The P50 auditory potential:

A potential neurophysiological marker of

Alzheimer's disease

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DEDICATIONS

I would like to dedicate this thesis to the most supportive team imaginable. I dedicate this thesis with a special feeling of gratitude to my Mom, Patricia Finn, whose well of support, love and wisdom runs deep. I dedicate this thesis to my dad, Patrick Green, who always encouraged me to pursue higher education (and tirelessly drove me to the beach to surf throughout graduate school). I would like to give special thanks to my brother, Dennis Green, sister, Kelly Finn, and stepdad, Thomas Finn, who truly comprise "the best blended family ever". I would like to thank my best friend, Lara Hughes, for being there for me through everything. And my heart would not be complete without the love I share with Joshua France, who makes the everyday act of living simply together something exceptionally beautiful.

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Abstract

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This auditory event-related potential (ERP) study investigated a candidate neurophysiological marker of Alzheimer's disease (AD) and symptom severity. ERPs are sensitive to disruption of cortical processing and therefore have been used to study cortical effects of AD. The P50 ERP component has been shown to increase in amplitude with healthy aging, with multiple-domain relative to single-domain mild cognitive impairment (MCI), and in MCI patients who convert to AD compared to those whose diagnoses remain stable. As "MCI due to AD" has recently been defined as the symptomatic predementia phase of AD, P50 amplitude may reflect changes underlying AD progression in the earliest stages and is expected to vary as a function of cognitive impairment. There is also evidence to support P50 amplitude decrease in AD compared to MCI patients and conflict in the literature as to whether P50 is larger in AD relative to age-matched controls. This study explores P50 amplitude as a marker to distinguish mild AD patients from healthy older controls as well as the relationship between P50 amplitude and symptom severity, as measured by the Mini Mental Status Exam (MMSE).

1. INTRODUCTION

Alzheimer's disease is a progressive neurodegenerative disorder characterized by cognitive, behavioral, and neurological impairments, especially memory loss, that impair the ability to perform activities of daily living. AD is a major health concern, as the most prevalent type of dementia among the elderly, accounting for 50-80% of all cases, and with reported costs of 183 billion dollars annually in the US (Alzheimer's Association, 2011; (Cummings, 2004; Jeong, 2004). Although the ultimate cause is unknown, age is the greatest known risk factor, with prevalence rates doubling every five years after age 60; therefore AD affects approximately 6% of 65-year-olds and nearly half (43%) of all individuals over 85 (Alzheimer's Association, 2011; Corey-Bloom, 2004). In 2011, an estimated 5.4 million Americans suffer from AD, with expected survival averaging 5-8 years from clinical diagnosis (Alzheimer's Association, 2011; Braco et al., 1994). AD research is increasingly important as the population of older adults is expected to rise sharply in the US, doubling to a whopping 70 million by 2070, with estimated yearly costs over $1 trillion (Alzheimer's Association, 2011).

1.1 Symptom Manifestation and Disease Progression

The cognitive and behavioral symptoms of AD are manifested insidiously and progressively worsen over time (Corey-Bloom, 2004; McKhann et al., 2011). AD is typically characterized by memory loss, impaired reasoning, language impairment, and visuospatial disturbances, in addition to behavioral and neuropsychiatric changes (Corey-Bloom, 2004; McKhann et al., 2011). AD symptom manifestation is heterogeneous, from typical (amnestic) to atypical (prominent language and visuospatial

deficits) presentations, which are distinguished by syndromic labels to denote their qualitatively different clinical expressions (Jack Jr. et al., 2011; McKhann et al., 2011). However, universally, impairments worsen and patients are increasingly unable to perform activities of daily living (Small, 1998).

Memory impairment is generally the primary manifestation of AD in the early stages of the disease while other cognitive abilities are relatively spared. At first, the patient has difficulty recalling new information, e.g., content of conversations, while remote memories are relatively preserved; symptoms include: repetitive questions or statements, forgetfulness, and getting lost on familiar routes (McKhann et al., 2011). As the disease progresses, amnesia worsens to include remote memory (Corey-Bloom, 2004).

Executive functions, such as problem solving, decision making, reasoning and judgment, are affected early in the disease progression and therefore immediately impact the ability to function and carry out activities of daily living (Amieva, Dellaa Sala, & Henry, 2004). These impairments are illustrated by difficulties planning, organizing, and driving, difficulty understanding safety risks, and affect performance at work, paying bills and managing finances (Alzheimer's Association [AA], n.d.; Corey-Bloom, 2004; McKhann et al., 2011). Later stages are accompanied by an increased difficulty in orienting to aspects of time and space and patients may become confused about where they are and unable to correctly tell the date, day of the week, or season (AA, n.d.).

Initial language failures are evident in the form of dysnomia, reduced fluency, and progressively worsen to include paraphasias, reduced output, poor speech comprehension, ultimately leading to receptive and executive aphasias, failure to speak in full sentences and near-mutism (Corey-Bloom, 2004; Victor & Ropper, 2001).

Visuospatial deficits are manifested in the misplacement of objects and difficulty driving (Corey-Bloom, 2004). In the intermediate stage patients are unable to recognize and reproduce figures, become lost, and are unable to comprehend directions or describe a route from place to place (Victor & Ropper, 2001). Eventually agnosic and prosopagnosic deficits are so extreme that patients forget how to use common objects and are unable to identify family members (Corey-Bloom, 2004, Victor & Ropper, 2001).

Motor functioning is relatively spared in the early stages, especially highly overlearned routine tasks, whereas more complex skills are susceptible to disruption (Corey-Bloom, 2004). Deficits progress to the level of ideational and ideomotor apraxia, in which commanded and demonstrated actions cannot be executed or imitated (Victor & Ropper, 2001). Advanced stages of AD result in motor impairment in the form of loss of gait and rigidity, similar to that exhibited by individuals with Parkinson's disease (Victor & Ropper, 2001). In advanced stages, individuals lose the ability to walk, sit without support and to hold their head up; they require help eating and suffer from incontinence (AA, n.d.).

The behavioral and psychological symptoms of dementia affect perception, thought content, mood and behavior (Finkel, et. al, 1998). Personality and uncharacteristic mood fluctuations occur, with depressed mood and agitation common

(Alzheimer's Association, 2011; McKhann et al., 2011). Other symptoms include: decreased motivation, initiation, and apathy, social withdrawal; compulsive or obsessive behaviors and socially unacceptable behaviors; purposeless motor activity; and even psychosis (Cummings & Back, 1998; McKhann et al., 2011).

1.2 Neuropathology

AD symptom manifestation and clinical course are thought to reflect the underlying and expanding neuropathology; however, the correspondence of the observable clinical syndrome and the underlying changes are not always consistent (Jack Jr. et al., 2011; Price, et al., 2009; Thal, Udo, Orantes, & Braak, 2002). Although the specific causes of the disease remain unknown, Alzheimer's disease alters the morphology and neurochemistry of the brain. The hallmark features of the neuropathology of AD are the existence of neuritic amyloid plaques and neurofibrillary tangles as well as diffuse neuronal cell loss, hypometabolism, and impairment of neurotransmitter systems (Jeong, 2004; Victor & Ropper, 2001). Amyloid plaques and neurofibrillary tangles are lesions that occur as a result of the abnormal accumulation of proteins in the cellular environment, namely, amyloid β-peptides in extracellular areas and tau proteins in intracellular areas, respectively (Corey-Bloom, 2004). Lesions tend to be found concentrated in vulnerable neural systems in the brain and are present in the cortex as well as in the limbic structures of the temporal lobes, disproportionately in the hippocampus, particularly the CA1and CA2 zones, parahippocampal regions of the entorhinal cortex and subiculum, and amygdala (Cummings, 2004; Victor and Ropper, 2001). These areas also consistently demonstrate marked hypometabolism in single

photon emission computed tomography (SPECT) and positron emission tomography (PET), especially in cortical association areas of the temporal and parietal lobes as well as prefrontal cortices (Small, 1998). Diffuse and progressive atrophy of cortical tissue, particularly a loss of hippocampal volume, accompanies the disease, with an overall loss of up to 20 percent or more of total brain weight (Victor & Ropper, 2001). It has been suggested that these pathophysiological changes occur in a time-ordered sequence such that beta amyloid pathology develops first during a long preclinical phase and is followed later by the development of neurofibrillary tangles and the resulting neuronal and synapse loss, which may accelerate more closely in time before the symptomatic phase is observable (Jack Jr. et al., 2011a).

The AD brain is also characterized by impairment of neurotransmitter systems, most notably acetylcholine (Nordberg, 2006; Victor & Ropper, 2001). A deficiency of acetylcholine occurs due to neuronal loss in the cholinergic neurons of the basal nucleus of Meynert (Victor & Ropper, 2001), which is involved in production of choline acetyltransferase, the enzyme responsible for the synthesis of acetylcholine (Cummings &Back, 1998). Choline acetyltransferase levels are notably reduced in the hippocampus and neocortex (Victor & Ropper, 2001). Additionally, selective degeneration of pre-synaptic cholinergic function occurs in AD patients with the number of pre-synaptic nicotinic acetylcholine receptors reduced throughout the cerebral cortex (Cummings & Back, 1998; Nordberg, 2006).

1.3 Differential Diagnosis

The new diagnostic criteria outlined by the National Institute on Aging (NIA) and Alzheimer's Association (AA) workgroup proposed several terms for classifying individuals: (1) probable AD dementia, (2) possible AD dementia (which are both for clinical use) and (3) probable or possible AD dementia with evidence of the AD pathophysiological process (which includes the use of biomarkers in the criteria and is only for research purposes) (McKhann et al., 2011). Patients who met the 1984 criteria for "probable AD" as described by the National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA), would meet the current criteria for probable AD dementia; however, those classified as "possible AD" by the 1984 criteria would need to be re-evaluated as the current standard is meant to capture individuals with an atypical course or who have an etiologically mixed presentation (McKhann et al., 2011).

Differential diagnosis is often difficult due to the lack of sensitive and reliable tests and markers of the disease, as well as other conditions resulting with similar presentations, such as vascular dementia, B-12 deficiency, infection, and exposure to toxins (Cummings 2004). The best clinical tools, such as neuropsychological evaluation, have relatively good diagnostic accuracy (positive predictive value of 85-90%), but fairly limited availability outside of university or research hospitals (Polikar et al., 2007). Also limited to these settings is the accessibility of tools examining other biomarkers, such as cerebrospinal fluid tau, β-amyloid, brain atrophy and volume loss detected by PET or MRI scan, which can be unreliable, invasive, and expensive (Polikar et al., 2007).

