CHANGES IN OLFACTORY EVENT-RELATED POTENTIALS AND
BEHAVIORAL MEASURES ASSOCIATED WITH THE
APOLIPOPROTEIN E4 ALLELE: A COMPARISON OF YOUNG,
MIDDLE AGE, AND OLDER INDIVIDUALS

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Middle Age, and Older Individuals

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ABSTRACT OF THE THESIS

Changes in Olfactory Event-Related Potentials and Behavioral Measures Associated with the Apolipoprotein ε4 Allele: A Comparison of Young, Middle Age, and Older Individuals
by
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The sense of olfaction declines with age and negatively impacts the lives of individuals. This decline can be seen in measurements of threshold, identification, and memory. Olfactory deficits beyond that of normal aging are present in Alzheimer’s disease (AD). The earliest stages of neurodegeneration occur in olfactory processing areas of the brain. The Apolipoprotein (ApoE) ε4 allele is a genetic risk factor for AD, and is associated with olfactory deficits. Presence of the allele is also associated with a more profound decrease in olfaction with age. This study examined differences in activation of olfactory and visual brain areas, reflected in event-related potential (ERPs) latencies and amplitudes, based on age group and ApoE ε4 status. This measure was also compared to traditional olfactory behavioral tests. Latencies and amplitudes were compared across the N1, P2, N2, and P3 peaks to explore which peaks are most effective at differentiating among groups. Significant differences were found in olfactory ERPs based on age, ApoE ε4 status, and their interaction. Visual ERPs were only significantly different by age. P3 was found to be the most effective peak to differentiate between ApoE ε4 positives and negatives for the young and middle age groups, whereas N1 was the most effective peak for the older age group. Overall, the results suggest that olfactory ERPs may be able to detect more subtle differences due to aging and AD risk than traditional behavioral tests.
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CHAPTER 1

INTRODUCTION

The sense of olfaction declines with age and negatively impacts the lives of individuals. Olfactory deficits beyond that of normal aging may be indicative of incipient Alzheimer’s disease (AD). The earliest stages of neurodegeneration occur in olfactory processing areas of the brain. The Apolipoprotein (ApoE) ε4 allele is a genetic risk factor for AD, and is associated with olfactory deficits. Presence of the allele is also associated with a larger decrease in olfaction with age. Event-related potentials can be significantly different based on age and ApoE ε4 status.

OLFACTION

This section is a review of olfactory processes and how they are measured.

Odorants

The study of olfaction is a complex endeavor because odorants themselves are complex. Odorants are most frequently identified by their chemical composition and molecular structure. However, two odors that are chemically and structurally very similar can be perceived as very different odors, while two structurally different odors can be perceived as very similar. Also, many odors are not comprised of a single chemical, but a combination of different odors to create an entirely new one. For example, there are up to 25 primary individual chemicals that create the odor for coffee. It is not perceived as combined individual odors, but rather as one unique odor (Mayer, Czerny, & Grosch, 2000). Alternatively, sometimes combining odors only makes one of them stronger.

Olfactory System

Olfaction is unique from senses such as audition and vision because it involves chemicals directly stimulating the receptor neurons. Odorant molecules in the air are dissolved in the mucus layer of the olfactory epithelium, and then bind directly with the G-protein-coupled-receptors in olfactory receptor neuron (ORN) membranes. ORNs are located
in the olfactory epithelium in the posterior area inside the nostrils. Each ORN responds to specific odorants. They project through the cribriform plate of the skull to the olfactory bulb. Specific ORNs project to groups of neurons called glomeruli in the olfactory bulb (Figure 1). Glomeruli amplify the signal of the particular odorant stimulus by exciting mitral and tufted cells while also inhibiting glomeruli with competing signals through inter-neurons (Christensen, Waldrop, harrow, & Hildabrand, 1993; Distler & Boeckh, 1997). This process makes stimuli more distinct, allowing for an increased ability to detect and discriminate odors (Christensen & Hildebrand, 1987; Laurent, 1999; Mori & Shepherd, 1994). Olfactory bulb neurons project directly to processing areas such as the entorhinal cortex, piriform cortex, olfactory tubercle, and amygdala, in contrast to the typical path of sensory processing through the thalamus prior to higher order processing. Neurons project from the amygdala to the hypothalamus and from the entorhinal cortex to the hippocampus. Many of these connections then converge on the thalamus and frontal cortex (Buck, 2000).

