI and cognitive testing showed no loss in diagnostic value with age, while the levels of CSF biomarkers became less diagnostic with age (Schmand B, et al., 2011). Both of these studies indicate that CSF biomarker levels change within individuals with AD over time, but the usefulness of this data on clinical analysis is not as robust as MRI or cognitive testing.

Cognitive Testing

Cognitive testing is the most widely used indicator for tracking progression, determining onset, and determining success of treatment in AD at this time. There are many methods of cognitive testing available both to the clinician and to the researcher. Examples are the mini-mental status exam, word list recall, story recall, mini-cog, clock drawing, trail making test, Stroop color-word test, and the Saint Louis University mental status exam.

This study used the latter three tests: the trail making test, Stroop color-word test, and the Saint Louis University mental status exam..

The Trail Making Test (TMT)

The TMT primarily assesses the areas of processing speed, sequencing, mental flexibility, and visual-motor skills (Bowie CR and Harvey PD, 2006). The TMT has been shown to be effected by age, education levels, and occupation (Amodio P, Wenin H, Del Piccolo F, Mapelli D, Montagnese S, Pellegrini A, Musto C, Gatta A, and Umilta C, 2002). The effects of normal aging result in an increase in time to completion, and individuals with increasing levels of education and more technically challenging occupations show a decrease in time for completion (Amodio P, et al., 2002). Some studies focus on the B/A ratio which is the comparison of the time it takes to complete TMT-B divided by the time it takes to complete TMT-A. This difference in time can be correlated with the flexibility of the brain. An increase in the B/A ratio would indicate a lack of flexibility compared to a lower B/A ratio (Bowie CR, et al., 2006). An overall increase in the time to complete TMT-A or B, either based on previous performance, or based on age-matched norms, may denote a deficit in cognitive processing. Many different cognitive problems can be measured including brain trauma, Parkinson's disease, schizophrenia, drug use, and AD. Studies have shown a correlation between AD and cognitive decline measured by the TMT.

One such study assessed various cognitive tests on a group of 551 individuals over a ten year period (Chen P, Ratcliff G, Belle SH, Cauley JA, DeKosky ST, Ganguli M, 2001). None of the study participants had a diagnosis of AD before the onset of the study and at the end of 10 years, 68 individuals were diagnosed with AD. Individuals with AD had a significant increase in time to completion on both TMT-A and TMT-B before the onset of symptoms,

compared to individuals who did not develop AD. In another study, both time to completion and amount of errors during the TMT-A/B were evaluated in normal aging controls, mild cognitively impaired patients, and in individuals with AD (Ashenndorf L, Jefferson AL, O'Connor MK, Chaisson C, Green RC, and Stern RA, 2008). Time to completion significantly increased from normal controls to mild cognitively impaired individuals, to AD patients, and the number of errors during the testing procedure also significantly increased. These studies add to the validity of using the TMT test as an indicator of cognitive decline in individuals with AD.

The Stroop Color-Word Test (SCWT)

The SCWT is one of the oldest, most reliable cognitive tests available (Van der Elst W, Van Boxtel MPJ, Van Breukelen GJP, and Jolles J, 2006). The original test developed by John Ridley Stroop in 1935 has been modified over time but is still based upon the Stroop paradigm (Seo EH, Lee DY, Choo IH, Kim SG, Kim KW, Youn JC, Jhoo JH, and Woo JI, 2008). Stroop noted that when an individual views the word for a particular color written in ink of a non-matching color, it takes longer for the individual to correctly state the color of the ink. For example, if the word “red” was written in green ink, it would be more difficult to name the color green when reading it compared to the word “green” written in black ink, or a color patch of green. The habitual response of stating the color seen is in conflict with the habitual response of reading what word is seen. By assessing the time it takes the brain to adapt to this situation, various mental faculties can be tested including processing speed, selective attention, cognitive flexibility, and executive function (Seo EH, et al., 2008).

Through the testing of the brain's inhibition we can gather information about its functional capacities (Seo EH, et al., 2008). The Stroop interference effect is a measure of cognitive function calculated by the difference in time it takes for individuals to complete the third phase of the test compared to the average of the first and second phases of the test (Van der Elst W, et al., 2006).

The time to complete the SCWT has been found to increase with increasing age and cognitive decline (Van der Elst W, et al., 2006). Other factors may have an effect on SCWT such as gender and education, but this is controversial. Normative data has been generated for the SCWT through several studies analyzing different age groups, gender, and educational levels. One such study performed in the Netherlands on 1,856 individuals aged 25-81 found that in normal populations, an increase in age correlated to an increase in the time to complete the SCWT, while an increase in education level decreased the time to complete the test (Van der Elst W, et al., 2006). Another study performed in Seoul, South Korea on 564 individuals aged 60-90 found that age and education level also affected the outcome of the test in normal populations (Seo EH, et al., 2008).

The SCWT has also been used to study individuals with AD (Amieva H, Phillips LH, Della Sala S, Henry JD, 2004, Bondi MW, Serody AB, Chan AS, Eberson-Shumate SC, Delis DC, Hansen LA, and Salmon DP, 2002, and Koss E, Ober BA, Delis DC, Friedland RP, 1984). Individuals with AD have displayed higher Stroop interference effects compared to normal age matched controls. The effects of AD cause a decrease in the inhibitory processing of the brain which increases the observed Stroop interference effect. The presence of larger

amounts of neurofibrillary tangles upon autopsy has also been positively correlated to an increase in the Stroop interference effect (Bondi MW, et al., 2002).

The Saint Louis University Mental Status (SLUMS) Exam

The SLUMS exam is a more recent cognitive test developed at Saint Louis University (Tariq SH, Tumosa N, Chibnall JT, Perry MH, and Morley JE, 2006). The test is widely used to assess cognitive impairment by the Department of Veterans Affairs, Neurology Service, specifically the Geriatric Research Education and Clinical Care center or GRECC. The test was designed to provide greater sensitivity/specificity in the screening of individuals with mild cognitive impairment (MCI) to mild AD. The test was initially compared to the mini mental status exam (MMSE), a test widely used to assess cognitive impairment, but the MMSE lacks the sensitivity/specificity to detect mild cognitive impairment (Tariq SH, et al., 2006).

The initial study to validate the test methods assessed the performance on 709 individuals age 60+ at the Saint Louis VA GRECC. The sensitivity/specificity was higher in mild cases of AD and MCI with the SLUMS exam than the mini mental status exam (Tariq SH, et al., 2006). Since this test is widely used within the Department of Veterans Affairs Neurology Services, it was incorporated into our study.

Glaucoma

Background

Glaucoma is another neurodegenerative disease that shares some of the same pathophysiological processes as AD. Glaucoma is a leading cause of blindness in the United States today along with age-related macular degeneration and diabetic retinopathy (Orcutt D, Monaco W, Lynch M, Penny J, Ingersoll S, Fingeret M, and Tielsch J, 2006). Glaucoma prevalence in the United States is reported to be over two million people (Friedman DS, Wolfs RCW, O'Colmain BJ, Klein BE, Taylor HR, West S, Leske MC, Mitchell P, Congdon N, and Kempen J, 2004). Due to the difficulty in diagnosing glaucoma, up to 50% of individuals that have the disease may go undiagnosed and untreated (Orcutt D, et al., 2006). It is estimated that by 2020 the number of people with glaucoma in the United States will reach three million, if prevention is not improved (Friedman DS, et al., 2004). The risk factors for glaucoma development include age, race, ocular hypertension, and myopia (Orcutt D, et al., 2006). African Americans develop glaucoma 4.3 times more often than age matched Caucasian individuals; The prevalence of normal tension glaucoma is higher in Asian populations, specifically Japanese individuals; Individuals with ocular hypertension, defined as intraocular pressure (IOP) of greater than 21 mmHg, have shown a sixteen times greater risk of developing glaucoma compared to age matched controls; Finally, individuals with myopia have a two to five fold increase in glaucoma development over non-myopic individuals, specifically for developing primary open angle glaucoma (POAG) (Orcutt D, et al., 2006).

Types of Glaucoma

Glaucoma is a family of ocular diseases with varying etiologies, but all having similar patterns of visual loss and optic neuropathy. Glaucoma can be separated into two main categories, open angle glaucoma (OAG) or angle closure glaucoma (ACG), based on the configuration of the anterior chamber angle. This can be estimated via the Von Herrick technique through slit lamp examination, but is mainly determined through indirect gonioscopy examination of the structures of the anterior chamber angle. These two forms of glaucoma can be further subdivided into primary or secondary glaucoma based upon the etiology of the disease. Secondary glaucomas occur secondary to another ocular disease, trauma, or pharmacological agent; whereas primary glaucoma is not. Many forms of glaucoma are typically accompanied by an elevation in IOP. Another type of glaucoma, normal tension glaucoma, manifests with similar signs as the rest of the glaucomas, except that the IOP is not considered to be elevated.

Diagnostic Testing for Glaucoma

Diagnosis of glaucoma can be very difficult. Various test methods are employed in the analysis of glaucoma such as tonometry, optic nerve head assessment, fundus photography, perimetry, and optical coherence tomography (OCT). Important findings to consider in a diagnosis of glaucoma are the presence of characteristic glaucomatous nerve head changes involving increased cup to disk ratios specifically vertically, changes in rim thickness that are uneven, such as the formation of notching, or changes that do not follow the ISNT rule, changes in the nerve head that are not symmetrical with respect to both eyes, and the

formation of hemorrhages on the optic disc known as Drance hemorrhages (Lewis TL, Barnebey HS, Bartlett JD, Blume AJ, Fingeret M, Lalle PA, and Mann DF, 2002). The ISNT rule is defines the proper variance in thickness of the ONH based on quadrant. A nerve that has a greater amount of rim tissue inferiorly, then superiorly, then nasally, and finally temporally is considered to follow the ISNT rule and by so doing makes a nerve less suspicious for glaucoma. Fundus photography can aid in the process of optic nerve head analysis and provides an image for comparison for future evaluations. This is especially important in monitoring progression of the disease. It is also possible to see changes in the retinal nerve fiber layer (RNFL) on fundus photography, indicating structural changes. Perimetry tests the patient's visual function. Static automated perimetry is very useful in identifying functional glaucomatous changes. Patients with glaucoma display characteristic visual field loss including the formation of nasal steps and pericentral loss of function within five to twenty degrees of fixation in an area known as Bjerrum's region (Lewis TL, et al., 2002). These eventually lead to a deficit that follows an arcuate pattern respecting the horizontal. Even with all of these methods of glaucoma screening, a diagnosis is very difficult to make and is often missed.

Pathophysiology of Primary Open Angle Glaucoma

Primary open angle glaucoma (POAG) accounts for greater than 75% of cases of glaucoma worldwide (Orcutt D, et al., 2006). The specific etiology of the disease is still controversial, though the changes still end in a loss of retinal ganglion cell (RGC) structure, characteristic remodeling of the optic nerve head, and characteristic loss of visual function. There are

many hypotheses as to the cause of the degeneration of the RGCs. Increased IOP leading to mechanical stress, ischemic conditions at the nerve head, oxidative stress, glutamate excitotoxicity, and autoimmunity are some of the more robust hypotheses at this time (Ray, K and Mookherjee S, 2009). There are also various genetic abnormalities linked to the disease, including the myocilin gene, optineurin gene, WD repeat domain 36 gene (WDR36), and the cytochrome P450 family 1 subfamily B polypeptide 1 gene (CYP1B1) (Ray K, et al., 2009).