1.4 EEG Research of AD

Research suggests that EEG, capable of detecting cortical neuronal and neurochemical dysfunction, is an important tool that may be used to address these and other important issues in understanding AD. EEG measures electrical potentials at the scalp from the summation of inhibitory and excitatory postsynaptic potentials. These potentials are the result of chemical communication among neurons via neurotransmitters. Therefore, EEG can directly measure the changes in cortical processing and indirectly capture the effects of subcortical processes on cortical functioning (Hegerl & Moller, 1997).

AD is a cortical dementia and EEG allows researchers to detect abnormalities that reflect the anatomical and functional deficits of the cerebral cortex damaged by the disease (Hegerl & Moller, 1997; Jeong, 2004). The characteristic EEG abnormalities in AD patients, slowed mean frequency, less complex activity, and reduced coherences among brain regions, are thought to be associated with the underlying neuropathology of AD. For example, neuronal cell death, axonal pathology, and cholinergic deficits are believed to contribute to functional disconnections among cortical areas, one proposed mechanism for the observed EEG abnormalities in AD patients (Jeong, 2004).

These EEG abnormalities, e.g., slowing and decreased coherence, have also been correlated with the severity of the disease, such as cognitive impairment as measured by the Mini Mental State Examination (Kowalski et al., 2001). However, other studies have reported only a weak correlation or no correlation between EEG changes and cognitive decline in patients with AD (Hughes, Shanmugham, Wetzel, Bellur, & Hughes, 1989;

Prinz & Vitiello, 1989 as cited in Jeong, 2004). This issue requires further study as the ability to detect disease severity and potentially predict disease course would be invaluable information for families to facilitate treatment and long-term care planning for loved ones suffering from the disease.

1.5 Event-Related Potentials

Event-related potentials (ERPs) are derived from EEG and represent the electrical activity synchronously produced by large groups of neurons time-locked to an event, such as a stimulus or a response. To obtain a robust ERP response, signal averaging is then used to aggregate the time-locked brain activity, averaging across trials to increase the signal-to-noise ratio. ERPs have high temporal resolution, therefore they measure the cortical response several milliseconds after the onset of a stimulus, allowing researchers to examine and quantify various levels of cognitive processing, such as stimulus perception, selective attention, and information and language processing. Therefore, ERPs are said to provide an on-line view of information processing and capable of revealing abnormalities in brain activity. Components of the ERP are the manifestation of the activity of specific brain regions/circuitry that is elicited to accomplish a specific information-processing task (Spencer et al., 1999). ERP components are identified by their polarity and latency from the stimulus onset.

2. The P50 ERP Component

The P50 ERP component is a positive waveform occurring 50 milliseconds after stimulus onset. It is produced in the primary and secondary auditory cortices in response to auditory stimuli, with the resulting amplitude thought to be moderated by

other brain regions (Korzyukov et al., 2007). The P50 has been shown to be a stable, reliable ERP component over time in healthy elderly individuals, bolstering rationale for the use of this component for detecting preclinical changes in at-risk elderly populations (Sandman & Patterson, 2000). It has been suggested that the P50 is more controlled by "preconscious", exogenous factors, such as the physical features of the stimuli, then by endogenous, higher-order cognitions, such as evaluation of the environment and decision-making (Sandman & Patterson, 2000).

2.1 Evidence P50 Amplitude Reflects Inhibitory Functions

Healthy individuals, in order to function efficiently, have the ability to inhibit responses to sensory stimuli in the environment. The term *sensory gating* refers to the internal process theorized by McGhie and Chapman (1961) that allows the selection of important information from diverse sensory input. Sensory gating has been observed in normal individuals using a suppression paradigm, in which two identical auditory stimuli (often clicks) are presented in quick succession (e.g., 500ms) at regularly spaced intervals (e.g., 10s). The amplitude of the P50 in response to the second click is reduced relative to the first (Boutros et al., 1995). The suppression of the P50 response to the second stimulus is thought to reflect inhibitory functions due to stimuli habituation. Changes in the normal response to auditory stimuli, i.e., failure to show normal P50 suppression, may reflect impairment of inhibitory processes (Boutros et al., 1995; Freedman et al., 1987; White & Yee, 2006).

P50 has been studied extensively in schizophrenia using sensory gating paradigms, with patients consistently demonstrating impaired suppression responses

(Freedman et al., 1987). These findings have been interpreted as failure to filter irrelevant material and therefore a tendency to sensory overload, consistent with symptoms of schizophrenia. P50 has also been found to be larger in response to auditory clicks in patients with lesions in the dorsolateral prefrontal cortex relative to age-matched controls (Knight, Scabini, & Woods, 1989). Knight and colleagues (1989) interpreted these findings as evidence that patients lacked inhibitory control over inputs to sensory cortex.

AD patients, who exhibit difficulties in executive control and inhibiting behavior, have also demonstrated reduced P50 suppression in sensory gating paradigms, whereby the ratio of P50 amplitude to both clicks successfully differentiated patients with AD from age-matched controls (Cancelli et al., 2006; Jessen et al., 2001; Thomas et al., 2010). Larger P50 amplitudes were found for the AD patients relative to age-matched controls as well, congruent with broader findings of cortical hyperexcitability present in AD; this hyperexcitability has been proposed as the result of impairment in inhibitory processes (Cancelli et al., 2006; Jessen et al., 2001; Thomas et al., 2010).

2.2 Evidence P50 Mediated by Cholinergic System

The cholinergic system is thought to modulate sensory gating mechanisms and be related to cortical hyperexcitability (Cancelli et al., 2006; Freedman et al., 2003; Lucas-Meunier, Fossier, Baux, & Amar, 2003; Thomas et al., 2010). In patients with schizophrenia and their relatives, impaired P50 suppression was found to be genetically linked to a nicotinic acetylcholine receptor (Freedman et al., 2003). However, the literature is conflicting regarding the effects of cholinesterase inhibitors on P50.

Cholinesterase inhibitors have been shown to influence P50 suppression in response to somatosensory but not auditory stimuli (Golob et al., 2005; Golob et al., 2007; Irimajiri, Michalewski, Golob, & Starr, 2007).

Whereas a cholinergic deficit is associated with AD, it has not been found to be sufficient to cause the cognitive symptoms of AD, as autopsy of individuals evincing AD symptomatology have shown no evidence of choline acetyltransferase or acetylcholinesterase deficiency (Jeong, 2004). However, the disruption of the cholinergic system affecting cognitive and electrophysiological findings may be subtler than previously thought. For example, in patients with mild cognitive impairment, there is evidence that cholinergic neurons are not regulated normally, despite an unchanged or even increased number of cholinergic receptors (Jeong, 2004). These findings suggest a subtler dysregulation of cholinergic neurons in the basal nucleus of Meynert early in the disease process, consistent with evidence that significant neuronal death and cholinergic disruption occurs later in the disease. The P50 potential, which has shown sensitivity to changes associated with AD and is thought to be mediated by the cholinergic system, may reflect this subtle dysregulation of basal forebrain neurons. This would make it a biomarker of early disease state.

2.3 Neural Correlates of P50 Response to Auditory Stimuli

The P50 is produced in the primary and secondary auditory cortices, areas spared until late in the Alzheimer's disease process (Golob et al., 2007). Sensory gating studies have implicated a network including the auditory cortex and areas of the frontal cortex that are involved in P50 suppression (Mayer et al., 2009; Oranje et al., 2006). Using

a double-click paradigm, researchers have found evidence for frontal cortical involvement in P50 suppression in normals and individuals with schizophrenia (Oranje et al., 2006; Weisser et al., 2001). Oranje and colleagues (2006) used auditory and visual stimuli in a double-click paradigm to show that (1) P50 suppression occurs in response to both auditory and visual stimuli and (2) source modeling indicated a shared origin of the P50 in the frontal cortex for both stimulus modalities. These findings suggest that the inhibitory processes that regulate sensory stimuli are not solely located in sensory cortical structures, such as the auditory cortex, but seem to be generated in frontal cortical areas. Invasive, subdural P50 recording in candidates for epilepsy surgery showed that sensory gating was related to areas involved in top-down control of sensory input in the lateral prefrontal cortex (Kurthen, Tautner, Rosburg, Grunwald, & Dietl et al., 2007). An event-related fMRI study of normals found a large network including dorsolateral prefrontal cortex and the thalamus involved in P50 sensory gating in response to identical (repeated) and non-identical (novel) tones (Mayer et al., 2009). The frontal cortex and nucleus basalis of Meynert are hypothesized to be involved in dampening the cortical response to auditory stimuli (Golob et al., 2007). These areas are also affected by the progressive cortical deterioration in AD. Therefore P50 amplitude increases may be a manifestation of reduced prefrontal inhibition of the auditory cortical response (Golob et al., 2007).

3. Auditory Target Detection

In an auditory target-detection, or *oddball paradigm,* participants are asked to identify infrequent, high-pitched tones (*targets,* p = .20) embedded in a series of frequent, lower-pitched tones (*standards,* p = .80), usually by pressing a button (Yamaguchi et al., 2000; Golob & Starr, 2000). Abnormal ERPs in auditory target detection have long been found in individuals with AD (Goodin et al., 1978). Using an oddball paradigm, P50 amplitude in patients with mild AD (n = 10) was significantly larger relative to age-matched controls (n = 12) (p <.001) (Golob & Starr, 2000). An oddball paradigm can also be used to investigate the brain response to task-irrelevant, *novel* sounds, which occur relatively infrequently and consist of unique sounds each of which is presented only once. Novel stimuli in the Yamaguchi et al. (2000) study were taken from a Disney movie and included environmental such sounds as a laugh, a door shutting, and a whistle (Yamaguchi, Tsuchiya, Yamagata, Toyoda, & Kobayashi, 2000).

3.1 Rationale for Inclusion of Novel Stimuli

ERP components in response to various types of auditory stimuli are thought to manifest activity of specific brain regions and circuits recruited to process different information (Spencer et al., 1999). ERPs in response to the different auditory stimuli are therefore generated in different areas of the cortex and are thought to represent separate underlying neural networks (Yamaguchi et al., 2000). For example, the novelty P300 (i.e., P300a) is distinguishable from the classical centro-parietal P300 (i.e., P300b) response to target sounds. The novelty P300a has been observed to occur in response to different experimental manipulations (novel tones) and with a more fronto-central scalp

distribution than the more posteriorly-distributed P300b component (Spencer et al., 1999). Using a variety of auditory stimuli may increase the sensitivity of EEG to detect the presence and severity of AD.