**Figure 1. Diagram of the olfactory system.**

**Measurement**
There are numerous ways of measuring olfaction. The most common types of behavioral test include thresholds, identification, and recognition memory. Different aspects of olfaction can also be tested using brain imaging such as functional Magnetic Resonance Imaging (fMRI), and electrophysiological measures such as Event-Related Potentials (ERPs).
NORMAL AGING

This section is a review of how aspects of olfaction change throughout the lifespan.

Olfactory Processing

A gradual decline in olfactory functioning is typical in normal aging. As many individuals are unaware of their olfactory deficits, the frequency reported in nasal clinics can underrepresent the actual number affected (Murphy et al., 2002). Significant differences in olfactory processing among age groups can be detected using measures such as traditional olfactory behavioral tests, brain imaging, and electrophysiology.

As individuals age, their ability to detect odors declines (Murphy, 1983). Also, with age the decrease in olfactory acuity is significantly related to decline in cognition (Dulay & Murphy, 2002). Research has shown that the ability to discriminate between tastes and between odors also declines with aging, even when higher concentrations are used in order for individuals with decreased detection abilities to detect the stimulus (Kaneda et al., 2000). Research that has implemented measures such as olfactory identification has also linked changes in olfaction to changes in cognition. In older individuals, olfactory identification is statistically significantly related to memory and language (Westervelt, Ruffolo, & Tremont, 2005).

Olfactory Memory

While many processing functions are a part of memory, there are differences in how working memory and long term memory change with age. Typically, odor memory strongly persists over time in young participants, however with age there is a decline in odor memory (Murphy, Cain, Gilmore, & Skinner, 1991; Murphy, Nordin, & Acosta, 1997). While some of these findings are due to deficits in odor memory, there are other factors that can contribute to differences due to aging; such as head trauma, medications, or the type of test used (Elsner, 2001). While some research has found no significant difference in retention of learned odors due to age (Westervelt, Carvalho, & Duff, 2007), other studies have found differences in overall odor memory scores based on age group and sex (Choudhury, Moberg, & Doty, 2003). This effect could be due to the method of measurement, as more recent studies have demonstrated that while item memory for odors can remain intact for older participants when the number of items to be remembered is small, source memory for odors
significantly declines (Gilbert, Pirogovsky, Ferdon, & Murphy, 2006; Herndanez et al., 2008).

**Quality of Life**

The evidence above suggests that older adults can experience a negative impact on quality of life as a result of normal sensory and cognitive decline. As olfactory thresholds increase, there can be a significant decrease in overall health of the participant (Griep et al., 1995). There is a strong decrease in quality of life in elderly groups who self report or are diagnosed with olfactory dysfunction (Miwa et al., 2001). There can also be increased reports of mild depression (Frasnelli & Hummel, 2005). In elderly persons, there are significant differences in quality of life associated with olfactory impairment. For example, safety becomes a concern due to a decreased ability to detect toxins. An additional concern is that social relationships can become impaired due to an inability to be aware of strong body odor.

**APOLIPOPROTEIN**

The Apolipoprotein gene has three common alleles, and has been studied in cognitive and olfactory research.

**Prevalence**

While there are multiple genetic factors that can be associated with Alzheimer’s disease, Apolipoprotein E (ApoE) is by far the strongest genetic risk factor found to date for the sporadic and late-onset familial variants of AD (Combarros, Alvarez-Arcaya, Sanchez-Guerra, Infante, & Berciano, 2002), and as such has been examined extensively. There are three common alleles in the population; nucleotide configurations labeled as epsilon 2, 3, and 4 (ε2, ε3, ε4). Of the alleles, ε2 is the least frequent, and ε4 is found in significantly more individuals with AD or mild cognitive impairment than the other two isoforms (van der Flier et al., 2008; Orsitto, et al., 2006). Carriers of the ε4 allele are not only at risk for the familial variant of AD, but also the sporadic type of AD (Saunders et al., 1993). The increased risk is specifically due to the presence of the ε4 allele (Teter, Raber, Nathan, & Crutcher, 2002).