Most often POAG cases present with an accompanied increase in aqueous production leading to elevated IOP of greater than 21 mmHg (Orcutt, D., et al., 2006). Increases in IOP could lead to increased mechanical stress on the RGC axons as they exit through the lamina cribrosa causing degeneration of the axons (Whitmore AV, Libby RT, John SWM, 2005). Decreases in intracellular trafficking due to mechanical stress leads to axonal loss, eventually leading to apoptotic cell death (Johnson EC, Deppmeier LM, Wentzien SK, Hsu I, and Morrison JC, 2000).

Ischemia in Glaucoma

Ischemic conditions can be found in various structures of the eye in patients with POAG, such as the retina, iris, optic nerve, and the choroid (Whitmore AV, et al., 2005). Increases in IOP can also cause ischemic conditions within the RNFL. Some of this loss of blood flow can be contributed to increases in IOP, in other circumstances, individuals will present with changes in blood flow in the absence of increased IOP, or even decreases to blood flow in other areas of the body (Whitmore AV, et al., 2005). In these cases, changes in retinal blood

flow are thought to occur due to a disregulation of the production of certain endothelium derived vasoactive compounds (EDVCs) (Ray K, et al., 2009). EDVCs are proteins that are released from the endothelial cells and control blood flow within the blood vessels of the nerve fiber layer and the inner nuclear layers of the retina. Endothelin-1 (ET-1) causes vasoconstriction of blood vessels and increases the viscosity of the blood (Flammer J, Orgul S, Costa VP, Orzalesi N, Krieglstein GK, Serra LM, Renard JP, and Stefiansson E, 2002). ET-1 is elevated in individuals with POAG (Flammer J, et al., 2002). The combination of vasoconstriction and increased viscosity leads to ischemic conditions within the retina, specifically within the RNFL. These transient ischemic events cause reperfusion injury to the retinal cells due to the formation of oxygen free radicals or reactive oxygen species (ROS) which lead to oxidative stress. ROS, such as singlet oxygen and hydrogen peroxide, hinder the normal cellular processes taking place within the RGCs as well as both genomic and mitochondrial DNA. Damage can specifically occur to the mitochondria, which will disrupt both regular energy production and the basal antioxidant production levels within the cell (Mozaffarieh M, Grieshaber MC, and Flammer J, 2008a). Antioxidants prevent the buildup of ROS. The eye is specifically susceptible to ROS build up due to the amount of light entering the tissues and from the oxygen that diffuses in from the tear film. To counteract this, the eye has high levels of vitamin C, a particularly effective antioxidant. The mitochondria also produce large amounts of NADPH, another antioxidant, during pentose phosphate shunt reactions. During the course of ageing, antioxidant levels start to fall. Within POAG patients, antioxidant levels are particularly low, and antioxidant treatment can be recommended (Mozaffarieh M, Grieshaber MC, and Flammer J, 2008b). The damage

from the ROS eventually and repeated reperfusion injury lead to apoptotic cell death of the RGCs (Mozaffarieh M, et al., 2008a). These same processes take place with neurons in AD.

Glutamate Excitotoxicity in Glaucoma

Glutamate excitotoxicity (GE) also plays a role in RGC apoptotic cell death. Glutamate is an excitatory central nervous system neurotransmitter. Increased levels of glutamate can cause excitotoxicity, which is a state of overstimulation of the neuron that leads to neurodegeneration (Olney JW, 1969). GE causes dysregulation of cellular ion concentrations, including calcium and chloride ions (Dreyer EB, 1998). This dysregulation causes neurodegeneration through various mechanisms including osmotic lysis due to depolarization of the cell by chloride ion influx, activation of calcium-sensitive proteolytic enzymes due to increased cellular levels of calcium ions and disruption of ATP synthesis reactions within the mitochondria (Dreyer EB, 1998). Mitochondrial disruption leads to increased levels of ROS within the cell. RGCs are specifically susceptible to these degenerative processes (Olney JW, 1969). A two-fold, or higher, increase in glutamate levels has been found, not only in animal models of glaucoma, but also in human populations with POAG (Dreyer, EB, 1998). Heightened glutamate levels are caused by a combination of the death of surrounding RGCs spilling their glutamate contents into the extracellular matrix, increased permeability of damaged RGCs to glutamate, and oxidative damage to surrounding Meuller cells, inhibiting their ability to decrease extracellular glutamate levels (Dreyer EB, 1998). A combination of these degenerative conditions and exposure to oxidative stress will

lead to apoptotic cell death (Ray K, et al., 2009, Quigley HA, 1999). These processes also take place within neurons in AD.

Autoimmunity in Glaucoma

Immunoregulation is also a key player in the fate of RGCs. Tumor necrosis factor alpha (TNF-α) is a cytokine secreted by immune cells that functions to attract and activate other immunological cells such as T-cells. When TNF-α binds to its receptor, TNF receptor-1, it causes apoptotic cell death. Glial cells within the retina produce TNF-α (Tezel G, Li LY, Patil RV, and Wax MB, 2001). TNF-α is elevated in patients with POAG and heightened levels lead to apoptotic cell death of RGCs (Wax MB, and Tezel G, 2009). Also, autoantibodies to normally produced RGC proteins have been found in POAG patients (Wax MB, et al., 2009). One group of proteins that seems to be affected by autoantibody targeting is heat shock proteins (HSPs). HSPs form when cells are put under stress to help regulate cellular processes. A specific HSP, HSP60 is regulated in POAG patients and increased levels of these antibodies are found in individuals with POAG (Wax MB, et al., 2009). ROS can also damage normal RGC proteins leading to antibody formation and immune system attack (Tezel, G, Yang X, Cai J., 2005). These antibodies can also interact with the undamaged RGC proteins as well (Ray K, et al., 2009). Lastly, abnormally high levels of T-cell build up have been reported in POAG patients (Ray K, et al., 2009). All of these autoimmune responses can eventually lead to apoptotic RGC death. Several of these autoimmune processes have also been reported in AD.

Genetics and Glaucoma

Up to 35 genes have been identified in the development or advancement of POAG. Four of the most studied genes are the myocilin gene, optineurin gene, WDR36, and CYP1B1. The myocilin gene encodes for a protein that carries an olfactomedin domain which is involved in developmental pathways. There are currently 74 mutations identified in this gene that can lead to glaucomatous pathology (Hewitt A, 2007). The presence of a mutated form of this gene is found in three to five percent of all POAG cases. Mutations in the gene cause protein misfolding which triggers apoptotic cell death within cells of the trabecular meshwork (TM). This breakdown of TM structure and a decrease in aqueous outflow cause elevated IOP. Myocilin mutations can also cause mitochondrial dysfunction leading to decreased ATP production and the increase of ROS (Ray K, et al., 2009). The function of the optineurin gene is not well understood. It has been found in certain familial forms of glaucoma, specifically normal tension glaucoma, and is involved with the protection of oxidative damage from ROS. Only one mutated form of the gene has been reported (Ray K, et al., 2009). The WDR36 gene is a multifunctional gene involved in areas such as T-cell activation, ribosomal rRNA processing, and brain and eye development. There are four identified genetic mutations to the WDR36 gene and deficits to its gene products lead to increased glaucomatous damage instead of initial development of the disease (Ray K, et al., 2009). The CYP1B1 is a cytochrome p450 gene product. It is involved in the metabolism of various cellular products and mutations in this gene cause malformation of the TM in mice models. This gene mutation is also thought to hasten progression of the disease instead of being involved in initial development of POAG (Ray K, et al., 2009).

Treatment for Glaucoma

Treatment for glaucoma involves lowering IOP. This can be achieved surgically or with use of pharmacological agents. Surgery involves either physical removal of a section of the TM through trabeculectomy, or laser perforation of the TM through trabeculotomy, the insertion of a filtering bleb with a shunt tube, or cyclophotocoagulation. Iridotomy, laser perforation of the iris, can also be effective in lowering IOP in cases of narrow anterior chamber angles. There are many pharmacological agents used in the treatment of glaucoma, many of which affect the autonomic nervous system. Parasympathomimetic agents stimulate the parasympathetic response causing relaxation of the ciliary body, opening up the TM leading to increased aqueous outflow and decreases IOP. Direct parasympathetic agonists like pilocarpine were the first line of treatment for POAG, but side effects such as miosis of the pupil have decreased its usage. Physostigmine, an acetylcholinesterase inhibitor, indirect parasympathetic agonist, is also effective, though not prescribed as often. Demecarium, another acetylcholinesterase inhibitor, is used in the treatment of angle closure glaucoma. B-receptor blocking sympatholytic drugs, such as timolol, decrease aqueous humor production, thereby lowering IOP. Acetazolamide, a carbonic anhydrase inhibitor, works by decreasing aqueous humor production. Prostaglandin class drugs are the most often prescribed and function by increasing aqueous humor outflow. Drugs in this class include Xalatan and Travatan Z. Future drug therapies are focusing on other areas of treatment in the development of glaucoma. This includes the use of memantine to inhibit glutamate excitotoxicity (Dreyer EB, 1998), and the development of vaccines to inhibit autoimmunity.

Various antioxidants are also being evaluated such as vitamin C and E (Mozeffarieh M, et al., 2008b).

Alzheimer's Disease and Glaucoma

Several aspects of the pathophysiology of AD and POAG are similar. Both are progressive neurodegenerative diseases that manifest with thinning of the RNFL due to changes in RGC axons. Both display patterns of apoptotic cell death. Neuronal damage can be linked to mitochondrial malfunction which leads to an increase in ROS and glutamate excitotoxicity in both diseases. Both diseases manifest neurodegeneration patterns throughout the visual system consistent with transneuronal degeneration models. Similarities between the two diseases have been the focus of several studies. One such study analyzed death certificates within the United States from all persons who died in 1978, and found individuals with AD had a higher rate of POAG than age-matched controls (Chandra V, Bharucha NE, and Schoenberg BS, 1986). A study in Germany noted a significantly higher population of AD patients with glaucoma (23.7%) compared to controls (6.5%) (Bayer AU, 2002a). In another study by the same group, individuals with POAG and AD had a greater rate of progression of POAG compared to controls (Bayer AU, 2002b). A third study by the same group found that individuals with AD had an occurrence rate of 25.9% compared to only 5.2% in controls (Bayer AU, Ferrari F, and Erb C, 2002). A study performed in Japan also supports this data as individuals with AD had a 23.8% rate of occurrence of POAG compared to only 9.9% in controls (Tamura H, et al., 2006). In contrast to these studies, a larger study in Denmark found no increased risk of developing AD in patients with POAG compared to individuals

without POAG (Kessing LV, 2007). Another larger study in the United States analyzed Medicare claims from 1994 to 2007 and also found no increased risk of developing AD in patients with POAG compared to individuals without POAG (Ou Y, Grossman DS, Lee PP, and Sloan FA, 2012). At this time more studies are needed to determine if in fact there is a link in occurrence of AD and POAG.