Novelty processing is complex and involves widely distributed neural circuitry. Topographical mapping in normals has shown activation of a neural circuit involving the prefrontal and posterior cortices in response to novel events (Fabiani & Friedman, 1995). Source localization of the novelty P300 also suggested its generation in the prefrontal cortex (Mecklinger & Ullsperger, 1995). Yamaguchi and colleagues (2000) found evidence that the P300 ERP component in response to novel tones, but not to target tones, involved the prefrontal cortex, as vascular dementia patients with known frontal lobe damage showed markedly reduced responses to novel tones, with the greatest reductions evident over frontal relative to posterior areas. This finding is consistent with the idea that the P50, which seems to be moderated by frontal brain regions, may differ in response to novel and target tones. If disruption of frontal neural circuitry results in less inhibition of the auditory cortical response, larger P50 amplitudes would be expected in response to novel tones.

4. P50 as a Marker of Cognitive Deterioration

Differences in P50 amplitude between healthy young adults and older controls, as well as differences among clinical groups categorized by severity of cognitive impairment, suggest that this ERP component may be a useful tool to evaluate cognitive deterioration. Evidence suggests that P50 amplitude reflects changes associated with cognitive decline, beginning with amplitude increases demonstrated with normal aging,

as observed in healthy older adults relative to younger controls when performing an oddball task (Golob et al., 2007). Severity of cognitive impairment has also been positively correlated with P50 using MMSE scores as a measure of cognitive status (Thomas et al., 2010). Thomas and colleagues found a correlation between auditory sensory gating and MMSE performance for their sample of AD patients, $r^2 = .25$ (2010). However, prior studies have failed to show such a relationship between P50 sensory gating and cognition (Cancelli et al., 2006; Jessen et al., 2001).

Patients with mild cognitive impairment (MCI), characterized by memory and cognitive deficits similar to AD but without impaired activities of daily living, show increased P50 amplitude relative to age-matched controls (Golob et al., 2002; Gobol et al., 2005; Golob et al., 2007). Severity of cognitive impairment within the MCI patients has also been related to P50 amplitude. Severity in MCI patients has been defined based on the number of cognitive domains affected, with the most common subtype being *amnestic* MCI, due to the presence of an episodic memory disorder, occurring alone (single domain, SD) or in the context of other cognitive impairments (multiple domain, MD) (Golob et al., 2007). This definition of disease severity seems warranted given that patients with amnestic MCI-MD convert to AD at a much higher rate than MCI-SD, as well as the recent addition of (research) criteria of *MCI due to AD* by the NIA-AA workgroup (as described below) (Albert et al., 2011; Golob et al., 2007). In a study by Golob and colleagues, P50 amplitude successfully differentiated amnestic MCI groups, with greater P50 amplitudes in MCI-MD relative to MCI-SD patients (2007).

The NIA-AA workgroup's criteria for the symptomatic predementia phase of AD is referred to as *MCI due to AD* (Albert et al., 2011). There has long been evidence that (1) a pre-clinical stage of AD exists, as neuropathological changes associated with AD may be present for years without producing a clinically detectable cognitive change; (2) AD and amnestic MCI patients show similar neuropathology in similar brain regions, such as the presence of beta-amyloid plaques; (3) amnestic MCI patients are at a greater risk for developing AD, six times greater than age-matched controls without memory impairment, with approximately 10% to 15% of MCI patients experiencing further cognitive decline to meet the criteria for AD (Morris & Price, 2001). Electrophysiological studies also suggest that many MCI cases are actually pre-clinical AD. In an oddball task, P50 amplitude was predictive of clinical course, with significantly larger P50 amplitudes demonstrated in MCI patients who later converted to a diagnosis of AD (up to five years later) than MCI patients whose diagnosis remained stable; discriminant analyses of MCI outcomes using P50 amplitude achieved 81% sensitivity and 73% specificity (Golob et al., 2002; Golob et al., 2007). Changes in cortical function and more widespread damage may be an important difference between the deficits evident in MCI SD relative to MCI MD and mild AD.

4.1 P50 in AD patients

The research concerning P50 amplitude in AD patients has been inconsistent and is in need of clarification. Some studies have suggested that the P50 amplitude increases are somewhat linearly related to severity of cognitive impairment, whereas other studies have suggested an inverted U-shaped function. However, no single study comparing

older controls, MCI, and AD patients has demonstrated significant findings across all groups to clarify this relationship.

The research consistently shows P50 amplitudes are greater in the elderly than the young, and greater in MCI patients than healthy older controls. Whether the P50 in AD increases as function of cognitive impairment, and therefore could be used to differentiate older controls from patients, or whether it returns to somewhat normal levels in AD patients is unclear. Findings from one oddball task revealed that AD patients had a significantly larger P50 response to both standard and target stimuli than healthy older controls (Golob et al., 2000). A follow-up study by Golob and colleagues (2007) showed the increased P50 amplitude in mild AD relative to controls was nonsignificant. Other studies have shown normal or only nonsignificantly larger P50 amplitudes in mild-moderate AD patients relative to healthy older controls (Pekkonen et al., 1994; Golob et al., 2007). These researchers found smaller P50 amplitudes in AD patients compared to MD MCI (Golob et al., 2007; Pekkonen et al., 1994). This inverted-U-shaped trend of P50 amplitude as related to severity of cognitive impairment (i.e., controls < MCI > AD) has also been shown in a P50 suppression study, where auditory P50 amplitudes to the second stimulus showed: young controls < older controls < MCI > AD (Golob et al., 2001).

Methodological differences across studies, such as different experimental paradigms, variability of definitions of "mild" AD among oddball paradigms, and heterogeneity of AD severity (e.g., collapsing across diagnostic subtypes AD probable and AD possible) have contributed to the inconsistencies in the research. Notably,

although Golob et al. (2007) were unable to replicate their prior findings showing P50 greater in mild AD relative to controls (2000), they noted substantial differences between the study cohorts, such that the controls in the latter study had a larger P50 relative to the first study cohort, and the AD cohort had a smaller P50 relative to the cohort in the first study. Therefore, further research is necessary to clarify whether P50 amplitude differentiates patients in the earliest stages of AD from age-matched controls.

5. Specific Aims

This research investigated a cortical measure of brain functioning, i.e., P50 amplitude, to further understand the neural correlates of AD symptom progression and potentially provide a tool for early diagnosis and the evaluation of cognitive decline. This study has two aims. First, this research expands on the study conducted by Golob & Starr (2000) which showed P50 amplitude differences differentiated mild AD patients from healthy older controls. The present study was conducted with a larger sample (three times greater) and expanded the scope to include novel stimuli in the target-detection paradigm.

Novelty processing is accomplished by widely distributed neural circuitry and evidence from ERP studies, topographical mapping, and source localization suggest that it involves more fronto-central brain regions than the processing of other auditory stimuli (Fabiani & Friedman, 1995; Mecklinger & Ullsperger, 1995). Thus, P50 amplitude, which seems to be moderated by frontal brain regions, may differ in response to novel versus target tones. Disruption of neural circuitry involving frontal regions may result in less functional connectivity, which may result in less inhibition of the auditory cortical

response, and therefore larger P50 amplitude in response to auditory stimuli. Thus, investigating the P50 response to novel tones in addition to target and standard tones may improve current techniques to differentiate AD patients from controls and study the neural correlates of cognitive decline associated with AD.

A greater understanding of the neural correlates involved in mild AD may provide information regarding the pathogenesis of the disease. The presence of neuropathological plaques and tangles, neuronal cell death, and disruption of the cholinergic system have been implicated in contributing to the cognitive impairment in AD. However, there is not clear evidence regarding the pathogenesis of the disease and the effect on cognition. For example, whereas cholinergic dysfunction contributes to cognitive impairment, it has not been found sufficient to cause the level of impairment evident in patients prior to autopsy studies. However, there is evidence from MCI patients that cholinergic neurons are not regulated normally, despite an unchanged or even increased number of cholinergic receptors (Jeong, 2004). These findings suggest a subtler dysregulation of cholinergic neurons in the basal nucleus of Meynert early in the disease process. The P50 potential, thought to be mediated by frontal circuitry of the cholinergic system, may reflect this subtle dysregulation of basal forebrain neurons.

Second, this study will investigate whether there is a relationship between the P50 component of auditory evoked potentials and neuropsychological measures of symptom severity in AD patients as measured by Mini Mental Status Exam scores. P50 amplitude increases with aging and has been shown to increase with cognitive impairment across MCI patients (MCI MD > MCI SD). P50 amplitude was also greater

for MCI patients who later converted to AD (up to five years later). Neuropsychological, neuropathological, and electrophysiological evidence suggest that some MCI cases may actually be pre-clinical AD. Sensitivity of the P50 to early, possibly prodromal state AD could help researchers identify at-risk individuals, before the irreparable damage to neural systems occurs that underlies AD symptom manifestation. Moreover, the P50 may help quantify the severity of the disease and cognitive impairment in patients with AD, providing invaluable information regarding clinical course to facilitate treatment planning and long-term care.

6. Method

6.1 Participants

Recruitment. AD patients and cognitively normal controls were recruited for a parent study conducted by researchers at the Penn Memory Center at the University of Pennsylvania in collaboration with Drexel University.

Inclusion and exclusion criteria. Inclusion criteria for the cognitively normal control group included: age 60 or older; and no indication of functional or cognitive decline two years prior to enrollment. Exclusion criteria for the cognitively normal control group included: evidence of neurological disease or the use of sedative, anxiolytic or anti-depressant medications within 48 hours of ERP data collection.

Inclusion criteria for the AD patients included: age 60 or older; functional or cognitive decline present over the past 12 months; and diagnoses were made by a neurologist using neurological and neuropsychological examinations, and family

interviews, meeting the requirements for NINCDS-ADRDA criteria for probable AD. Exclusion criteria for the AD patient group were the same as for the controls.

All participants signed Drexel-approved IRB consent forms (in addition to those for the parent study for the University of Pennsylvania). The protocol was approved by the Drexel University and University of Pennsylvania IRBs. Participants were paid $25 for completing the study.

6.2 P50 Issues Relevant in Participant Selection

First-degree relatives. There is some evidence that first-degree relatives of individuals with AD may have larger P50 amplitudes. Individuals with first-degree (sibling or parent) relatives meeting criteria for AD definite, AD probable, and AD possible formed three levels of *at risk* groups in a study comparing ERP differences among those at risk to a similarly aged control group with no family history of AD. Mean differences in P50 amplitude were statistically significant among the groups ($p <$ .009) (Boutros et al., 1995).