**Antagonistic Pleiotropy**

ApoE allelic variations and their association with changes in olfactory and cognitive abilities have also been studied. Excluding the presence of dementia, individuals positive for
the ε4 allele have an increased rate of cognitive decline compared to those without the ε4 allele (Small, Basun, & Bäckman, 1998; Swan, Lessov-Schlaggar, Carmelli, Schellenberg, & La Rue, 2005). In non-demented elderly participants, those positive for ApoE ε4 took significantly longer to acquire a conditioned eye-blink response to an olfactory stimulus than ApoE ε4 negatives. This effect was not demonstrated when the conditioned stimulus was auditory (Moore, Bondi, Salmon, & Murphy, 2005). When studying ApoE and cognitive functioning alone, cross sectional studies tend to not detect significant effects; however longitudinal designs do so consistently (Small et al., 2000). As olfactory tests are able to detect these differences in cross sectional and longitudinal designs, they could be a more sensitive measure of ApoE allele effects. A strong predictor of increased rates of cognitive decline is olfactory impairment, especially if the ε4 allele is also present. In fact, tasks requiring odor identification can predict cognitive decline better than global cognitive functioning tests (Graves et al., 1999). Before other cognitive functions such as memory decline, participants positive for the ε4 allele have a significantly worse ability to identify odors compared to those negative for the ε4 allele (Handley, Morrison, Miles, & Bayer, 2006; Murphy, Bacon, Bondi, & Salmon, 1998).

Research suggests that the same allele that impairs older adults could be related to superior cognitive performance in younger adults (Alexander et al., 2007; Mondadori et al., 2006; Puttonen, Elovinio, Kivimäki, Lehtimäki, & Keltikangas-Järvinen, 2003; Scarmeas et al., 2005). However, if there is a period of time where the ε4 allele is beneficial, there is still little empirical evidence demonstrating at what age the effect of the ε4 allele shifts to being detrimental to the individual. The theory of cognitive benefit from the ε4 allele is also weakened by other studies that have demonstrated lower cognitive performance for children from eleven to sixteen years old who have the ApoE ε4 allele and a family history of AD (Bloss, Delis, Salmon, & Bondi, 2008). It seems likely that the influence of the ε4 allele on cognitive performance will depend on the brain areas involved in performing the test and thus may vary with different cognitive functions.

Proposed mechanisms for this effect include benefits during infancy that protect developing neurons (Alexander et al., 2007), increased efficiency in neural networks of young ε4 positives (Mondadori et al., 2006), or that the ε4 allele does not instigate cognitive or olfactory decline, but instead exacerbates an unknown primary underlying disease process.
(Scarmeas et al., 2005). This is supported by research that has found the ApoE ε4 allele is not a direct link or cause for AD, but probably works in conjunction with other genes or biological, environmental, or life style factors with the end result being the AD disease (Strittmatter et al., 1993). This is known as antagonistic pleiotropy.

**ALZHEIMER’S DISEASE**

While diagnoses of Alzheimer's Disease are increasing each year, applying olfaction research has shown benefits in understanding the pathology of this disease.

**Prevalence**

For a growing number of adults, changes in chemosensory and cognitive functioning are greater than what is characterized as a part of normal aging. As longevity increases, the rate of Alzheimer’s disease (AD) continues to increase. As of 2009, the Alzheimer’s Association estimates that AD accounts for 60%-80% of dementia cases. The current estimate for number of Americans with AD is 5.3 million, and is expected to increase to at least 7.7 million by 2030. Also, while deaths attributed to many other causes have been declining, death from AD has been increasing. There are currently no medications or medical programs that significantly slow or stop the disease process.