AD Pathology in the Eye: Animal Studies

Though the occurrence of AD with POAG may be controversial, there are many studies that have demonstrated AD type pathology within the eye in both mice models and human patients. One study assessed mutant APP gene expression and Aβ plaque formations within the retina of AD model mice (Ning A, Cui J, To E, Ashe KH, and Matsubara J, 2008). Two different AD mouse model strains, one expressing a mutant human APP gene and a mutant human PSEN1 gene, and the other strain expressing mutant human PSEN1 gene and a chimeric mouse/human APP gene. Both strains are well established mouse models for studying the effects of AD (Ning A, et al., 2008). The study found mutant APP within the RGC layer, the RNFL, and within the retinal and choroidal vasculature within the test groups, but not in the control groups, and the amount increased with age. The study also showed that apoptosis occurs within the retina more often in experimental AD models and also with increasing age. Another mouse model study analyzed the presence of APP and Aβ within the eyes of experimentally induced glaucoma mice. A strain of mouse was used that develops elevated IOP and glaucomatous optic nerve changes after 6 months of life. Both mutant APP gene products and Aβ-40 peptides were found in the old and young test mice

within the RGC layer, the optic nerve head, and within the pia/dura of the optic nerve, with old mice having significantly higher levels than young mice. The study findings indicate a correlation between mutant APP/Aβ accumulation and glaucoma, and that disruption of the homeostatic properties of APP with consecutive Aβ cytotoxicity might be a contributing factor in sustaining apoptotic cell death in glaucomatous mouse retinas (Goldblum D, Kipfer-Kauer A, Sarra G, Wolf S, and Frueh BE, 1994).

AD Pathology in the Eye: Human Studies

Human studies have also shown the presence of characteristic AD pathology within the eyes of patients with glaucoma. One such study analyzed the aqueous humor of patients following its removal during routine cataract surgery (Janciauskiene S and Krakau T, 2001). The study found that 40% of cases with glaucoma were positive for Aβ peptide, while only 18% were found in controls. The same group found Aβ peptides within the aqueous humor of individuals with macular degeneration and diabetic retinopathy, along with again demonstrating it within the eyes of patients with glaucoma (Janciauskiene S, Westin K, Grip O, and Krakau T, 2011). Another study analyzed the vitreous humor for the presence of both Aβ and tau within patients with glaucoma (Yoneda S, Hara H, Hirata A, Fukushima M, Inomata Y, and Tanihara H, 2005). Vitreous humor samples were gathered from patients with glaucoma that underwent vitrectomies due to the presence of either central retinal vein occlusion, age-related macular degeneration, macular holes, cataract with phacodenesis, uveitis, or branch retinal vein occlusion. The study found that there was a decrease in the amount of Aβ present in test samples and an increase in the presence of tau compared to the

control group, because free Aβ levels decrease due to the formation of the Aβ senile plaques, and tau levels increase due to the formation of NFTs. This indicates that Aβ and tau pathology is present in glaucoma and suggest that the neurodegeneration processes may share a common mechanism with AD. A third study analyzed human retinas of glaucoma patients for the presence of hyperphosphorylated tau (Gupta N, Fong J, Ang LC, and Yucel YH, 2008). The study found that normal tau can be found in all layers of the retina and within the optic nerve. Hyperphosphorylated tau was found only within the test group and within the outer boarder of the inner nuclear layer, the inner plexiform layer, and within the horizontal cells of the retina. The presence of AD type pathology within the eye is not unexpected as the eye develops from the same early cellular populations, and changes from this pathology to retinal structures may affect various aspects of the visual system.

Alzheimer's Disease, Glaucoma and the Visual System

AD is known to affect the visual system. Areas of vision affected by AD are a decrease in visual acuity (Uhlmann RF, 1991), decrease in contrast sensitivity (Lakshminarayanan V, Lagrave J, Kean ML, Dick M, and Shankle R, 1996 and Bassi CJ, Solomon K, and Young D, 1993), decrease in color vision (Pache M , Smeets CHW, Gasio PF, Savaskan E, Flammer J, Wirz-Justice A, and Kaiser HJ, 2003), loss of motion perception (Gilmore GC, Wenk HE, Naylor LA, and Koss E, 1994), oculomotor deficits (Verheij S, Muilwijk D, Pel JJ, Van der Cammen TJ, Mattace-Raso FU, and Van der Steen J, 2012), decreases in both visual evoked potentials and electroretinograms (Katz B, Rimmer S, Iragui V, and Katzman R, 1989 and Partanen J, Hartikainen P, Konomen M, Jousmaki V, Soininen H, and Riekkinen P, 1994),

visual field loss (Trick GL, Trick LR, Morris P, and Wolf M, 1995), and the formation of β-amyloid cataracts (Goldstein LE, Muffat JA, Cherny RA, Moir RD, Ericsson MH, Huang X, Mavros C, Coccia JA, Faget KY, Fitch KA, Masters CL, Tanzi RE, Chylack LT Jr., and Bush AI, 2003).

Optic nerve head changes in POAG, and RNFL drop out in POAG and AD are the predominant retinal changes observed in the eye in these two neurodegenerative diseases. Recent studies have shown that retinal neurodegeneration continues further into the visual system. The RGCs that reside within the ganglion cell layer of the inner sensory retina project their axons along the inner retina, where they travel along as the RNFL, and then pass down through the lamina cribrosa, bundled together as the optic nerve. As the axons travel out of the eye and out of the orbit through the optic canal, about half of them, the nasal portion, decussate to the contralateral side at a junction known as the optic chiasm, located superior to the pituitary gland, on the ventral surface of the brain. Most of the axons, here forward known as the optic tract, continue on to synapse onto the lateral geniculate nuclei (LGN) located within the lateral thalamus, posterior to the ventral tier, at the level of the uppermost cross-section of the midbrain. The LGN is the first relay within the visual system. Visual information arrives with retinotopic organization with information from the retina synapsing onto one of the six layers within the LGN. The LGN is further subdivided by the type of visual information that is processed. Layers one and two contain larger nuclei known as magnocellular nuclei and are involved in the processing of movement and contrast. Layers three through six contain smaller nuclei known as parvocellular nuclei which are involved in mostly color and form perception of red/green colors. A third type of nuclei

known as koniocellular nuclei process color and form perception of blue/yellow colors and are located throughout all six layers of the LGN. Information then moves out from the LGN through relay neurons, known as optic radiations, that course through the internal capsule of the cortex. They then synapse in the striate cortex in the occipital lobe, otherwise known as the primary visual cortex, for further processing before heading off to either the parietal lobe or the temporal lobes.

Transneuronal Degeneration

Transneuronal degeneration or transsynaptic degeneration (TD) is a process by which post-synaptic neurons are adversely affected by a disease state or loss of function in their pre-synaptic, afferent neurons (Su JH, Deng G, Cotman CW, 1997). Loss of the afferent signal causes malfunction, atrophy, and death of the efferent neuron. This pattern of loss can continue throughout the entire neuronal tract. TD is found in many central nervous system (CNS) diseases particularly because of the difficulty in repair and renewal of CNS axons (Yucel Y and Gupta N, 2008). There is evidence that TD takes place in both POAG and AD. One study analyzed human brains for the presence of hyperphosphorylated tau proteins, post-mortem via immunohistochemistry (Su JH, Deng G, and Cotman CW, 1997). The study found hyperphosphorylated tau that began within the entorhinal cortex, a section of the limbic region known to be first affected by AD, and carried on throughout the limbic region. Individuals with more severe forms of AD had further penetrating and more developed hyperphosphorylation. A "toxic substance" was released by the pre-synaptic neurons to cause the damage to the post-synaptic neurons. This "toxic substance" could be glutamate as

per the glutamate excitotoxicity model of neurodegeneration found in both AD and POAG. Another study performed by quantified characteristic AD changes consisting of both senile Aβ plaques and NFTs via gross examination within sections of the visual system (Leuba G and Saini K, 1995). The study analyzed the LGN, lateral inferior pulvinar, superior colliculus, and the primary visual area 17. The study found senile plaque formation within the LGN, with greater number in the parvocellular layers, the lateral inferior pulvinar, in greater numbers than within the LGN, the superior colliculus, and within the primary visual area 17. The study found NFTs within only the superior colliculus and the primary visual area 17, with a predominance of pyramidal neurons of layers two through six. Lastly, the study also noted the appearance of senile plaques within the fiber tracts between the LGN and the lateral inferior pulvinar. The study noted no senile plaques or neurofibrillary tangles within the visual system in control patients with the exception of some small senile plaques in the visual area 17 in older patients. This pattern of damage within the visual system is consistent with TD and progresses from the occipital cortex back to the LGN in a retrograde manner.

Studies have also analyzed the presence of TD within individuals with glaucoma. One such study reported an overall neuronal loss in the magnocellular and parvocellular layers of the LGN in experimentally induced glaucoma in primates that positively correlated to a decrease in the RNFL (Yucel YH, Zhang Q, Gupta N, Kaufman PL, and Weinreb RN, 2000 and 2001). The study also noted a deafferentation related directly to the amount of atrophy showing that there was a direct relationship to neuronal loss in the retina and neuronal loss in the nuclei of the LGN. This provides evidence to support the loss of visual system neurons

by anterograde TD. Another study found similar results in rhesus monkeys. The study found no preference for which type of cells were lost, both magnocellular and parvocellular cells were affected the same way (Weber A, Chen H, Hubbard W, and Kaufman P, 2000).

Studies have also shown TD in glaucoma within humans. A case report of a post-mortem examination of the visual pathway of a 79-year-old white male with POAG had severe glaucomatous hemifield loss and very large cup to disc ratios of 0.9 with inferior notching (Gupta N, Ang L, Noel de Tilly L, Bidaisee L, and Yucel YH, 2006). Compared to two age-matched controls, the POAG patient had pronounced optic nerve atrophy, marked axonal loss, LGN atrophy, shrunken LGN relay neuron axons, and cortical ribbon thinning within the visual cortex. The same group also found atrophy of the LGN within human glaucoma patients, *in vivo*, using magnetic resonance imaging (MRI) (Gupta N, Greenberg G, de Tilly LN, Gray B, Polemidiotis M, and Yucel YH, 2009). The study found a significant decrease in LGN size and volume indicating atrophy compared to controls. The study also performed post mortem studies and reported similar sizes and amounts of atrophy of the LGN upon physical examination. These studies indicate that the effects of POAG and AD can be found throughout the visual areas of the brain. The apparent method of degeneration throughout the visual system is TD, with an anterograde pattern in POAG and a retrograde pattern in AD. This neurodegeneration found throughout the visual system should indicate that retinal ganglion cells in particular can be affected in AD and if so, the RNFL should also be affected.

Measuring Retinal Ganglion Cell Degeneration in Alzheimer's Disease

Histological Studies

Evidence for RGC degeneration in AD was first reported in 1986. RGC loss and RNFL thinning was first observed upon post-mortem analysis of retinal tissue via electron microscopy (Hinton DR, Sadun AA, Blanks JC, and Miller CA, 1986). The study found that the axons of the RGCs as well as the cells themselves were fewer in number with an overall decrease of two- to three-fold, and they were more dispersed in the AD group compared to the control group. The study concluded that the findings showed a clear evidence for a distinctive histopathologic process in the retinas of AD patients. Another study also demonstrated similar results in RGC axon degeneration, decreased number of RGCs, and RNFL thinning using similar techniques (Sadun AA, and Bassi CJ, 1990). The study also noted that degeneration appeared to follow a retrograde axonal route from the optic chiasm towards the optic nerve head suggesting that degeneration may start further back within the visual system and move towards the retina, and the specific type lost were magnocellular RGCs. Neither of these two studies reported NFT formation or Aβ plaque formation within the retina or optic nerve (Hinton DR, et al., 1986, and Sadun AA, et al., 1990). Other studies performed around the same time do not show significant differences in RGCs in AD compared to controls, though these reports do state that different tissue preparation methods were used (Curcio CA, and Drucker DN, 1993 and Davies DC, McCoubrie P, McDonald B, and Jobst KA, 1995). The conflicting findings indicate that there is no consensus on this

matter from the histological studies. None of the groups specifically looked at severity of the disease, and this likely affected the study outcomes.