Gender differences. The P50 response has not been found to differ as a function of gender. In a study of 31 cognitively healthy adults, excluded on the basis of self-identified attention and psychiatric problems, there were no significant differences between the P50 amplitude for the 16 men and 15 women included in the study (Brinkman & Strauder, 2007).

Cholinesterase inhibitors. In studies of both MCI and AD patients taking cholinesterase inhibitor medications, such as donepezil, there were no significant main effects of medication or interaction effects between group and medication found on P50 amplitude and latency (Golob et al., 2007; Golob et al., 2005).

6.3 Apparatus & Measures

128-channel EEG caps with digitally-linked mastoid reference electrodes manufactured by Electro-Cap International were used to record data from 19 channels placed according to the International 10-20 system. The MANSCAN recording system was used to amplify, digitize, and record the EEG.

The Mini-Mental State Exam (MMSE) is a widely used measure to evaluate cognitive functioning. The MMSE assesses orientation, attention, immediate and short-term recall, language, and the ability to follow simple verbal and written commands. The total score has been used as a measure to classify severity of cognitive impairment in patients with AD. MMSE score is inversely related to years of education and age. The MMSE has good psychometric properties and is widely used. Concurrent validity was examined by correlating MMSE scores with the verbal ($r = .776$, $p < .0001$) and performance ($r = .660$, $p < .001$) subscales of the Wechsler Adult Intelligence Test (Folstein, Folstein, & McHugh, 1975). The 24-hour test-retest reliability was examined, correlating pretest and posttest scores with a single examiner ($r = .887$) and multiple examiners ($r = .827$) (Folstein et al., 1975).

6.4 Procedure

All participants underwent two phases of the experiment. The first phase included participant screening, diagnosis, and neuropsychological testing, conducted at the Penn Memory Center at the University of Pennsylvania. The second phase included EEG recording at the EEG Laboratory at Drexel University. All participants gave informed consent for both phases of the experiment. All data were de-identified; participants were assigned an identifying number associated with their demographic data, neuropsychological test results, diagnosis and EEG data. De-identified data used in the current analysis are stored on a password protected computer at Drexel University's the EEG Lab in accordance with the Drexel IRB-approved protocols for the parent study.

EEG preparation. Participants were instructed to eat before their appointment to not be hungry during the session, sleep well the night before, and to abstain from alcohol, anxiolytic or antidepressant medications within 48 hours of the appointment. Electrodes were placed according to the international 10-20 system.

Participant instructions. Participants were instructed to stay relaxed, though alert, throughout the EEG recording. Participants were seated three feet from a computer screen and asked to keep their eyes focused on the fixation cross at the center of the screen. Participants were asked to identify high-pitched tones among a series of low-pitched tones and to and press the left mouse button as quickly as possible when the target was heard. Participants were also informed that other sounds that were not plain tones would be present occasionally but they were to only respond to target tones. Volume levels were individually adjusted to a comfortable, audible level for each

participant. Hearing aide devices were worn during the experiment if necessary. A practice session, consisting only of standard and target tones, was conducted to ensure the procedure had been understood.

EEG Data collection. A total of 864 computer-generated stimuli were presented for a duration of 100 ms with an interstimulus interval of 1.0 to 1.3 seconds. High-pitched target tones (n = 172) were randomly interspersed among low-pitch tones (n = 560). Novel tones (n = 132) were unique, environmental sounds presented only once during the session for duration of 200ms. Stimulus presentation took place in six 3-minute experimental blocks. A mandatory minimum 30-second break followed each 3-minute experimental block.

Nineteen-channel EEG (sampling rate: 256 Hz, bandpass: .02-100 Hz, digitally-linked mastoid reference) was recorded for the duration of the experiment.

7. Data Analysis

7.1 Group Demographics

A total of 69 participants were included in the study, 31 AD patients (17 Male, 14 female) and 38 controls (20 Male, 18 Female) as shown in Table 1. There were no significant group differences in sex, $\chi^2(1) = .033$, $p = .86$, age (AD: M = 74.29, SD =7.41; control: M = 75.76, SD = 7.21), $t(67) = .702$, $p = .48$, or years of education (AD: M = 15.74, SD = 2.82; control: M = 16.89, SD = 2.91), $t(67) = 1.66$, $p = 1.02$ (see Table 2).

Table 1

Number of Males and Females for AD and Control Groups

	Males	Females	Total
Controls	20	18	38
AD	17	14	31
Total	37	32	69

Table 2

Mean Age, Education, and MMSE Scores for AD and Control Groups

	Age		Education		MMSE	
	Mean	SD	Mean	SD	Mean	SD
Controls	75.76	7.20	16.89	2.91	29.24	1.17
AD	74.29	7.40	15.74	2.81	24.26	2.74

7.2 Behavioral Data

In the oddball task, the control group performed significantly better than the AD patients, correctly recognizing targets with 98.73% accuracy (SD = 1.64) relative to patients' 90.55% accuracy rate (SD = 12.53), $t(67) = 3.61$, $p = .001$. There was one outlier in the AD group whose mean target accuracy (36.63%) was three standard deviations below the AD group mean; however, this participant's performance was within 3 standard deviations in response to novel (86.36%) and standard (83.39%) tones. There was no significant difference in accuracy rate responding to novel tones (Control: M = 90.45%, SD = 8.95; AD: M = 89.42%, SD = 15.21), $t(67) = .33$, $p = .74$. There was one outlier in the AD group whose accuracy to novels (16.67%) was 3 standard deviations below the

AD group mean; however, this participant's performance was within 3 standard deviations in response to target (76.16%) and standard (90.89%) tones. The two AD participants with outlying performance values did not have any outlying P50 amplitude values at any electrode for any condition. The control group performed significantly better than the AD patients responding to standard tones with 99.16% accuracy (SD = 2.69) relative to patients' 96.94% accuracy rate (SD =4.75), $t(67) = 2.32$, $p = .025$. Mean target accuracy rates and number of false alarms to standard and novel tones are presented in Tables 3 and 4.

Table 3

Percent Accuracy on the Oddball Task

	Standard*		Target*		Novel	
	Mean	SD	Mean	SD		
AD	96.94	4.75	90.55	12.53	89.42	15.21
Control	99.16	2.69	98.73	1.64	90.45	8.95

Note. *Group differences significant at $p < .05$.

Table 4

Number of False Alarms on the Oddball Task

	Standard		Novel	
	Mean	SD	Mean	SD
AD	17.13	26.58	13.97	20.08
Control	4.71	15.06	12.61	11.81

7.3 P50 Amplitude

EEG data was processed for artifact rejection, eye-blink correction, and spatial and temporal and filtering. The data were high-pass filtered at 0.2 Hz and notch-filtered from 59-61 Hz to remove artifact. Post-stimulus ERPs were computed for the standard, target and novel tones and averaged across all six data blocks to increase signal to noise ratio. ERP epochs were 1000 ms, including a 200 ms prestimulus baseline. The average amplitude (in μV) across the 34-77 ms poststimulus P50 epoch was computed for each participant. This epoch was selected to approximate prior studies, many of which define P50 as occurring 30-65ms, 40-75ms, or 40-80ms after stimulus onset. In the present case, epoch selection was limited by the available samples (256 per second).

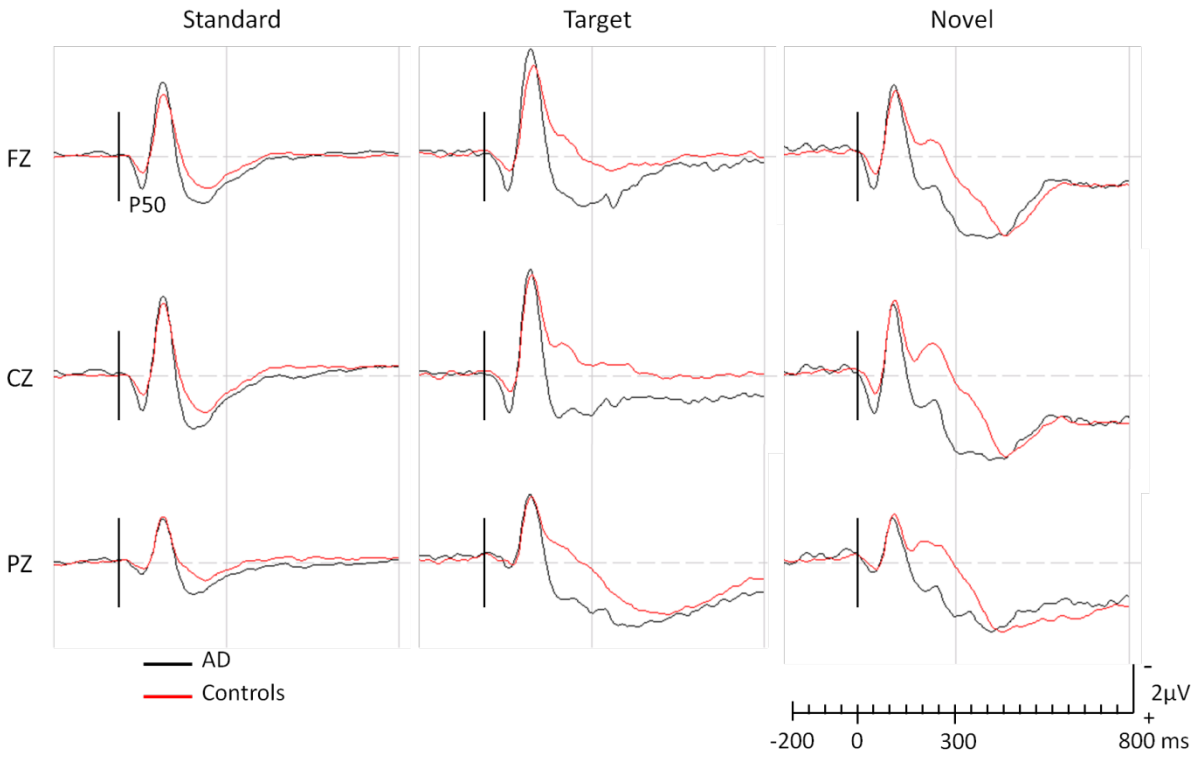

Figure 1. ERP Grand average waveforms at FZ, CZ, and PZ to standard, target, and novel stimuli. ERP grand average waveforms were lowpass filtered at 30Hz for display in this figure. The P50 component is indicated in the Standard ERP at electrode FZ.