**Diagnosis**

An official diagnosis of Alzheimer’s disease can only be obtained after post-mortem confirmation that the histological pathology is consistent with AD. The three main histological characteristics of AD neurodegeneration are neurofibrillary tangles (NFTs), β-amyloid plaques, and atrophy. NFTs are formed when tau proteins that bind microtubules to form axons destabilize, causing the cell to break apart (Price, 2000). NFTs are also commonly found in healthy aging brains. However, β-amyloid plaques are much less common in healthy aging (Braak & Braak, 1997). The combination of these three pathological markers, along with the exclusion of other neurodegenerative diseases, is the current method of diagnosing definite AD.

Pre-mortem classifications of diagnosis are labeled as possible and probable AD. Possible or probable AD is evaluated using multiple measures. The diagnostic guidelines for AD were published by the National Institute of Neurological and Communicative Diseases
and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (McKhann et al., 1984), and are currently being revised. The diagnostic criteria are based on the presence of symptoms after ruling out all other causes of dementia. Common symptoms of dementia can include memory deficits, inability to learn new information, language disturbance, inability to perform motor movements (even though the client is physically capable of movement), and the inability to identify objects, plan, organize, sequence, or abstract (DSM IV). The original criteria allowed that neuropsychological tests may be implemented to assist in ruling out alternative explanations for symptoms. Tests to help establish the presence of these symptoms include the Dementia Rating Scale (DRS) and the Mini Mental State Exam (MMSE). However, by the time they detect possible AD there has already been substantial brain damage due to the disease process.

**Early Detection**

There is an increasing body of research focusing on brain imaging as a method of diagnosis. However, differentiating between normal aging and the earliest stages of AD using this method is still expensive and under development (Brewer, Magda, Airriess, & Smith, 2009; Matsuda, 2007; Wang et al., 2003). Additionally, cognitive tests may not be sensitive to the earliest areas damaged in the disease process. Intervention could be improved if begun before extensive brain damage has already occurred, requiring the use of tests that are sensitive to the earliest damage. Before impairment in cognitive function, such as memory, is noticeable there are often olfactory impairments (Serby, 1986). Olfactory thresholds correlate with degree of dementia (Murphy, Gilmore, Seery, Salmon, & Lasker, 1990). There are multiple behavioral tests available to evaluate olfaction, and three types of tests frequently used are thresholds, identification, and recognition memory.

The increased olfactory impairment in Alzheimer’s disease and elderly at risk for AD might be due to physiological changes during early stages of the disease (Braak & Braak, 1997; Getchell, Shah, Buch, Davis, & Getchell, 2003). A seminal article categorizing the physiological changes in Alzheimer’s disease was published in 1997 by Braak and Braak, who demonstrated that olfactory processing areas such as the perirhinal and entorhinal cortex developed β-amyloid plaques and neurofibrillary tangles in the preclinical stages of the disease. In order to test the association of olfactory impairment and neurodegeneration,
behavioral olfactory measures have been compared to changes detected with structural or functional magnetic resonance imaging (MRI or fMRI). In a study by Murphy, Jernigan, & Fennema-Notestine (2003), threshold and identification scores compared to brain volume yielded different results depending on dementia status. The normal control group had lower olfactory scores associated with volume loss in the amygdala; however AD patients demonstrated the association with a different area of volume loss, namely the left hippocampus. Similar results have also been found by Wang et al. (2003), with normal aging characterized by decreased volumes in the head and scattered throughout the body of the hippocampus, however there was heavy deterioration of the CA1 region of the hippocampus found in Alzheimer’s disease patients.