Fundus Photography

During the 1990's, studies were performed using fundus photography. One such study analyzed RNFL thickness using fundus photography (Tsai C, Ritch R, Schwartz B, Lee SS, Miller NR, Chi T, and Hsieh FY, 1991). The study found that there was a significant amount of diffuse RNFL thickness loss and found no significant difference in retinal pallor. The study is the first to suggest that loss of RGCs and thinning of RNFL could be a means to measure disease progression. Another study found conflicting results (Hedges T, 1996). The study determined that although there was some significant data supporting the loss of RNFL thickness in AD patients, the lack of quality of fundus photographs left it difficult to reach a definitive conclusion.

Scanning Laser Ophthalmoscopy

As retinal imaging technology advanced, so did the methods used to study RNFL thickness in AD patients, *in vivo*. During the early to mid-2000's, studies were performed using scanning laser ophthalmoscopy (SLO) to assess RNFL loss. One such study analyzed RNFL thickness using the Laser Diagnostics Technologies Nerve Fiber Analyzer with GDx software (Kergoat H, 2001). The study found no significant difference in the RNFL thickness compared to controls. Another study found conflicting evidence (Danesh-Meyer HV, 2006) The study found a decrease in RNFL thickness globally that was approaching significance. Although the study concluded that SLO was the most advanced technology for

in vivo diagnostic assessment of RNFL at the time, newer emerging technologies such as optical coherence tomography may be better equipped to assess RNFL thickness.

Other studies

Other studies have used immunohistochemistry to assess neurodegeneration *in vivo* at the level of the neuron (Cordeiro MF, Guo L, Coxon KM, Duggan J, Nizari S, Normando EM, Sensi SL, Sillito AM, Fitzke FW, Salt TE, and Moss SE, 2010). One group used florescent antibody tags to assess the processes of necrosis and apoptosis within RGCs and their axons both in glaucoma and in AD in animal models. The study found that in the glaucoma group, early apoptotic markers were present after three weeks of elevated IOP and by eight weeks the cells were gone, indicating apoptotic cell death. They then used the same model to assess cell death within triple transgenic mice positive for both Aβ and tau pathology. After comparing the data with normal wild type mice the study found that there was a significant increase in early apoptotic cell death in test mice compared to controls. The study concluded that temporal resolution and quantification of the early and late phases of apoptosis and necrosis of single nerve cells can be viewed *in vivo* and by using this method, clinicians may be able to assess RGC death, *in vivo*, as a method of monitoring disease progression and treatment efficacy. This study provides evidence that neurons in both glaucoma and AD are undergoing apoptotic neurodegeneration.

Functional loss can also be assessed in AD in a similar fashion as in glaucoma, via visual field analysis. One such study assessed visual fields in individuals with mild AD and age-matched controls (Trick GL, et al., 1995). Visual field analysis was performed using the

Humphrey Visual Field Analyzer using a 30-2 full threshold STATPAC test. The study found that within the test group, 79.4% had visual field defects compared to only 34.1% of controls. Within the AD group the most predominant visual field loss was both an overall decrease in sensitivity and inferior arcuate-like scotomas. This study confirms a functional visual loss in some cases of AD and helps to classify the specific type of loss, diffuse overall loss of sensitivity and inferior arcuate-like scotomas.

These early forms of RNFL and RGC assessment yielded mostly controversial results. A more sensitive method of analyzing the retinal anatomy was required. Optical Coherence Tomography can accurately assess the retinal anatomy and is sensitive enough to find structural changes within the retina to explain functional changes.

Optical Coherence Tomography (OCT)

OCT is a widely accepted, noninvasive, *in vivo* ocular imaging technique. OCT uses low-coherence light to produce 3-D representations of live tissue, *in vivo*. Near infrared light is sent into the tissue at which point it permeates and scatters off of different areas within the tissue and reflects back at different rates (Sakata LM, Deleon-Ortega J, Sakata V, and Girkin CA, 2009). Based on the time it takes for the light to return to the machine, compared to the time it takes light to return from a reference mirror at a known distance, the computer can determine a 2-D structure (Huang D, Swanson EA, Lin CP, Schuman JS, Stinton WG, Chang W, Hee MR, Flotte T, Gregory K, and Puliafito CA, 1991). These depth scans are known as A-scans. Through multiple scans across the surface of the tissue, known as B-scans, a composite picture can be generated. These first generation OCTs are known as time-domain OCT (TD-OCT). The first use of OCT in both optical and non-optical analysis was

performed in 1991. The study imaged the peripapillary region of the retina including the optic disc with higher depth resolution than can be obtained from clinical ultrasound or confocal scanning systems and it provided information on the contour and thickness of important retinal structures that would later become the basis of new clinical diagnostic procedures. The scans reached a resolution of approximately nine microns in the post mortem sample. The first study to analyze the living human retina in came in 1993 (Fercher AF, Hitzenberger CK, Drexler W, Kamp G, and Sattmann H, 1993). The study imaged a healthy individual's ocular fundus at the site of the optic disc and reported the ability to clearly delineate between various structural layers of the retina including the internal limiting membrane, retinal pigment epithelium, the choroid, and part of the optic nerve head. The scans reached a resolution of approximately twenty microns in the live human eye.

With new innovations in OCT technology, the more recent spectral-domain or Fourier-domain OCT has emerged. Spectral-domain OCT (SD-OCT) uses a spectrometer to detect varying wavelengths of light during scanning and Fourier transformations are then performed allowing for the analysis of more A-scans giving faster results and higher resolution (Chen TC, Cense B, Pierce MC, Nassif N, Park BH, Yun SH, White BR, Bouma BE, Tearney GJ, and de Boer JF, 2005). The faster scanning also allows for better correction from even small amounts of peripheral eye movement during image acquisition. Live videos of the retina can also now be recorded (Chen TC, et al., 2005). Newer models also incorporate confocal SLO within the unit for better overall fundus imaging and eye tracking and even angiographers for better vascular imaging. These newer technologies are allowing more detailed imaging of the photoreceptor outer and inner segment layers and the ganglion cell layer. There is better

visualization of macular pathologies, macular holes, and photoreceptor drop out (Sakata LM, et al., 2009). OCT provides the best visual imaging of the posterior pole of the eye to date (Sakata LM, et al., 2009). This technology can vastly improve our capability to not only diagnose ocular disease but also to track its progression and evaluate treatment effectiveness.

Optical Coherence Tomography Studies in Glaucoma

OCT is quickly becoming the gold standard to track the early progression of glaucoma. RNFL thickness measurements can be acquired and changes tracked over time to determine both the severity of disease progression and the effectiveness of treatment. The first reported use of OCT in glaucoma was in 1995 (Schuman JS, Hee MR, Puliafito CA, Wong C, Pedut-Kloizman T, Lin CP, Hertzmark E, Izatt JA, Swanson EA, and Fujimoto JG, 1995a). The study compared the average RNFL thickness globally and by quadrant around the optic nerve head in patients with glaucoma to age matched control eyes. The study found that diagnosis of glaucoma was associated with a significantly thinner RNFL compared to controls and there was a significant decline in RNFL thickness with aging in both groups. Many other studies have been reported since then lending support for the use of OCT in glaucoma diagnosis and tracking (Huang J, Pekmezci M, Mesiwala N, Kao A, and Lin S, 2010, Li G, Fansi AK, Boivin J, Joseph L, and Harasymowycz P, 2010, Nakatani Y, Higashide T, Ohkubo S, Takeda H, and Sugiyama K, 2010, Parisi V, 2003, and Sihota R, Naithani P, Sony P, and Gupta V, 2010.)

Studies have also found that SD-OCT scans are not affected by pupil dilation when acquiring images compared to TD-OCT scans, which require a dilated pupil for optimum results

(Massa GC, Vidotti VG, Cremasco F, Lupinacci APC, and Cost VP, 2010). A recent study also found that the use of SD-OCT/SLO instruments can provide more detailed scans of glaucomatous eyes compared to TD-OCT based on the higher resolution acquired and the ability to track the eye movements during image acquisition due to the SLO component (Cho JW, Sung KR, Hong JT, Um TW, Kang SY, and Kook MS, 2010). Another study showed that results of RNFL thickness measured by OCT are also reproducible not only by different analysts, but also by the same analyst over multiple images (Lee SH, Kim SH, Kim T, Park KH, and Kim DM, 2010). This new technology allows for a more objective form of measurement, free of analyst bias, for the analysis of possible glaucomatous changes and is highly reproducible between different clinics. SD-OCT advances have brought OCT to the forefront of retinal imaging technology and have provided the most powerful tool in identifying glaucomatous changes *in vivo*.

Optical Coherence Tomography Studies in Alzheimer's Disease

The analysis of glaucoma by OCT has provided a great model for the identification of other retinal diseases *in vivo*. With earlier studies have shown similarities in transneuronal degeneration models between AD and glaucoma, and loss of RGC and RNFL thickness in AD, new studies have adapted this technique for the study of AD. A summary of the following studies and their outcomes is available in Figure 1. The first study of AD via OCT was performed in 2001 (Parisi V, Restuccia R, Fattapposta F, Mina C, Bucci MG, and Pierelli F, 2001). The study used the Humphrey TD-OCT to analyze the RNFL of patients with AD versus age-matched controls, following pupil dilation. The study found that RNFL

thickness was significantly thinner in AD patients compared to controls both globally and in all four quadrants. There was no significant correlation between RNFL thickness and age or mini-mental status exam scores.

The next study to analyze RNFL thickness in AD was performed in 2006 using the Zeiss Model 3000 TD-OCT (Iseri PK, Altinas O, Tokay T, and Yuksel N, 2006). The study analyzed both RNFL thickness and macular thickness in mild to moderate AD patients compared to age-matched controls, following pupil dilation. The study found that RNFL thickness was significantly thinner in AD patients compared to controls both globally and in three of the four quadrants, excluding the temporal quadrant. The study also found the mean total macular volume was significantly decreased in AD patients compared to controls and this correlated to performance on the mini-mental status exam.

The next study was published in 2007 (Berisha F, Feke G, Trempe C, McNeel JW, Schepens C, 2007). The study used Stratus TD-OCT to analyze the RNFL thickness, and laser Doppler flowmetry to analyze overall blood velocity and flow rate, within mild to moderate AD and age-matched controls. The study found a significantly lower blood flow rate in patients with AD compared to controls, and found that RNFL thickness was significantly thinner in AD patients compared to controls, but only in the superior quadrant. The study was the first to suggest that thinning of the RNFL may take place within the superior quadrant first and may correlate to findings of inferior visual field deficits found in previous studies (Trick et al, 1995).