Grand averages of P50 amplitude were computed for each group separately in response to target, standard, and novel tones. Grand averages for the AD and control groups are displayed in Figure 1 in response to standard, target, and novel tones at electrodes FZ, CZ, and PZ. Subtraction of group grand average waveforms for target minus standard, novel minus standard, as well as target minus novel waves are shown in Figure 2. Visual inspection shows the largest between-group difference in P50 amplitude for the novel minus standard conditions at electrodes FZ and CZ.

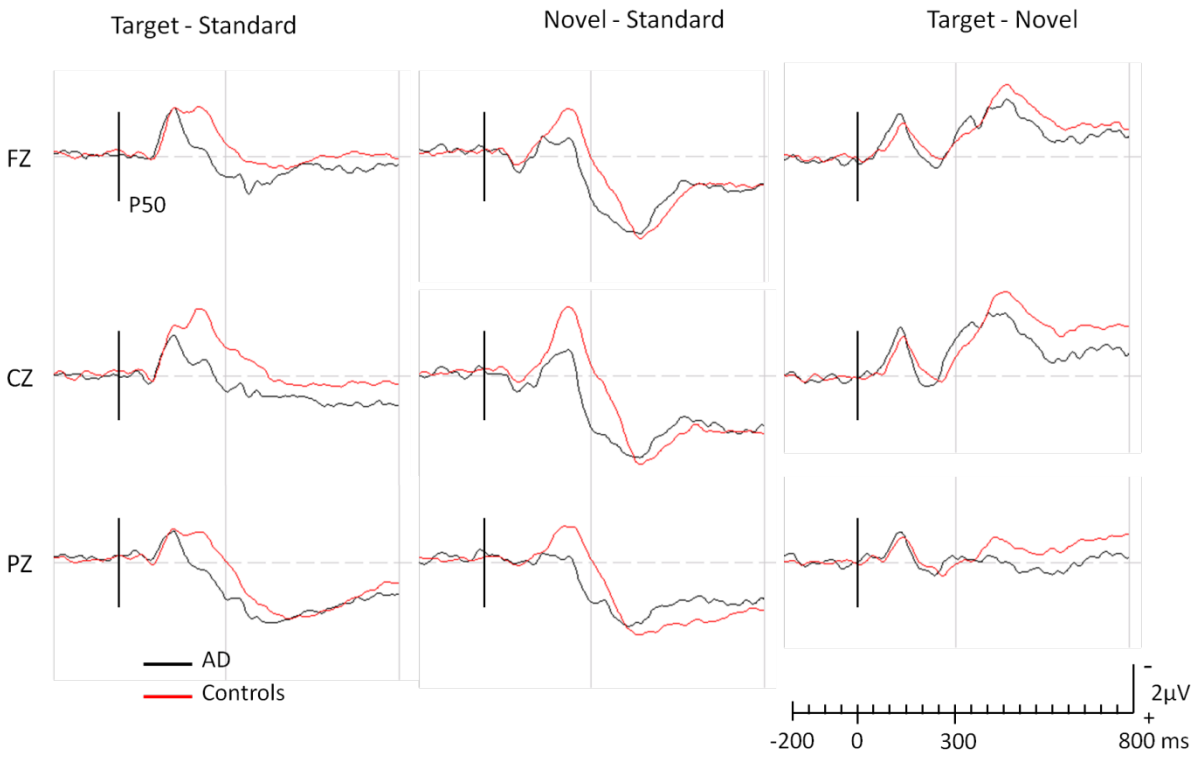

Figure 2. Subtractions of ERP grand average waveforms at FZ, CZ, and PZ. ERP grand average waveforms were lowpass filtered at 30Hz for display in this figure.

The frequency distributions of subjects' P50 amplitudes illustrate differentiation between the AD and control groups and are shown in Figures 3-5 for group responses to standard, target and novel tones at electrode CZ. Generally, P50 amplitudes above 3μV capture AD patients. For standard tones, values above 2μV capture most AD patients, although some control participants had values above that cutoff as well (Figure 3). For target and novel tones, only AD patients showed amplitudes above 3μV, with the exception of two outliers in the control group, who had P50 amplitudes that were three standard deviations greater than the control group's mean (Figures 4-5).

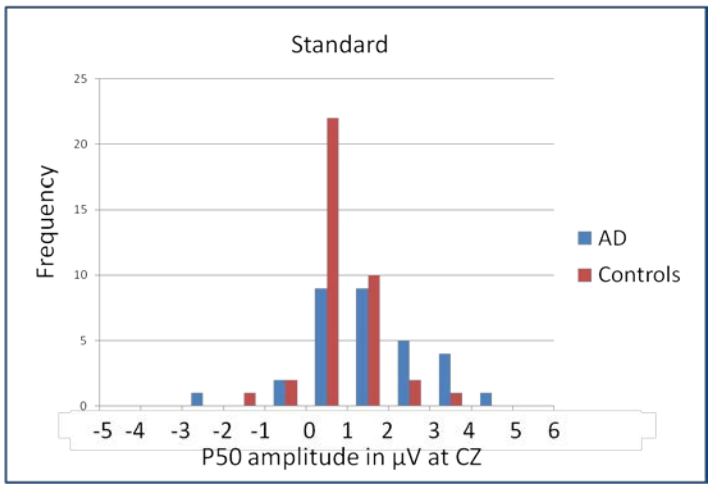

Figure 3. Frequency distribution of P50 amplitude at electrode CZ in response to standard tones, showing the number of AD and control participants with values within each 1µV bin. No outliers present.

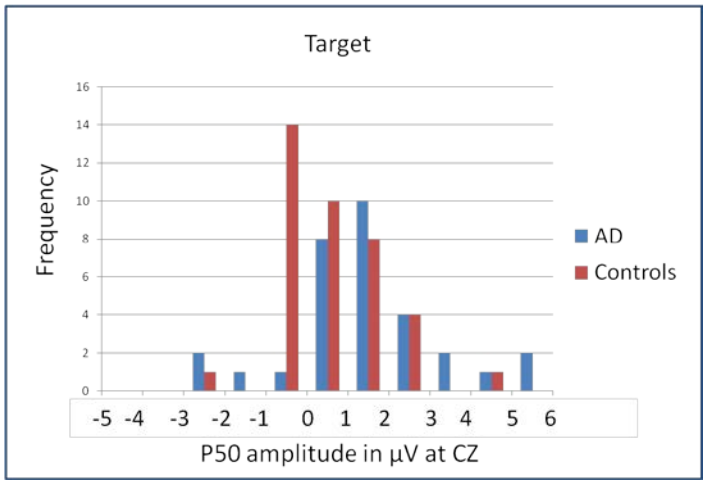

Figure 4. Frequency distribution of P50 amplitude at CZ in response to target tones, showing the number of AD and control participants with values within each 1µV bin. One control group outlier above 3µV (participant 69).

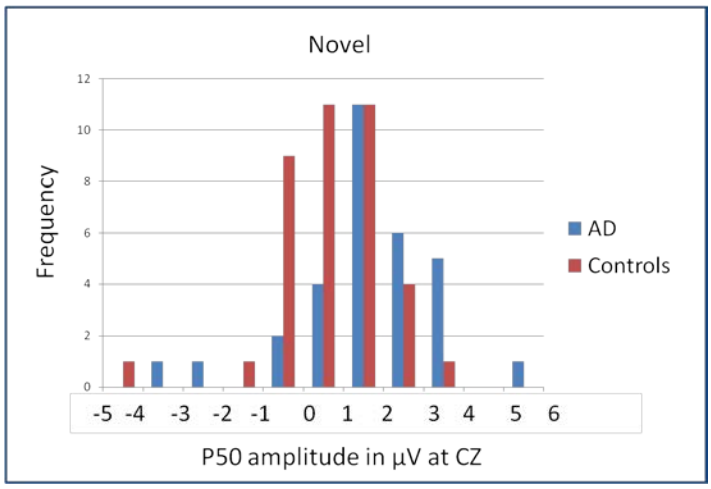

Figure 5: Frequency distributions of P50 amplitude at CZ for novels, showing the number of AD and control participants with values within each 1µV bin. One control group outlier above 3µV (participant 50).

The frequency distributions of the condition subtractions for target minus standard, novel minus standard, and target minus novel are shown in Figures 6-8. Among these comparisons, the target minus standard condition shows mostly AD patients showing a condition difference greater than 1µV (Figure 6). Whereas the AD group demonstrated greater P50 amplitudes in response to targets versus standard tones, relative the control group, the frequency distribution for novel minus standard condition showed much overlap between the groups, despite the fact that the overall mean group difference for novel minus standard was larger for the AD group relative to controls (Figure 7 and Table 5). The AD group showed larger P50 in response to novels versus targets, with a subtle shift in the distribution evident relative to controls (Figure 8).

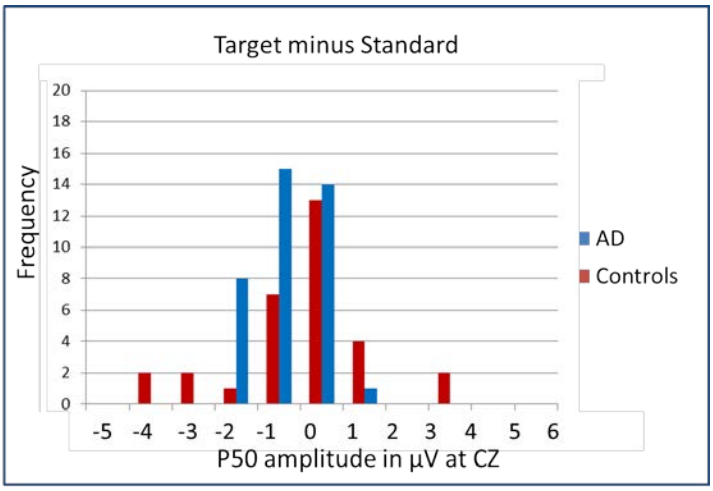

Figure 6: Frequency distribution of P50 amplitude for target minus standard stimuli for AD and Controls.