**ALZHEIMER’S DISEASE VERSUS PARKINSON’S DISEASE**

Olfactory threshold, identification, and recognition tests are highly associated with ApoE status as significantly if not better than purely cognitive measures (Calhoun-Haney & Murphy, 2005; Schiffman, Graham, Sattely-Miller, Zervakis, & Welsh-Bohmer, 2002). The effect of olfactory decline in AD has been replicated many times with these tests as well. However Parkinson’s disease (PD) patients show similar deficits (Hawkes, 2003; Mesholam, Moberg, Mahr, & Doty, 1998). These similarities in deficits highlight a weakness in using olfactory measures alone to assess risk or diagnosis of AD (Doty, 2007; Lötsch, Reichmann, & Hummel, 2008). However, the neurodegenerative processes of AD and PD are distinctly different (Braak & Braak, 1997; Braak et al., 2003). Doty (2007) reviewed and compared the patterns of olfactory degeneration differed for AD compared to PD. While amyloid plaques and neurofibrillary tangles have different origins and progression in their pathology (Price, Davis, Morris, & White, 1991), stages of clinical AD tend to be characterized by both (Braak & Braak, 1997). In general, amyloid plaques and neurofibrillary tangles in AD affect areas around the perirhinal and entorhinal cortices prior to detection using traditional cognitive tests in stage 1. They then spread through the hippocampus, then farther out in the cortex (Braak & Braak, 1997) (Figure 2). In PD, the first areas impacted are in the medulla oblongata; then it continues in an anterior direction through the pontine tegmentum, substantia nigra, and in stage 4 reaches the area near the entorhinal cortex and CA2 section of the hippocampus. Over the course of the final two stages of PD lesions proliferate through
Given this difference in progression, one promising assessment technique is the olfactory event related potential (OERP) that may be able to detect more subtle differences between the two diseases compared to traditional olfactory measures. A study conducted by Sakuma, Nakashima, and Takahashi (1996) studied the differences in OERPs among anosmic, AD, and two groups of PD patients, along with a group of control participants. PD patients were separated into a group of people claiming olfactory deficits, and another group claiming no olfactory deficits. The first, second, and third positive peaks (P1, P2, P3) were analyzed and found that all clinical patients had similar significant differences from the control group with longer latencies and lower amplitudes for P1 and P3. For P2, only the PD group with self-reported olfactory deficits was significantly different from controls. While the AD, PD, and anosmic groups were not statistically compared to each other, further research should continue to examine the possible tasks or peaks that might reliably differentiate between Alzheimer’s disease and Parkinson’s disease.

**EVENT-RELATED POTENTIALS**

This section is a review of the application of Event-Related Potentials to olfactory research, and how they are impacted by factors such as age and the ApoE gene.

**Electroencephalography**

The event-related potential (ERP) technique can be implemented using electroencephalogram (EEG) technology. The first demonstration of an electroencephalogram recorded from the human brain was conducted by Hans Berger in 1929 (Berger, 1929). Electroencephalograms measure the electrical activity of the brain. The EEG records post-synaptic potentials on the dendrites of the neurons receiving stimulation from the axons of other neurons. Each neuron has a dipole, or direction of electromagnetic current.

When stimulated by another neuron the dipole shifts as the electrical polarity of the cell shifts. When a group of neurons are stimulated their dipoles shift almost in unison. By shifting almost in unison, the electrical fields across all of the dipoles summate, creating a much stronger signal than any individual cell. This makes the electrical signal strong enough to be picked up by electrode sensors on the scalp and, once amplified, be recorded for analysis (Nunez, 1981). This concept is the basis for the electroencephalogram. Depending
on the research question, specific waves (delta, theta, alpha, beta, gamma, and mu) and time points in relation to the stimulus can be examined.