Another study published in 2007 was the first to analyze mild AD patients, severe AD patients, and those with MCI, compared to age-matched controls (Pacquet C, Boissonnot M, Roger F, Dighiero P, Gil R, and Hugon J, 2007). The study found progressively thinner RNFL between controls, to the MCI group, to the mild AD group, and then to the severe AD group. The differences were significant between all groups except for between the mild AD and MCI groups. The study suggested that the involvement of the retina is an early event in the course of the disorder in which global cognition is still momentarily preserved.

The next study published in 2010 used Stratus TD-OCT to analyze the RNFL thickness and overall health of the RNFL via fundus photography (Lu Y, Li Z, Zhang X, Ming B, Jia J, Wang R, and Ma D, 2010). The study compared mild to moderate AD and age-matched controls and reported a significant decrease in RNFL thickness in AD patients in the superior and inferior quadrants and globally. The study also found that the pattern of RNFL dropout based on fundus photography varied in different AD patients. The study concluded that RNFL thinning is found in patients with AD and seems to be preferential to the superior and inferior quadrant, especially early on in the disease progression, the second to comment on the superior quadrant.

The next study was published in 2011 (Kesler A. Vakhapova V, Korczyn AD, Naftaliev E, and Neudorfer M, 2011). The study also used Stratus TD-OCT and examined individuals with MCI, AD, and age matched controls. The study found thinning of the RNFL that increases between the MCI and then the AD groups and found it first within the inferior

quadrant, versus other studies that implicated the superior quadrant first. The study found no correlation between the RNFL thickness and cognitive testing.

The next study published in 2012 evaluated RNFL thickness and also total macular thickness and included multifocal electroretinogram (mfERG) analysis of the fovea and perifoveal areas (Moschos MM, Markopoulos I, Chatziralli I, Rouvas A, Papageorgiou SG, Ladas I, and Vassilopoulos D, 2012). The study recruited individuals with AD and age-matched controls. The study found that macular thickness and RNFL thickness within the inferior and superior quadrants were lower and the mfERG response was decreased both at the fovea and at the perifovea areas in individuals with AD compared to controls.

Lastly, a study published in 2013 analyzed the average RNFL thickness in individuals with different types of dementia including AD, dementia with Lewy bodies, and Parkinson's disease (Moreno-Ramos T, Benito-Leon J, Villarejo A, and Bermejo-Pareja F, 2013). The study found that in all of the different dementia groups there was a decrease in average RNFL thickness globally compared to age-matched controls. The study also found a correlation between RNFL thickness and cognitive performance using both the MMSE and the Mattis Dementia Rating Scale between all dementia groups.

To date, there are only two studies that have employed the SD-OCT technology to analyze the RNFL thickness of individuals with AD, both of which were published in 2013. The first study analyzed the average RNFL thickness of participants with early untreated AD and age-matched controls and found that there was a significant thinning in the RNFL of AD patients compared with controls, both globally and within the superior quadrant (Kirbas S,

Turkyilmaz K, Anlar O, Tufekci A, and Durmus M, 2013). The second study analyzed elderly individuals over a 25 month period to determine if loss of cognitive function correlated to RNFL thinning (Shi, Z, Yujie W, Meijuan W, Jing C, Wei F, Yan C, Chunbo L, and Yuan S, 2013). Over the 25 month period, 18 of these individuals developed AD, and within this group there was a significant decrease in RNFL thickness within the inferior quadrant compared to age-matched controls. The study was the first to demonstrate that RNFL thickness changes over time in AD patients.

Purpose of the Study

There are no studies that compare RNFL thinning in glaucoma patients and in AD patients. There are also only two studies that use SD-OCT technology to analyze the RNFL in AD and further studies are required to understand any potential benefit to using this technology compared to TD-OCT. Our study aimed to fill in these gaps in knowledge. We postulated three hypotheses: First, patients with AD would show thinner RNFL compared to age-matched controls, which has been documented previously. Second, the pattern of RNFL thinning in AD patients would be different than that of patients with glaucoma. Third, RNFL thickness would correlate to cognitive function in AD patients such that individuals with thinner RNFL would correlate with poorer performance on cognitive tests. The TD-OCT and the SD-OCT have enough resolution to accurately measure the RNFL within the retina and we hypothesized that there would be no difference in the RNFL findings between the TD-OCT studies and the SD-OCT studies. It is likely that the SD-OCT will be more valuable in longitudinal studies as the eye tracking software is much more robust within the SD-OCT

units and the increased resolution may aid in finding smaller differences between scans in future studies.

Chapter Two: Materials and Methods

Study Design

In this cross-sectional pilot study, participants were recruited from Jamaica Plain Veterans Affairs Hospital (JPVA) within the Neurology, Geriatric Research Education and Clinical Centers (GRECC), and JPVA Optometry Section clinics. Patients were assigned to one of three groups matched for age, gender, and race. The groups consisted of patients with early to moderate POAG (based on the Bascom Palmer Glaucoma Severity Scale) (Mills RP, et al., 2006), mild to moderate AD, and a healthy control group, with a recruitment goal of twenty participants in each group for a total of 60 participants. Nineteen participants were recruited into the POAG group, five in the AD group and seventeen in the control group. Participants were recruited into the POAG group based on prior diagnosis and treatment by staff optometrists and/or ophthalmologists, many of whom were on IOP lowering medications. Participants were recruited into the AD group based on prior diagnosis and treatment by staff neurologists and determined to be in the mild to moderate range. AD group participants showed no signs of glaucoma suspicion or glaucoma including ocular hypertension or

asymmetric nerve head cupping. All participants had comprehensive eye exams within the VA within the past year, most within weeks of study testing. All AD group study participants were tested within one month of their last VA Neurology or GRECC clinical exam to ensure that their ability to provide consent was not compromised. All individuals provided informed consent before the onset of any study testing and all protocols were authorized by VA Boston Healthcare System Institutional Review Boards.

Participants were excluded if they had a history of any of the following conditions: macular degeneration; macular edema, uncontrolled diabetes mellitus (defined as level of non-proliferative diabetic retinopathy that was greater than mild and/or HbA1c levels of greater than 8.0%), uncontrolled hypertension (defined as most recent blood pressure reading of > 140/90), ocular trauma, ocular surgery (except for uncomplicated cataract surgery), best corrected visual acuity worse than 20/60, or refractive error measured by spherical equivalent greater than ±6D. Participants with AD did not have a non-Alzheimer primary dementia. Controls were excluded if diagnosed with dementia, cognitive impairment, glaucoma or glaucoma suspicion, or any other issue known to affect RNFL thickness. Participants were excluded if unable to provide informed consent.

Following informed consent, the participant's visual acuity was assessed using the Snellen acuity chart. Visual acuity was recorded for both eyes individually. Cognitive assessment was performed using the St. Louis University mental status exam (SLUMS), the Delis-Kaplan Executive Function System (D-KEFS) including only the Trail Making Test (TMT), and the Color Word Interference Test (CWIT). SD-OCT scans were acquired on both eyes

for all participants using the Spectralis SD-SLO/OCT (Heidelberg Engineering, Germany). Scans were taken for RNFL thickness in four quadrants (Superior, Inferior, Nasal, and Temporal) as well as six sectors (Superior Nasal, Superior Temporal, Inferior Nasal, Inferior Temporal, Nasal, and Temporal) and also globally. Cup to disc ratio and disc size were determined using SLO images of the ONH. Ratios were then calculated based on number of pixels vertically/horizontally for the cup compared to the disc measured in Microsoft Paint. Data was collected from the VA computerized patient record system (CPRS) including the age, race, gender, refractive error, date of diagnoses, central corneal thickness, number of concurrent diagnoses, number of current medications, intraocular pressures, and visual field status. All tests were performed within the JPVA optometry clinic .

Optical Coherence Tomography Methods

Participants underwent OCT analysis using the Spectralis SD-OCT (Spectralis, Heidelberg Engineering). Participants were comfortably positioned at the device and were given any breaks that may have been necessary during the testing to help eliminate any possible discomforts. Artificial tears were also offered to the participants before, during, and after imaging if necessary. All necessary aseptic techniques were followed during testing. Imaging was performed using the scanning laser ophthalmoscope to acquire images of the ONH for cup to disc ratio analysis. Several images were acquired and the best saved for analysis. The ONH was then scanned using the patented eye tracking software and three circular scans were acquired for average RNFL thickness around the ONH. These images were analyzed for quality and the best was saved for analysis. This process was repeated for

the second eye. Following the procedure, thickness data was gathered from the machine and recorded onto the relevant testing pages. Thickness measured in microns was taken globally, in each of four quadrants (superior, nasal, inferior, and temporal) and in six sectors (superior nasal, nasal, inferior nasal, inferior temporal, temporal, and superior temporal). Cup to Disc Ratios were analyzed by taking the raw image, opening it within the paint program and measuring the number of pixels vertically for the cup and then the disc and also horizontally for the cup and then the disc and then divided to get the cup to disc ratio for both eyes. All images and OCT scan data were transferred off of the SD-OCT machine via a government controlled encrypted jump drive to the government controlled database. All raw data sheets were contained in a locked cabinet within a locked office to protect patient.

Cognitive Testing Methods

Delis-Kaplan Executive Function System (D-KEFS) Trail Making Test (TMT)

The TMT test is actually separated into two main sections, TMT-A and TMT-B. TMT-A involves the sequential tracing of numbers/letters on a large 11 in x 17 in piece of paper. Both numbers and letters are displayed in a mixed array around the test page and the subject must trace a path sequentially around the paper using a pen following either numbers, 1, 2, 3, etc. or letters, A, B, C, etc. The time it takes the subject to complete the test is recorded in seconds. This section is predominantly used to assess visual search and motor speed skills (Bowie CR, et al., 2006). TMT-B involves a similar tracing on a similar test page, but numbers and letters are alternated as the sequence is followed. For example, the subject would start at the number “1” and then move to the letter “A” and then to the number “2” and

then to the letter “B”, and so on around the page. Again, time is measured in seconds. This section is considered to be more of a test of mental flexibility (Bowie CR, et al., 2006). In the event the subject is unable to complete TMT-A, TMT-B is usually not tested, as it is determined that the cognitive demand increases from test A to test B, due to the larger distance between, and increased amount, of interfering stimuli (Bowie CR, et al., 2006). Errors made during the test are not directly tallied. In the event that the subject incorrectly traces the path, the examiner will redirect the subject back to the previously correct answer and then allow the subject to try again. The time that it takes to correct the subject and for them to continue on to the next correct sequential step factors into the overall time to complete the test, allowing for an indirect way of factoring errors into the final test completion time (Bowie CR, et al., 2006).

Delis-Kaplan Executive Function System (D-KEFS) Color Word Interference Test (CWIT)

During the test procedure, subjects are first given an 8½ in x 11 in piece of paper with color patches of red, blue, and green on it, in various sequences. The subject then reads off the colors seen. All sections of the test are timed. Next, the subject is given another piece of paper with the words “red”, “blue”, and “green” written on it in various sequences, all written in black ink. The subject proceeds to read through the words. The average of the first two times is then used as the baseline for the ability to recognize and read colors and words. The subject is then given a third piece of paper with the words “red”, “blue”, and “green” written on it, but the words are written in one of the three colors. The color is not matched with the

word. The subject then proceeds to name the colors of the ink each word is written in. The last section's time is then compared to the averaged baseline from the first two tests yielding the Stroop interval.