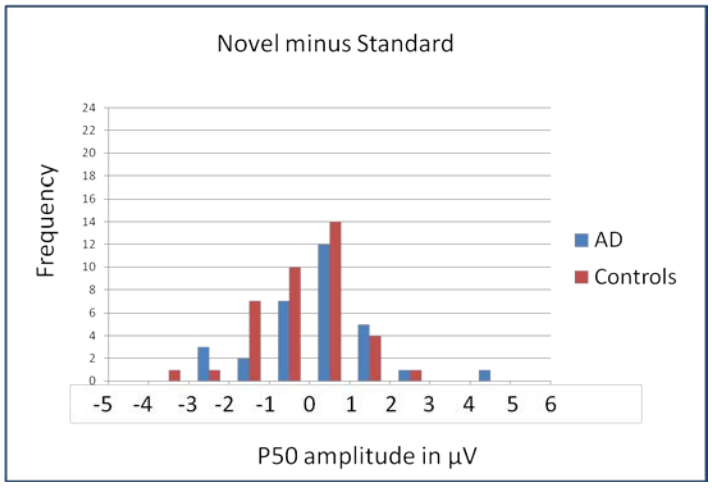

Figure 7: Frequency distribution of P50 amplitude for novel minus standard stimuli for AD and Controls.

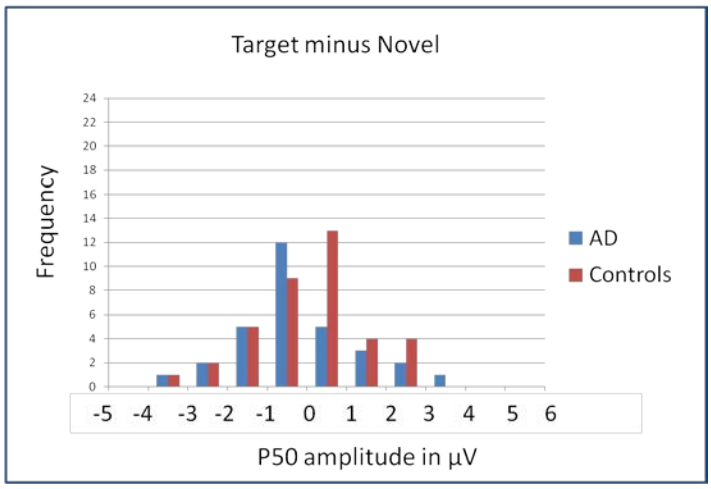

Figure 8: Frequency distribution of P50 amplitude for target minus novel stimuli for AD and Controls.

A mixed-model repeated measures analysis of variance (ANOVA) was used to investigate the relationship between P50 amplitude, stimulus type, and scalp location for the AD and control groups. Analyses included the between-group factor of diagnosis (AD, controls; 2 levels) and the within-group factors of electrode location, anterior/posterior (AP, 4 levels) x dorsal/ventral (DV, 2 levels) x hemisphere (H, 2 levels) as well as the within-group factor of stimulus type (target, standard, novel; 3 levels). The electrodes included in this analysis were: FP1/2, F3/4, F7/8, C3/4, T7/8, P3/4, P7/8, and O1/2. The Huynh-Feldt correction was applied to control for Type-I error, and the adjusted *p*-values were reported. Two-tailed differences of $p < .05$ were considered significant. Post-hoc testing with independent samples *t*-tests was used to compare P50 amplitude at all 19 electrodes between AD patients and controls, separately for the standard, target, and novel conditions. Significant main effects were found for factor AP (4), $F(3, 67) = 13.46$, $p < .001$ and stimulus type, $F(2, 67) = 6.43$, $p = .003$. There was a

significant interaction effect found for the factors AP(4) x DV(2) x stimulus(3) x diagnosis (2), $F(6, 67) = 3.85$, $p = .007$. The interaction of the electrode factors, stimulus, and diagnosis are of primary interest, as this indicates a different spatial pattern of neural activity was measured across the scalp for each group that varied as a function of stimulus type.

This ANOVA was performed again with outliers removed. Outliers were defined as any participant with at least two P50 amplitude values three standard deviations above or below their respective group mean for all electrodes and conditions. Six AD and six control participants were removed, many with multiple outlying values at various electrodes and across conditions. With the outliers removed, the interaction found between location, stimulus and diagnosis [(AP(4) x DV(2) x stimulus(3) x diagnosis (2)], was no longer significant, $F(6, 55) = 0.00$, $p = 1.00$. There were still significant main effects for factors anterior/posterior, $F(3, 55) = 52.13$, $p < .001$, and stimulus type, $F(2, 55) = 5.55$, $p = .006$.

A second mixed-model repeated measures analysis of variance (ANOVA) was performed to include midline electrodes, FZ, CZ, and PZ. This analysis included the between group factor of diagnosis (AD, controls) and the within group factors of electrode location AP (3 levels) x stimulus type (3 levels). A significant main effect was found for factor anterior/posterior, $F(2, 67) = 56.41$, $p < .001$. There was a significant interaction between anterior/posterior x diagnosis, $F(2, 67) = 4.88$, $p = .011$. This ANOVA was repeated with the 12 outliers removed as described above. Again, a significant main

effect was found for factor anterior/posterior, $F(2, 55) = 61.35$, $p < .001$ and a significant interaction was found between anterior/posterior x diagnosis, $F(2, 55) = 5.43$, $p = .007$.

Post-hoc testing with independent-samples *t*-tests was used to compare P50 amplitude at all 19 electrodes between AD patients and controls, separately for the standard, target, and novel conditions. The *t*-scores allow for more precise information about the topographical differences between the two groups' brain activation in response to the different stimuli. As these were computed as follow-up tests to clarify the patterns driving the significant ANOVA interaction, corrections for multiple comparisons were not made.

Overall, the AD group demonstrated significantly greater P50 amplitudes over frontal and central regions relative to controls in response to target, standard, and novel tones (see Figure 9). This difference was greater over the right hemisphere, particularly in response to target and standard tones. Left parietal amplitudes were greater in the AD group relative to the controls across all stimulus types, with the most marked difference observed in response to novel tones. Relative to the AD group, there was a nonsignificant trend for the control group to show greater activation in left temporal and occipital regions across all stimuli types; in response to target tones, activity at electrode O1 was significantly greater relative to the AD group, $t(67) = -2.09$ $p = .041$.

With the 12 outliers removed, the overall topographic trends remained similar, as seen in Figure 10. Across all conditions, the between group differences at electrode CZ were marginally stronger across all conditions. For the standards, the group differences are less noted in frontal areas and more focused around central and right

temporal-parietal areas. For targets, the maps are comparable but somewhat less robust. For novels, frontal areas are more broadly recruited for the AD patients, with larger differences notable at CZ.

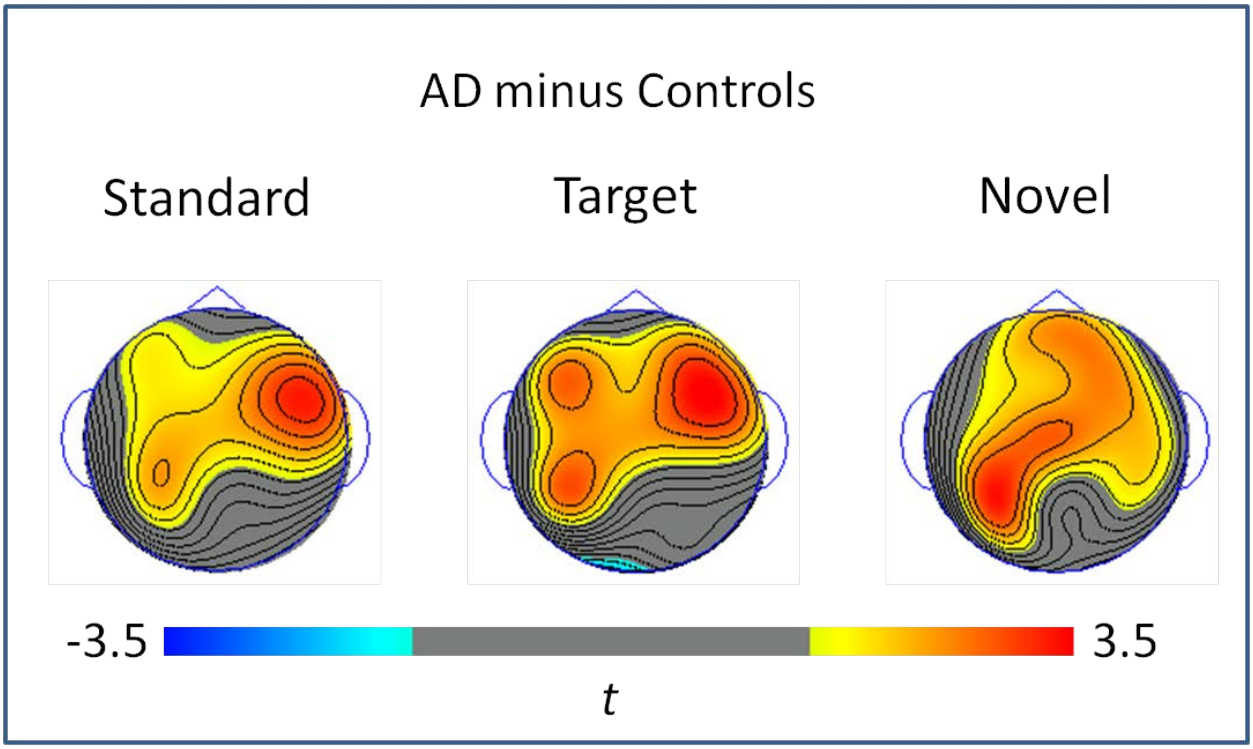

Figure 9: Topographical maps of significant between group t-score differences (AD – controls) at each electrode where $p < .05$, two-tailed.