The Saint Louis University Mental Status (SLUMS) Exam

The test combines many smaller test questions into a comprehensive cognitive assessment. Various questions are asked such as the day of the week, present year, and present state of residence to test the subject's orientation to time and place. There is a memory retention test in which the subject is asked to remember five objects and then asked to repeat them near the end of the test. There are simple math problems and an animal naming exercise. There is a clock drawing exercise where the subject must draw a clock and indicate a specific time using the hour and minute hands. Lastly there is a small paragraph of text that is read to the subject, followed by some questions about the text. The test is administered and scored based on correct answers out of 30 points. Once the test is completed the subjects score is compared to a chart identifying the subject as either normal, having MCI, or as having AD (dementia). The highest level of education attained by the subject can affect the test and the table is therefore adjusted for this variable. If the subject did not complete a high school level of education, the breakdown is a score from 1-20 AD, 21-26 MCI, and 27-30 normal. If the subject completed a high school level of education, or above, the breakdown is a score from 1-19 AD, 20-24 MCI, and 25-30 normal.

Statistical Analyses

Patient Demographics

Age (years) and refractive error (Spherical Equivalent) were analyzed between each of the groups (Control, Glaucoma, and Alzheimer's disease) using one factor ANOVA. The visual acuities were taken via Snellen acuity charts. In order to perform statistical analysis on this data set the Snellen fractions were converted into LogMar notation and then chi squared tests were performed and the final numbers then converted back into Snellen fractions.

Optical Coherence Tomography

Vertical and Horizontal cup to disc ratios, as well as global RNFL thickness (um) were analyzed between each of the groups (Control, Glaucoma, and Alzheimer's disease) using one factor ANOVAs. RNFL thickness (um) was also analyzed between the four quadrants and four of the sections, in relation to each group using one factor ANOVAs. (The Heidelberg Spectralis SD-OCT system gives RNFL thickness scans based on quadrant (4) and sections (6), but the temporal and nasal quadrant and section data are the same. For calculation purposes, the temporal and nasal section data were left out in order not to duplicate results.) Following the ANOVA analysis, *ad hoc* analysis via student t-tests, unpaired two sample with unequal variance and with two tails were performed on control vs. AD data groups for both sector and quadrant to further evaluate statistical significance within each section.

The data for the glaucoma group was also analyzed both as a whole group, and then based on severity of glaucoma using the Bascom Palmer Glaucoma Severity Scale and selecting for only mild glaucoma. Of the 19 glaucoma participants studied, 3 of them fit into Grade 0 (indicating early glaucoma), 13 of them fit into Grade 1 (indicating mild glaucoma), and 3 of them fit into Grade 2 (indicating moderate glaucoma). The Bascom Palmer Glaucoma Severity Scale determines grading of glaucoma severity by the amount of mean deviation and type, quantity, and depth of visual field defects on the Humphrey Visual Field (HVF). The worse of the two eyes is then graded accordingly. A participant with Grade 0 glaucoma indicates that the patient has no visual field defects, but may be diagnosed and treated for glaucoma due to documented glaucomatous loss on optical coherence tomography.

Cognitive Testing

Each separate condition (2,3,4, and 5) of the TMT, as well as B/A Ratio, SLUMS Exam scores, and each separate condition (1,2, and 3) as well as the Stroop Interval (seconds) were analyzed between each of the groups (Control, Glaucoma, and Alzheimer's disease) using one factor ANOVA. Education level was analyzed by chi square test for each group. Pearson correlation coefficients (r values) were calculated for each test variable in relation to the global RNFL thickness in order to look for a trend between the two data sets. p values were calculated and statistical significance was established as $p < 0.05$.

Chapter Three: Results

Patient Demographics

A total of seventeen control, nineteen glaucoma, and five Alzheimer's disease participants were recruited into the study. The demographics of each group is summarized in Table 2 including the age, race and gender of each test group, along with each group's mean VA (Snellen) and refractive error (Spherical Equivalent). The results of the statistical analysis is also summarized in Table 2 for each group in relation to age (years), VA (Snellen), and refractive error (Spherical Equivalent).

The control group was comprised of 16 males and one female, and 16 Caucasians and one African American. The glaucoma group was comprised of 17 males and no females, and 11 Caucasians and eight African Americans. The Alzheimer's disease group was comprised of

five males and no females, and five Caucasians and no African Americans. The mean ages of each group were 69, 70, and 71 respectively. There was no statistically significant difference in mean age between all of the groups (p = 0.89) The mean VA (Snellen) of each group were 20/24, 20/23, and 20/28, respectively. There was no statistically significant difference in VA (Snellen) between the three groups (p = 0.47). The mean refractive error (Spherical Equivalent) of each group were +0.25, plano, and -0.50, respectively. There was no statistically significant difference in mean refractive error (Spherical Equivalent) between all of the groups (p = 0.63).

Optical Coherence Tomography

The cup to disc ratio and global RNFL thickness analysis of each group is summarized in Table 3 including the Vertical and Horizontal cup to disc ratios, as well as global RNFL thickness (um). The results of the statistical analysis is also summarized in Table 3 for each group in relation to Vertical and Horizontal cup to disc ratios, as well as global RNFL thickness (um).

The mean vertical cup to disc ratio of the control, glaucoma, and AD groups were 0.40, 0.65, and 0.45 respectively. There was a statistically significant difference in mean vertical cup to disc ratio between the three groups (p = 2.73E-12). *Ad hoc* analysis shows the difference to be between the POAG group and the control and AD groups. There was no significant difference between the control and AD groups. The mean horizontal cup to disc ratios of each group were 0.40, 0.60, and 0.50 respectively. There was a significant difference in the mean horizontal cup to disc ratio between the three groups (p = 3.25E-10). *Ad hoc* analysis

shows the difference to be between the POAG group and the control and AD groups. There was no significant difference between the control and AD groups. This indicates that the POAG group has the characteristic glaucomatous cupping observed in glaucoma, not like the control and AD groups. This identifies a key difference between glaucomatous nerves and the nerves of individuals with AD. The mean global RNFL thickness (um) of each group were 92, 78, and 92, respectively. There was a statistically significant difference in mean global RNFL thickness between the three groups (p = 3.62E-07). *Ad hoc* analysis shows the difference to be between the POAG group and the control and AD groups. There was no significant difference between the control and AD groups as was found in several earlier studies.

The analysis of RNFL thickness by quadrant and sector are summarized in Table 4. The analyses indicate that there is a statistically significant difference between the RNFL thickness (um) in all areas with the exception of the superior nasal quadrant which was approaching significance (p-values listed in Table 4). These data were further analyzed via *ad hoc* analysis using student t-tests, unpaired two sample with unequal variance and with two tails as stated above. The glaucoma group had a statistically significant thinner average RNFL compared to both the control and the AD groups. The difference in thickness were more statistically significant in the superior temporal and inferior temporal areas. Results from the *ad hoc* analysis between the control group and the AD group are listed in Table 5. The RNFL thickness (um) was statistically significantly thicker in the temporal quadrant and inferior temporal sector in the AD group compared to the control group (p = 0.05 and p = 0.02 respectively.) Also, the RNFL thickness (um) approached statistical significance within

the nasal quadrant and the superior nasal sector with the control group having a thicker RNFL than the AD group ($p = 0.08$ and $p = 0.07$ respectively.)

Cognitive Testing

The cognitive data analyses of each group is summarized in Table 6. The mean time to completion of condition 2 (seconds) of the TMT was 51, 51, and 82. The mean time to completion of condition 3 (seconds) of the TMT was 49, 52, and 102. The mean time to completion of condition 4 (seconds) of the TMT was 118, 122, and 279. (All written as control/glaucoma/AD group.) There was a statistically significant difference in all three of these conditions between each of the groups ($p = 0.05$, $p = 0.001$, and $p = 0.0002$, respectively). *Ad hoc* analysis shows that there is no statistically significant difference between the control and glaucoma group for all conditions, and there is a statistically significant difference between both the control and glaucoma, and the AD group with the AD group having a longer time to completion for each condition. This means that the AD group took longer to navigate the test page with both numbers and letters (Cond 2 and 3), and also took longer to complete the number/letter switching exercise (Cond 4) which implies a difficulty with cognitive flexibility and decision making.

The mean time to completion of condition 5 (seconds) of the TMT was 39, 35, and 52. There was no statistically significant difference between the three groups ($p = 0.16$). This means that all participants were able to trace the lines at the same speed. This is important because it indicates that physical dexterity was not a factor in the completion of the other three conditions, and that AD participants were still able to trace straight lines. The mean B/A

Ratio of each group were 2.39, 2.38, and 2.94 respectively. There was no statistically significant difference in mean B/A Ratios between the three groups ($p = 0.23$). Despite the significant differences in conditions 2, 3, and 4, the B/A ratios were not significantly different though the AD participants did have higher B/A ratios than the other groups.

The mean SLUMS Exam scores of each group were 25, 25, and 18 respectively. There was a statistically significant difference between the groups ($p = 0.0002$). *Ad hoc* analysis concluded that there was no statistically significant difference between the control group and the glaucoma group, but there was a statistically significant difference between both of these groups with the AD group, with the AD group having a lower SLUMS exam score. The mean education level of the three groups were 2, 2, and 2. There was no statistically significant difference between the three groups ($p = 0.79$). This is important because it indicates that the educations levels were similar between all participants and therefore education is not a confounding factor within this study.

The mean time to completion of condition 1 (seconds) of the CWIT was 37, 34, and 50. There was a statistically significant difference between the three groups ($p = 0.004$). There was no statistically significant difference between the control and glaucoma group, but there was a statistically significant difference between these groups and the AD group with the AD group having a longer time to completion. This indicates that AD participants took longer to read the words and may suggest a reading deficit in individuals with AD.

The mean time to completion of condition 2 (seconds) of the CWIT was 28, 26, and 31. The mean time to completion of condition 3 (seconds) of the CWIT was 78, 89, and 131. There

was no statistically significant difference between the three groups within these two conditions ($p = 0.28$ and $p = 0.10$, respectively). The mean Stroop interval (seconds) of each group were 46, 59, and 91, respectively. There was no statistically significant difference between all of the groups ($p = 0.16$).

The Pearson correlation coefficients for each group for B/A Ratio, Stroop Interval, and SLUMS exam score vs. global RNFL thickness are summarized in Table 7. The coefficients were 0.13, 0.39, and -0.44 for B/A Ratio in relation to global RNFL thickness with p values for the control group of $p = 0.46$, glaucoma group of $p = 0.02$, and AD group of $p = 0.20$. The coefficients were -0.60, -0.12, and 0.70 for SLUMS exam scores in relation to global RNFL thickness with p values for the control group of $p = 0.0002$, glaucoma group of $p = 0.48$, and AD group of $p = 0.02$. The coefficients were 0.14, 0.30, and -0.66 for the Stroop Interval in relation to global RNFL thickness with p values for the control group of $p = 0.44$, glaucoma group of $p = 0.07$, and AD group of $p = 0.07$. Scatter plots of the data are available in Figures 1 - 3 with each group designated separately along with r squared values and best fit lines. The data was also analyzed for each cognitive test outcome with each group combined and the r values and p values are found in Table 8.

Chapter Four: Discussion

Study Hypotheses

In this study, individuals with AD had significantly thicker RNFL in the temporal quadrant and inferior temporal sector. It is possible that there is some process taking place that causes this area of the RNFL to thicken. We also found a trend toward thinning of the nasal quadrant and the superior nasal sector in the AD group compared to controls. This is similar to previous studies' findings. Several studies indicate that the superior quadrant seems to be affected first in AD, though the cause of this is not understood. We did not find significant global RNFL thinning, which has been widely reported in the literature (Parisis et al., 2001, Iseri et al., 2006, Pacquet et al., 2007, Lu et al., 2010, Moreno-Ramos et al., 2013, and Kirbas et al., 2013). This may be because our participants had very early AD, and therefore the changes have yet to affect the whole of the optic nerve, which would support the thought that

changes in the neuronal structure in AD patients happen first within the posterior part of the visual system and then move retrograde towards the optic nerve and the RGCs. The process by which neurons die off in AD is through apoptosis (Cordeiro MF, 2010), and RGCs were found to incorporate Aβ into the cytoplasm before initiation of apoptosis (Cordeiro MF, 2010). The swelling of the inferior temporal area may indicate incorporation of Aβ into the RGCs causing swelling, just before RGC death leading to thinning of the RNFL. It may also be that the superior nasal area shows RNFL thinning because it is the first area to be affected by the AD pathology. This would indicate that individuals with early AD show first superior nasal loss of RNFL and then inferior temporal loss both of which are preceded by a swelling of the RNFL due to Aβ. All study participants were found to be at a very early stage of AD and therefore could all be showing the same stage of AD pathology within the eye. More studies would be necessary specifically analyzing the process of apoptosis or levels of Aβ within the RGCs at different stages of AD with close attention to the area of the retina the RGCs reside to confirm this hypothesis. Due to the small nature of our sample size, it is also possible that the AD group participants had tilted insertions of their ONHs. A study of tilted nerve heads found that eyes with tilted ONH insertions had thicker RNFL within the temporal quadrant via SD-OCT (Brito PN, Vieira MP, Falcão MS, Faria OS, Falcão-Reis F, 2013). This does not appear to be the case though based on the SLO images taken of the optic nerve heads for cup to disc ratio and there were no high myopes included in the study.

We also hypothesized that the pattern of RNFL thinning would be different between individuals with AD compared to individuals with glaucoma. This is because the damage from glaucoma happens from the inside of the eye outward in an anterograde course

throughout the visual system as discussed earlier (Yucel YH, et al., 2000 and 2001), in contrast to the retrograde loss found in AD (Su JH et al., 1997 and Leuba G et al., 1995). Our study showed that individuals with glaucoma show overall RNFL thinning both globally and in every quadrant, with the most significant thinning in the superior temporal and inferior temporal sectors. This differs from the superior nasal thinning and inferior temporal thickening found in individuals with AD. The glaucoma group also showed significant cupping both vertically and horizontally compared to controls. The AD group did not show this cupping. These differences are important because they can help to distinguish early RNFL thinning in AD compared to glaucoma. The fact that glaucoma participants showed cupping of the ONH and AD participants did not indicates some difference in the disease. Many of the studies that analyzed RNFL thickness in AD patients compared to age-matched controls did not measure cup to disc ratios. One study that did, did not find any differences between controls and AD patients, similar to our findings (Berisha et al., 2007). It may be that the changes happening in AD are so subtle that the overall rim tissue is not affected at first. It may also be that only a certain section of ganglion cells are affected in AD. Studies have indicated that motion perception can be affected early in AD, as discussed earlier, and it may be that ganglion cell populations that carry this type of information are affected first in AD, leaving other nearby ganglion cells intact, preserving the overall rim tissue. Future studies could be performed analyzing RNFL thickness and motion perception in AD patients.

Lastly, we hypothesized that RNFL layer thickness would correlate with cognitive testing within our AD participants. We found that there was a positive correlation between the global RNFL thickness and the SLUMS exam score showing that individuals with AD have

both a decrease in cognitive function and a decrease in global RNFL thickness. This is the first time that this has been reported with the use of the SLUMS exam. This correlation did not extend to the other cognitive test parameters and also did not extend across the glaucoma or control groups. This may indicate that cognitive function is only correlated to RNFL thickness in regards to patients with AD.

Biases, Limitations, and Anomalies

One limitation to this study is the large percentage of male participants. Only one female was recruited into the study within the control test group. A second limitation was the racial makeup of the participants (57% Caucasian, the rest being African Americans).The vast majority of the patients seen within the VA are Caucasian males (Department of Veterans Affairs National Center for Veterans Analysis and Statistics, 2014). Future research should attempt to recruit an equal number of males and females with more racial diversity in order to determine if the findings of this study are similar in these other groups.

An area of potential bias lies in the analysis of the cognitive test data. The Neurology and GRECC clinics from which the study recruited the AD participants use the SLUMS exam as part of the identification criteria for an AD diagnosis. This would indicate that the AD test group would have a lower SLUMS exam score than both of the other groups. This is the case when comparing both the control and glaucoma groups with the AD group.

One final area of potential bias could involve the data collection and interpretation were performed by the same individual, who was not masked to the participants' diagnoses.

As stated in the beginning of the results section, the goal for the number of participants recruited within the AD group was not met, and as such, limits the power of this study. Problems were found during recruitment. First, fewer than half of the potential study participants had eye exams within the Boston VA healthcare system, and this was a required component of the recruitment process in order to screen out individuals who fit the exclusion criteria. This may indicate that many of these individuals either receive eye care outside of the VA, or that they receive no eye care at all. This is concerning since there are many documented ocular problems that can occur in AD patients, first of which is a decrease in visual acuity. Second, it is very difficult to diagnose early AD and therefore the number of patients with definitive AD diagnoses who are followed by the Neurology clinic can be limited. This makes finding individuals with early AD difficult. Lastly, some of the prospective AD participants were listed as glaucoma suspects and could not be included in the AD study group as this could confound the results. This further limited the individuals that could be recruited into the AD group.

Some anomalies existed within our study. One such anomaly involved the cognitive test data analyses. The SCWT requires the ability to distinguish between red, blue, and green colors in order to perform the test. The presence of red-green color deficiency can taint the data from this test, and with enough deficiency can make the test impossible to complete for some individuals. This was overlooked from the initial study design, and as such, two study participants were included within the study, but could not perform the test. Their study data were used in analysis of all study parameters, but left out of the SCWT data groups because they could not perform the test.

Another anomaly found during testing was that four of the study participants did significantly better/worse than expected based on the study group they were included in and may be considered outliers for the purposes of this study. One of the individuals from the AD group performed far better on the cognitive tests than his counterparts did despite being diagnosed as having AD. After administering the cognitive tests it was determined that this individual worked in a neurology clinic performing cognitive function tests, and therefore had a learning bias for each of the three cognitive tests used. Three individuals, one from the control group, and two from the glaucoma group, did far worse than their counterparts on the cognitive tests. None of these individuals had a diagnosis of dementia, but these individuals may have AD, or another dementia type, if assessed by neurology, though these cognitive tests alone cannot diagnose AD. The removal of these outliers from the testing data did not show a significant difference in the outcome of the data analyses.

Future Research

With the instance of AD on the rise, and the overall population living longer, new research into early detection and treatment of AD is of paramount importance. Current therapies rely on preservation of current levels of cognitive function, and on prolonging the cognitive faculties of the individual affected with the disease. Without a means to reverse cognitive decline, or prevent disease formation to begin with, the treatment strategy must be to identify the signs of disease processes as early as possible, particularly before loss of cognitive function, and to begin treatment when first detected.

Future avenues for research would involve a larger, prospective, longitudinal study that would include SD-OCT scans of RNFL thickness in each quadrant and sector with particular attention to the superior nasal and nasal areas to asses for changes over time in normal individuals, as well as in those individuals who convert to AD over time. The SLUMS exam should be included in the determination of AD and should be performed at regular intervals to correlate RNFL change with cognitive decline, as this test is more sensitive to early changes in cognitive function. Another recommendation would be the use of cognitive preserving medications such as galantamine or donepezil and to determine if this technology could be used to track efficacy of treatment based on RNFL loss as well as cognitive decline.

Our study demonstrated RNFL thinning within the superior and superior nasal quadrants of AD patients compared to controls as well as RNFL thickening within the inferior and inferior temporal areas of the optic nerve head. This is the first time RNFL thickening was described in AD patients and further studies to confirm this finding are warranted. Our study also demonstrated that individuals with glaucoma show a different pattern of RNFL thinning compared to AD patients, implicating the possibility of a different mechanism for RNFL loss between these two neurodegenerative diseases. Future studies aimed at better understanding of the pathophysiology of these changes throughout the visual system may lead us to earlier methods of detection and therefore earlier treatment of this devastating disease.

Tables

Author	Year	Significant area of Thinning	Type of OCT	Correlation to Cognitive Testing	Other Results
Parisi et al.	2001	Global and all 4 Quadrants	Time Domain	No with Mini Mental Status Exam (MMSE)	n/a
Iseri et al.	2006	Global and all Quadrants	Time Domain	Yes with MMSE	Macular thickness was decreased in AD patients.
Berisha et al.	2007	Superior Quadrant only	Time Domain	n/a	Blood flow rates were lower in AD patients vs. Controls.
Pacquet et al.	2007	Global only	Time Domain	n/a	n/a
Lu et al.	2010	Global, Superior, and Inferior Quadrants	Time Domain	n/a	There was no significant difference in fundus photos in AD patients
Kesler et al.	2011	Superior and Inferior Quadrants	Time Domain	No with MMSE	n/a
Moschos et al.	2012	Superior and Inferior Quadrants	Time Domain	n/a	Multifocal Electroretinograms were decreased in AD patients.
Moreno-Ramos et al.	2013	Global only	Time Domain	Yes with MMSE and Mattis Dementia Rating Scale	RNFL thickness also decreases in patients with Lewy bodies and Parkinson's Disease.
Kirbas et al.	2013	Global and Superior Quadrant	Spectral Domain	n/a	n/a
Shi et al.	2013	Inferior Quadrant only	Spectral Domain	Yes with MMSE	First group to demonstrate a longitudinal pattern of RNFL loss in patients with AD.