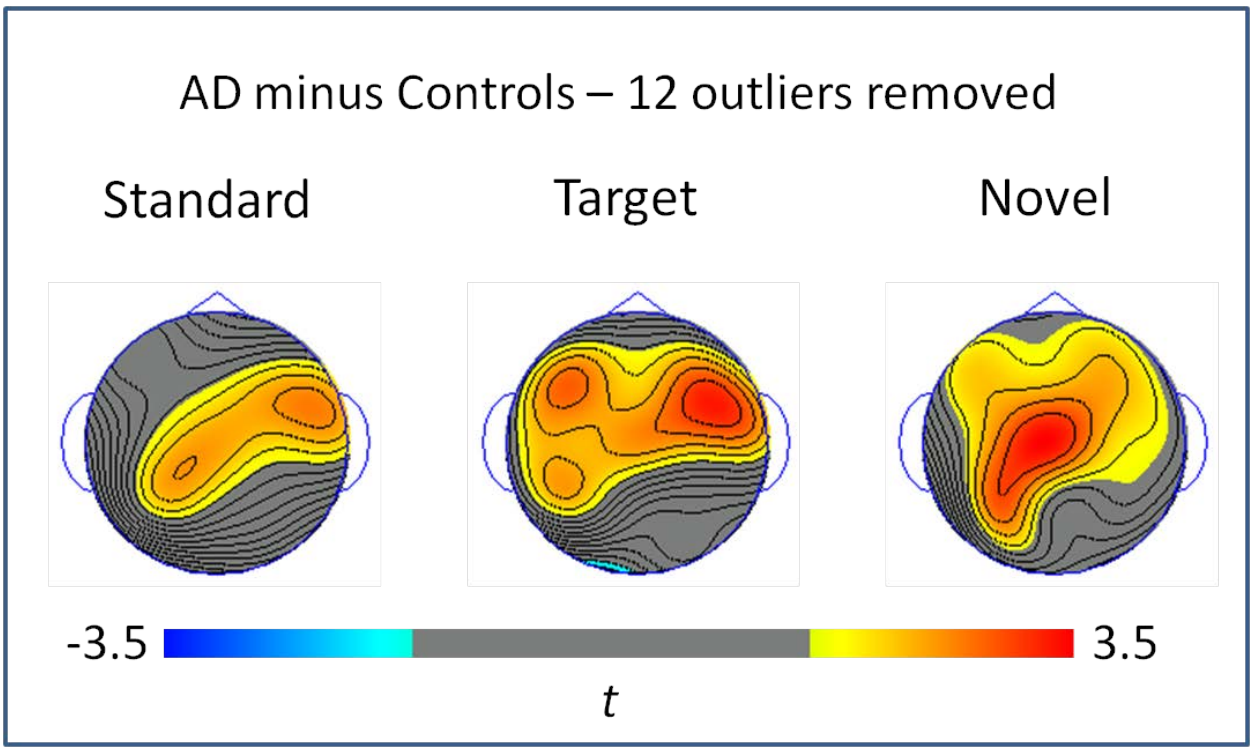

Figure 10: Topographical maps of significant between group t-score differences with outliers removed (AD – controls) at each electrode where $p < .05$, two-tailed.

At electrode CZ, P50 amplitude was greatest in the AD group in response to novel > target > standard tones; for controls, P50 amplitude was greatest for standard > novel > target tones (see Table 5). When the outliers were removed, this trend remained for the controls but changed for the AD group, who then showed novel > standard > target (see Table 6). Among midline electrodes FZ, CZ, and PZ, P50 was maximally produced in response to targets and standards at electrode CZ for both groups. Whereas the AD group's P50 amplitude in response to novel tones was also greatest at CZ, it was maximally produced at FZ for controls (Table 5). When the outliers are removed, P50 is still maximally produced at CZ for the AD group under all conditions. Whereas the

control group's maximal responses to standards and targets are unchanged, the maximal P50 in response to novels, like targets, is seen at electrode FZ.

Table 5

Mean P50 Amplitudes (in μV) at Midline Electrodes for all Stimuli

		FZ		CZ		PZ	
		Mean	SD	Mean	SD	Mean	SD
Standard	AD	1.29	1.57	1.42	1.53	0.46	1.33
	Control	0.69	0.86	0.80	0.82	0.26	0.70
Target	AD	1.36	1.70	1.44	1.82	0.04	1.40
	Control	0.57	1.20	0.58	1.25	0.00	0.82
Novel	AD	1.37	1.37	1.62	1.71	0.38	1.40
	Control	0.64	1.36	0.60	1.35	0.19	1.21

Table 6

Mean P50 Amplitudes (in μV) at Midline Electrodes for all Stimuli after 12 Outliers Removed

		FZ		CZ		PZ	
		Mean	SD	Mean	SD	Mean	SD
Standard	AD	1.42	1.52	1.52	1.25	0.54	0.97
	Control	0.37	0.68	0.84	0.68	0.28	0.57
Target	AD	1.19	1.20	1.38	1.41	-0.02	1.26
	Control	0.60	0.93	0.56	1.08	0.00	0.77
Novel	AD	1.37	0.97	1.67	1.08	0.48	1.04
	Control	0.71	1.36	0.61	1.35	0.20	1.23

7.4 P50 and MMSE Scores

MMSE scores ranged 18-30 for AD patients and 25-30 for controls (see Figure 11). As expected, MMSE scores for AD patients (M = 24.26, SD = 2.74) were significantly lower than controls (M = 29.24, SD = 1.17), $t(67) = -9.42$ $p < .001$.

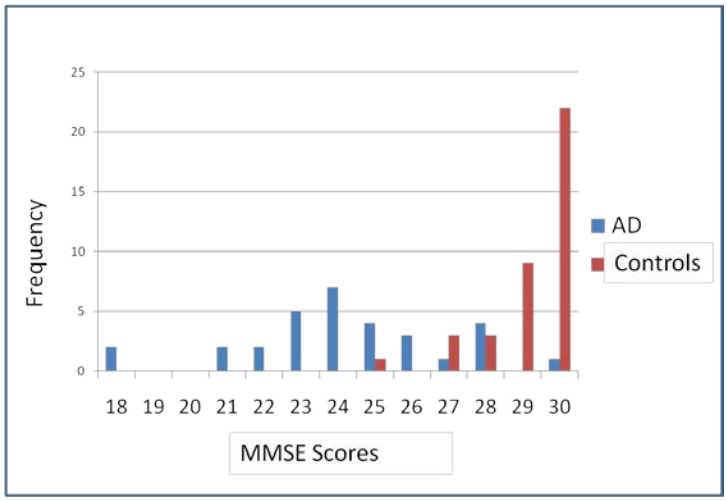

Figure 11: Frequency distribution of MMSE scores for the AD and control groups

To assess whether MMSE scores were predictive of P50 amplitude for the AD group, P50 amplitude at electrode CZ was regressed on MMSE scores separately for target, novel and standard tones. Curve estimation was used to investigate the hypothesized negative quadratic relationship between the variables (see Figure 12).

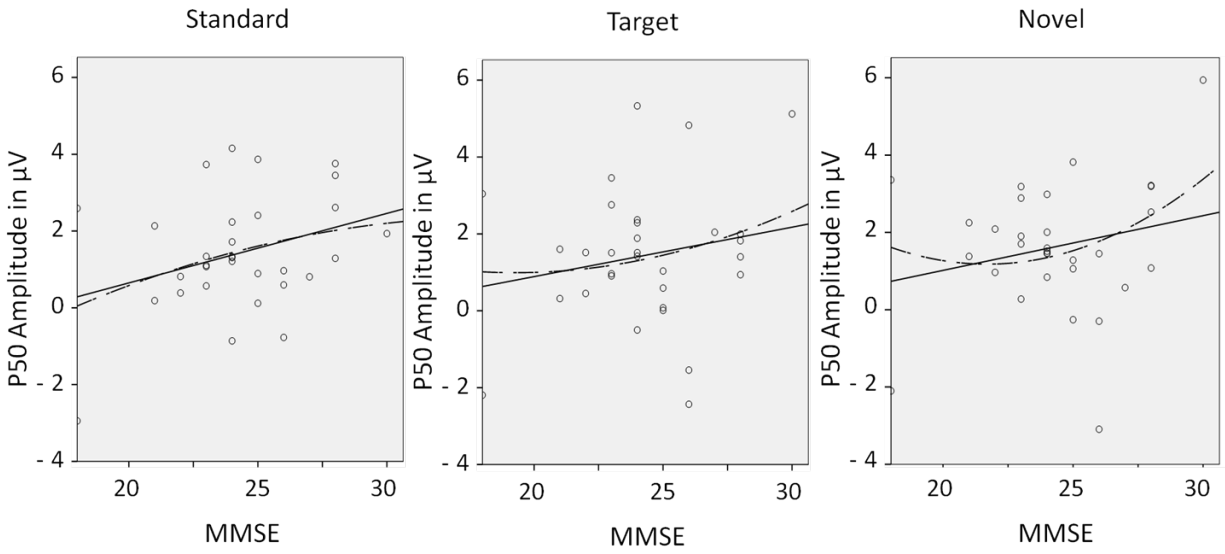

Figure 12: Curve Estimation of MMSE scores as potential predictors of P50 amplitude at electrode CZ for the AD group.

Contrary to the hypothesis, The AD group's MMSE scores were not found have a significant negative quadratic relationship with P50 amplitude at electrode CZ for targets, b = 1.38E-08, $t(30)$ = .45, p = .66, novels, b = 3.19E-08, $t(30)$ = 1.13, p = .27, or standard tones, b = -8.67E-09, $t(30)$ = -.35, p = .73. P50 amplitude at CZ was also linearly regressed on the AD group's MMSE scores for all three conditions. There was not a significant linear relationship between MMSE and P50 amplitude at CZ for targets, b = 1.29E-07, $t(30)$ = 1.06, p = .30 or novels, b = 1.42E-07, $t(30)$ = 1.26, p = .22, however it approached significance with standard tones, b = 1.81E-07, $t(30)$ = 1.85, p = .07. There were no significant relationships found between MMSE score and P50 amplitude when the outliers were removed.

8. Discussion

The concept of Alzheimer's disease has changed substantially since the diagnostic criteria were proposed in 1984 by the NINCDS-ADRDA. The biggest changes in the recently released NIA-AA diagnostic guidelines are (1) the contention that AD exists on a continuum with three defined stages that include: the dementia phase; a symptomatic, pre-dementia phase; and an asymptomatic pre-clinical phase, and (2) the inclusion of biomarkers in the diagnostic criteria (AA, n.d.; Jack et al., 2011). Given that irreversible brain damage has occurred by the dementia phase, early diagnosis of AD is necessary for treatment to intervene and potentially slow the disease progression. With regards to diagnosis, the consensus is moving towards giving more weight to neuropathological changes rather than focusing mainly on clinical criteria (AA, n.d.; Jack et al., 2011). EEG is capable of capturing abnormalities in brain activation that reflect these disease-related structural and functional changes. In line with these modern conceptualizations of the disease and its diagnosis, the present study used ERP techniques to investigate a cortical measure of brain functioning, i.e., auditory P50 amplitude, to further understand the neural correlates of AD symptom progression and potentially provide a tool for early diagnosis.