Table 1: Summary of Various Studies on RNFL Thickness and AD

Participant Demographics				
	Control (n=17)	Glaucoma (n=19)	AD (n=5)	p value (p<0.05)*
Mean Age in Years (SD)	69 (7)	70 (9)	71 (12)	0.89
Mean Visual Acuity in Snellen	20/24	20/23	20/28	0.47
Mean Ref Error in Spherical Equivalent (SD)	+0.25 (2.25)	0.00 (1.50)	-0.50 (1.50)	0.63
Gender (males/females)	16/1	19/0	5/0	n/a
Race (Caucasian/African American)	16/1	11/8	5/0	n/a

Table 2: Table of Participant Demographics

Cup to Disc Ratio and Global RNFL Thickness				
	Control (n=34)	Glaucoma (n=38)	AD (n=10)	p value (p<0.05)*
Mean Vertical C/D Ratio (SD)	0.40 (0.15)	0.65 (0.10)	0.45 (0.10)	2.73E-12*
Mean Horizontal C/D Ratio (SD)	0.40 (0.15)	0.60 (0.10)	0.50 (0.10)	3.25E-10*
	Control (n=34)	Glaucoma (n=37)	AD (n=9)	p value (p<0.05)*
Mean Global RNFL Thickness in um (SD)	92 (9)	78 (12)	92 (6)	3.62E-07*

Table 3: Table of Cup to Disc Ratio and Global RNFL Thickness

RNFL Thickness by Quadrant and Sector				
Area Mean RNFL thickness in um (SD)	Control (n=34)	Glaucoma (n=37)	AD (n=9)	p value (p<0.05)*
Superior	112 (15)	97 (18)	108 (9)	0.001*
Temporal	65 (15)	59 (11)	75 (12)	0.003*
Inferior	117 (13)	93 (20)	119 (11)	2.59E-08*
Nasal	73 (15)	62 (15)	66 (7)	0.007*
Superior Temporal	122 (18)	103 (21)	130 (19)	3.74E-05*
Inferior Temporal	129 (20)	101 (25)	142 (11)	4.56E-08*
Inferior Nasal	105 (22)	85 (20)	97 (20)	0.0005*
Superior Nasal	101 (23)	91 (20)	87 (19)	0.08

Table 4: Table of RNFL Thickness by Quadrant and Sector

Ad Hoc Analysis of RNFL Thickness (um)			
Quadrant	Control (n=34)	AD (n=9)	p-value (p<0.05)*
Superior	112	108	0.43
Temporal	65	75	0.05*
Inferior	117	119	0.59
Nasal	73	66	0.08
Sector	Control (n=34)	AD (n=9)	p-value (p<0.05)*
Superior Temporal	122	130	0.28
Superior Nasal	101	87	0.07
Inferior Temporal	129	142	0.02*
Inferior Nasal	105	97	0.33

Table 5: Table of *Ad Hoc* Analysis of RNFL Thickness between Control and AD Groups

Cognitive Tests				
Trail Making Test	Control (n=17)	Glaucoma (n=19)	AD (n=5)	p value (p<0.05)*
Mean value Cond 2 in seconds (SD)	51 (14)	51 (19)	82 (29)	0.05*
Mean value Cond 3 in seconds (SD)	49 (17)	52 (21)	102 (61)	0.001*
Mean value Cond 4 in seconds (SD)	118 (35)	122 (45)	279 (183)	0.0002*
Mean value Cond 5 in seconds (SD)	39 (20)	35 (13)	52 (24)	0.16
Mean B/A Ratio (SD)	2.39 (0.48)	2.38 (0.39)	2.94 (1.63)	0.23
SLUMS Exam	Control (n=17)	Glaucoma (n=19)	AD (n=5)	p value (p<0.05)*
Mean SLUMS Exam Score (SD)	25 (3)	25 (3)	18 (5)	0.0002*
Mean Education Level (SD)	2 (1)	2 (1)	2 (1)	0.52
Stroop Color Word Test	Control (n=16)	Glaucoma (n=19)	AD (n=4)	p value (p<0.05)*
Mean value Cond 1 in seconds (SD)	37 (7)	34 (7)	50 (15)	0.004*
Mean value Cond 2 in seconds (SD)	28 (6)	26 (6)	31 (5)	0.28
Mean value Cond 3 in seconds (SD)	78 (19)	89 (54)	131 (51)	0.10
Mean Stroop Interval in seconds (SD)	46 (18)	59 (54)	91 (41)	0.16

Table 6: Table of Cognitive Test Data

Correlation of Cognitive Testing with Global RNFL Thickness		
	Correlation Coefficient	p value ($p<0.05$)*
B/A Ratio		
Control (n=17)	0.13	0.46
Glaucoma (n=19)	0.39	0.02*
AD (n=5)	-0.44	0.2
SLUMS Score		
Control (n=17)	-0.6	0.0002*
Glaucoma (n=19)	-0.12	0.48
AD (n=5)	0.7	0.02*
Stroop Interval		
Control (n=16)	0.14	0.44
Glaucoma (n=19)	0.3	0.07
AD (n=4)	-0.66	0.07

Table 7: Table of Correlation of Cognitive Testing with Global RNFL Thickness

Correlation of Cognitive Tests and Global RNFL Thickness	
D-KEFS Trail Making Test	r value
Condition 2	-0.04
Condition 3	0.003
Condition 4	0.04
Condition 5	0.17
B/A Ratio	0.11
D-KEFS Color Word Interference Test	r value
Condition 1	0.17
Condition 2	0.17
Condition 3	0.15
Stroop Interval	0.13
SLUMS Exam	r value
SLUMS Exam Score	-0.24

Table 8: Table of Correlation of Cognitive Tests and Global RNFL Thickness Across all Groups

Figures

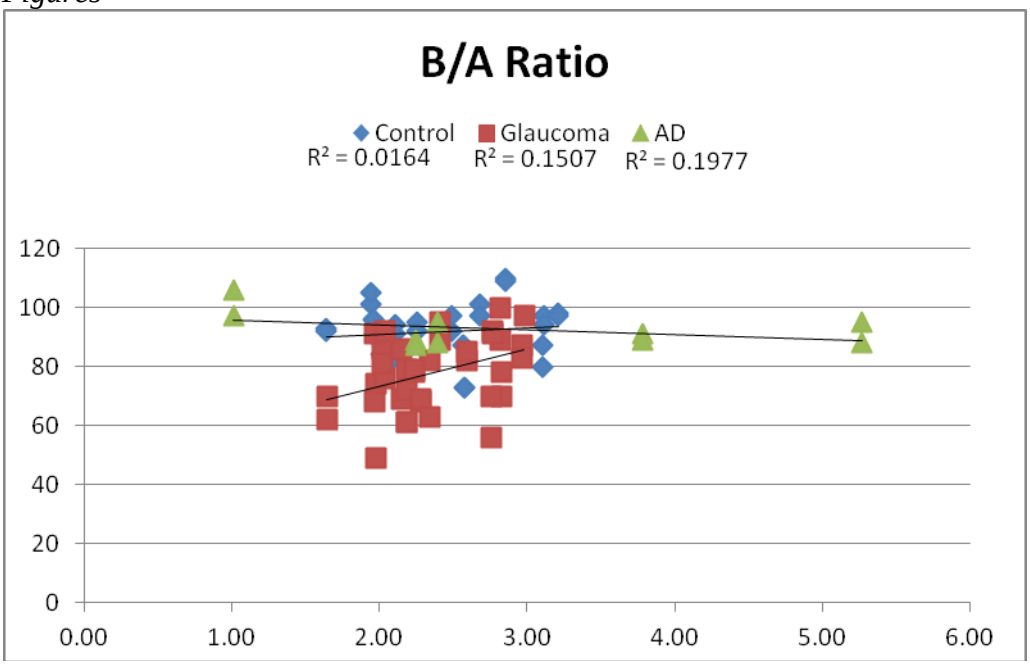


Figure 1: Scatterplot B/A Ratio vs. Global RNFL Thickness

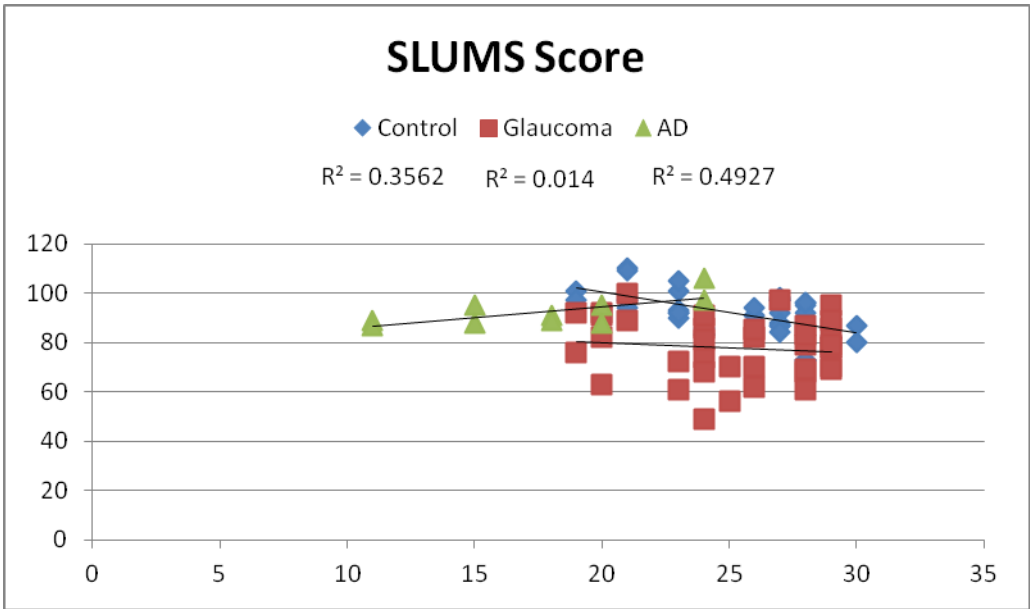


Figure 2: Scatterplot SLUMS Exam Scores vs. Global RNFL Thickness

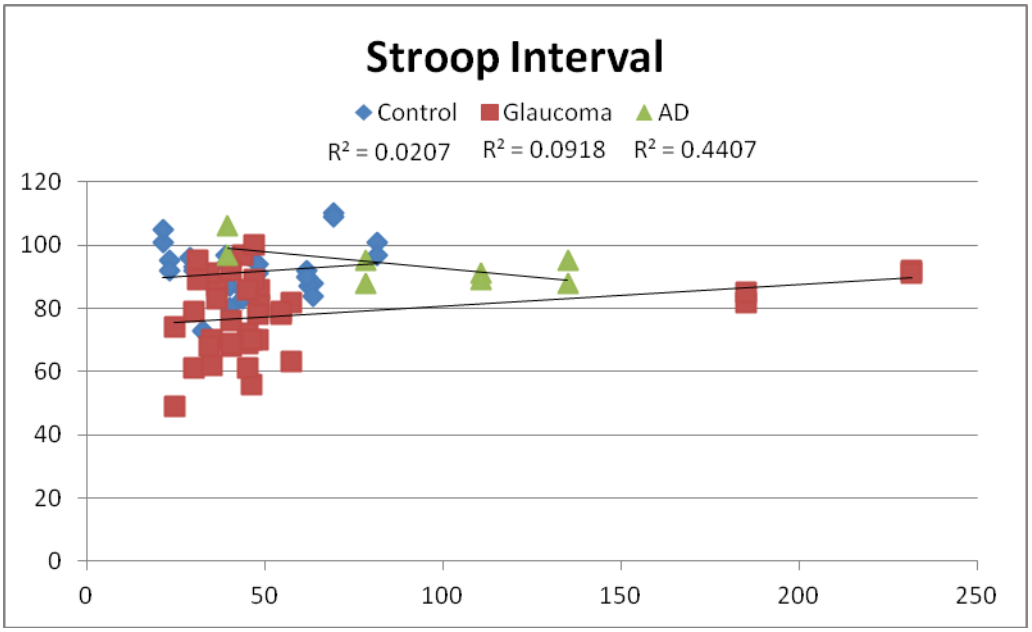


Figure 3: Scatterplot Stroop Interval vs. Global RNFL thickness

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