One of the aims of this study was to clarify conflicting reports in the literature that auditory P50 amplitude differentiates AD patients from healthy older controls. Methodological differences and differences in statistical reporting make the comparison of prior studies to each other as well as to the present study difficult, including: (1) different experimental paradigms that may not include novel tones; (2) some studies do

not report P50 amplitude results for all stimuli, even if they were included in the study; (3) studies vary in their statistical analyses (e.g., data transformations); (4) studies vary on the electrodes used; and (5) the electrodes for which statistics are reported.

The present study found, as hypothesized, that the AD group demonstrated significantly greater P50 amplitude at electrode CZ in response to all stimulus types. These findings are consistent with Golob et al. (2000) who found significantly greater P50 amplitude in AD patients in response to both target and standard tones at electrode CZ. Although a follow up study by Golob and colleagues (2007) failed to find significant differences in P50 amplitude between the AD and age-matched control groups, the number of AD participants was much smaller (n = 14) than the group in the present study. More importantly, while the 2007 AD group was comparable in age and education to the group in the present study, their mean MMSE score (M = 21.7, SD = 3.0) was almost one standard deviation (.9) below the mean for the present study (M = 24.3, SD = 2.7). However, Golob's 2000 AD group's MMSE (M = 23.0, SD = 0.9) was comparable to the present study. Based on MMSE scores, it is possible that the 2007 group was further along in disease progression than both the 2000 cohort and present study group and that they passed the point of an early stage dementia, during which P50 amplitudes would still be expected to be significantly greater than controls. Taken together, these findings are consistent with the hypothesized inverted-U-shaped relationship between P50 amplitude and disease severity.

That AD patients showed greater P50 amplitude relative to controls may reflect deficient inhibitory functions, which is consistent with studies showing impaired

inhibition of P50 associated with the prefrontal cortex as well as known prefrontal pathology in AD that results in observable deficits in behavioral inhibition (Knight et al., 1989). However, behavioral differences were not observed in the ability to inhibit responding to the novel tones, as the number of false alarms were comparable between the groups.

The frequency distributions of subjects' P50 amplitude values show differences between the groups, with only AD patients tending to show P50 amplitudes greater than 3μV in response to any particular stimuli. This is most strikingly the case for responses to targets and novels. However, there are no clear cutoff points at which diagnostic sensitivity and specificity much above 70% would be achieved. This is consistent with a hypothesized inverted-U-shaped function relating P50 amplitude and disease severity, according to which P50 amplitudes would be expected to return to normal levels in more advanced disease states and be difficult to differentiate from controls. Whereas low or normal P50 amplitudes do not differentiate between the groups, it is the case that values at the high end of the distribution seem to only to belong to AD patients. Given the tendency for P50 amplitude to increase in MCI relative to healthy aging as well as in the transition from MCI to AD, values at the higher end of the spectrum may reflect neurophysiological degradation. The implications for clinical use of P50 amplitude as a biomarker are potentially as a screening measure to identify at-risk individuals. It may be the case that control subjects with P50 amplitudes in the upper range represent a pre-symptomatic group for whom neurophysiological degradation is already underway. Although it is usually the manifestation of memory and cognitive difficulties that

precipitate seeking medical care, the underlying neuropathology for AD is prevalent for years before symptoms emerge. These pathophysiological changes seem to be reflected in altered P50. Therefore, it may be possible to use P50 amplitude to screen asymptomatic older adults in order to detect those in an early disease state.

The present study found that relative to controls, AD patients demonstrated significantly greater P50 amplitude in frontal, central, and left parietal brain regions in response to target, novel, and standard tones. These differences were greatest over right frontal areas in response to target and standard tones and greatest over electrode CZ and the left parietal region in response to novels. This is the first study to report topographical differences in P50 amplitude between AD and age-matched controls using this experimental paradigm.

The between group topographical differences were found to be dependent on stimulus type. In the overall ANOVA, there was a significant interaction among location factors (AP and DV), stimulus, and diagnosis on P50 amplitude. Differentiation between the groups was stronger in response to certain stimuli, depending on the location of measurement. Therefore, nonspecific cortical hyperexcitability in the AD group relative to controls does not easily account for the group differences. However, there was no significant interaction between stimulus and diagnosis in the midline ANOVA (sites FZ, CZ and PZ), which was consistent with Golob et al.'s 2000 study, although they also included electrodes C3, C4, T3 and T4 in their statistical analysis. That the present study differed from Golob et al. (2000) and found a stimulus interaction effect in the overall ANOVA is likely due to (1) the inclusion of novel stimuli in the current study, which

show the largest between group differences at CZ, and (2) the analysis in this study included more frontal electrode sites, including F3 and F4, which showed some of the greatest between-group differences across stimulus types.

Although novelty processing is accomplished by widely distributed neural circuitry, evidence from ERP studies, topographical mapping, and source localization suggest that it involves more fronto-central brain regions than the processing of other auditory stimuli (Friedman et al., 1993; Fabiani and Friedman, 1995; Mecklinger and Ullsperger, 1995). The inclusion of novel tones highlighted significant topographical differences in maximal P50 amplitude in AD patients relative to controls which varied as a function of stimulus type.

Among midline electrodes (FZ, CZ, and PZ), P50 amplitude was maximally produced at different electrode locations for the groups in response to different stimulus types. For the AD group, P50 amplitude was maximally produced at CZ in response to all stimuli. The controls also showed maximal P50 at CZ in response to standards. Together, this is consistent with previous findings of maximal P50 at CZ in response to standard tones (Yamaguchi et al., 2000). However, in response to novels, P50 amplitude was greatest at CZ for patients and at FZ for controls. This posterior shift of maximal P50 amplitude in the AD group suggests that patients' neural circuitry for novelty processing is functioning differently than controls'. Given that fronto-central areas are affected in AD patients, it is possible that other, more central and posterior brain regions are being recruited to process novel stimuli. Likewise, once outliers were removed,

maximal P50 was measured at FZ for controls, suggesting a posterior shift in the AD group's brain activation in response to target tones.

8.1 P50 and MMSE

Contrary to the hypothesis, the present study did not find MMSE scores to be predictive of the AD patients' P50 amplitude. Curve estimation was used to investigate the hypothesized negative quadratic relationship between the MMSE scores of the AD patients and P50 amplitudes; no significant relationships were found between the variables in response to target, novel, or standard stimuli. Additionally, no significant relationship was found when P50 amplitude was linearly regressed on MMSE scores separately for all 3 stimulus types.

Given that (1) MCI is now conceptualized on a continuum of disease severity with AD, and (2) given the trend shown by Golob et al. (2007), that P50 amplitude was larger in an arguably a more severe form of amnestic MCI (multiple domain > single domain), and (3) that P50 was larger in MCI patients who later converted to AD, these data suggest a relationship between disease severity and P50 amplitude. There are caveats to the present study in the examination of this potential relationship. Most notably, there is a very limited range of MMSE scores, which makes finding a statistically significant relationship more difficult. Also, the novelty P50 showed the strongest differentiation between the groups' topographical differences, with a posterior shift of maximal P50 for the AD group; if this is suggestive of frontal abnormalities in novelty processing or a reduction in the ability to inhibit the cortical response to auditory stimuli, it is possible that a cognitive task for which performance is dependent

on more frontal regions that underlie inhibitory functions might show a relationship to P50 amplitude in response to novels.

Although the data in the present study do not support a relationship between MMSE scores and P50 amplitude, the data do still support the hypothesized negative quadratic relationship between disease severity and P50 amplitude. This is illustrated in the frequency distribution histograms, which show between group differentiation at the highest values of P50 amplitude, associated with early and transitioning disease state, versus the low end of the spectrum, where P50 values are expected to return to normal.

Control participants who show P50 amplitude comparable to AD participants in the present study may be in an early, pre-symptomatic disease state. Also, the recent NIA-AA diagnostic guidelines now make a semantic and conceptual distinction between AD pathophysiological processes and the various clinically observable syndromes that result, because there often is not a clear relationship between clinical presentation and underlying neuropathology (Jack et al., 2011). These variations in the qualitative and quantitative presentations of the disease may be suggestive of the presence of diagnostic subgroups with the Alzheimer's dementia type, which may account for the extreme heterogeneity observed across AD patients.

Overall, the distinction between AD and controls in the present study is consistent with expectations given (1) empirical findings of larger P50 amplitude in arguably greater disease states from MCI to mild AD and (2) the modern conceptualization of these previously distinct illnesses now conceptualized as existing together on a single continuum, as outlined in the NIA-AA diagnostic guidelines (Golob

et al., 2001; Golob et al., 2002; Golob et al., 2007; Jack et al., 2011; Irimairi et al., 2005). Furthermore, the participants in the current study were selected for being in an early stage dementia, therefore further along in the disease state than MCI patients but whose neuropathology had not progressed to impair the source of P50 generation in the primary auditory cortex. As early diagnosis is meant to identify individuals at risk or in the earliest stages of AD in order to implement interventions, P50 amplitude distinctions would be more valuable if they capture these individuals, as seems to be shown by data in the present study. That P50 has more potential diagnostic utility in early and symptomatic pre-dementia states bodes well for the development of screening measures of pre-symptomatic individuals for whom the underlying pathophysiological process has already begun.

8.2 Conclusions and Directions for Further Research

As cortical abnormalities present in AD can be detected by EEG, EEG may serve as a useful tool to elucidate the changes that occur in the brain during the earliest stages of disease, possibly leading to new insights into the pathogenesis of AD, identifying at-risk individuals, better predicting the clinical course, and developing more effective interventions. The data in the present study suggest that both P50 amplitude and its scalp distribution may be useful in discriminating between healthy older adults and AD patients in an early, possibly pre-symptomatic disease stage. The differences in the sample of the present study compared with the Golob et al. (2007) follow-up study, namely, that the current sample was twice as large and possibly in an earlier disease state, may account for the ability to make a statistically significant distinction between

the diagnostic groups. However, despite the fact that that the present study found significant group differences, the data also suggest that it is more pertinent to focus on pre-symptomatic healthy older adults and follow them to determine whether P50 predicts not only decline from MCI to AD but also decline from a pre-symptomatic stage.

Future research should also consider the NIA-AA diagnostic conceptualizations and consider diagnostic variables that are continuous, rather than dichotomizing between AD, MCI, and pre-clinical participants. Although there is heterogeneity in the AD population that prevents a 1:1 correlation with pathophysiological changes and cognitive functioning, neuropsychological measures that rely on fronto-central brain circuitry may elucidate the relationship between P50 amplitude and the underlying circuitry responsible for the group differences present in this study, allowing for a deeper understanding of the changes in neural circuitry that occur and progress throughout the disease.

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