**Extracting ERP Waveforms**

While the natural EEG rhythms of participants can be appropriate for analysis, it is more common in chemosensory research to investigate the effects specific stimuli have on waves relevant to the processing of that stimulus. When EEG recordings are analyzed at a
specific interval of time surrounding a pre-determined stimulus, it is called an Event Related Potential (ERP). At different times after the stimulus, there are different peaks of electrical activity which correspond to different aspects of processing. The latency and amplitude of the peaks vary based on a number of factors, including the stimulus. Some of the most common peaks examined in ERP research include P1, N1, P2, N2, and P3. P and N correspond to the direction of the peak with respect to the baseline EEG activity (Figure 4). Each of these components occur after peripheral sensory neurons detect, begin processing, and relay information to the central nervous system. As such they reflect the sensory and cognitive processing of stimuli. Cognitive expectations and memories can influence any of these peaks. The time between the stimulus onset and the ERP of interest is the latency. Amplitude is defined as the difference between the peak and baseline activity in strength of the electrical signal being sent. Olfactory event-related potential (OERP) latencies and amplitudes can be used to measure levels of olfactory functioning (Kobal & Hummel, 1991). If adaptation is controlled through longer inter-stimulus intervals, OERPs can be stable enough to obtain sufficient data for analysis with three to twenty trials (Wetter & Murphy, 2003). The waveforms resulting from averaging across trials for each individual are reliable over test sessions (Thesen & Murphy, 2002). This was found for both latencies and amplitudes of positive and negative peaks, although latencies were more reliable. In AD participants, ERP group differences are larger when examining olfaction than audition (Morgan & Murphy, 2002). OERP latencies are equally if not more accurate than traditional olfactory tests in differentiating between AD and normal controls (Morgan & Murphy, 2002).

Components

All ERPs represent sensory and cognitive processing; however different components of the waveform correspond to different aspects of processing. Some components, such as P1, N1, P2, and N2 are frequently labeled exogenous due to their strong association with stimulus qualities. However, individual components are highly variable and do not reflect one single process, but the summation of all processes occurring at a given moment in time. For example, increasing odor intensity results in significantly shorter latencies for P2 and N2 (Covington, Geisler, Polich, & Murphy, 1999). N2 can also be manipulated by varying the
difficulty of detecting the stimulus and classification task (Ritter, Simson, Vaughn, & Macht, 1982). N2 had significantly longer latencies for a more difficult classification task such as an oddball design compared to a single stimulus design. Thus N2 is not processing one specific quality of the stimulus, but is a measurement of the overall processing of stimulus quality happening at that time.

Later components such as the third positive peak (P3) are considered to reflect cognitive processing or decisions based on the stimulus. For example, probability influences P3 amplitude when the stimulus is task-relevant (Donchin, 1981; Sutton, Tueting, Zubin, & John, 1967; Tueting, Sutton, & Zubin, 1971). Rather than measuring attention in general, P3 reflects updating the context of the situation or schema. In their review, Donchin and Coles (1988) do not consider P300 to be informing the individual of the current stimulus per se, but instead of whether or not that stimulus environment will change future responses. This requires processing of the current stimulus compared to memory schema. Depending on the task, different memory systems are used leading to different waveform permutations (Polich, 2007). Different characteristic P3’s are elicited by paradigms that test different forms of memory; however these are all summations of the same stage of processing (Polich, 2007). Another review by Katada, Sato, Ojika, and Ueda (2004) supports the theory that P3 is
related to cognition by demonstrating similar findings across studies varying in modality and paradigm.

**Aging**

As OERPs reflect cognitive processing of olfactory stimuli, cognitive changes can be studied using this method of assessment in addition to traditional olfactory measures. Changes in cognition and olfaction occur as a normal part of aging. When comparing groups of older participants to young participants, significant group differences are not always found for all OERP components. Across studies examining the N1 OERP component, group differences in amplitudes and latencies are just as often significantly different as they are not (Evans, Cui, & Starr, 1995; Hummel, Barz, Pauli, & Kobal, 1998; Morgan, Covington, Geisler, Polich, & Murphy, 1997; Morgan, Geisler, Covington, Polich, & Murphy, 1999; Murphy et al., 2000; Murphy, Nordin, de Wijk, Cain, & Polich, 1994; Thesen & Murphy, 2001). Latencies and amplitudes for P2 are significantly different based on age group more often than not (Evans et al., 1995; Hummel, Barz, Pauli, & Kobal, 1998; Morgan et al., 1997; Morgan et al., 1999; Murphy et al., 1994; Murphy et al., 2000; Thesen & Murphy, 2001). N2 has rarely been studied in the context of OERPs and aging, however the few studies that did examine them consistently found significant differences for latencies and amplitudes (Evans et al., 1995; Morgan et al., 1997; Morgan, Geisler, Covington, Polich, & Murphy, 1999). The P3 component has been studied with olfactory changes with aging more than N2, but not as frequently as N1 and P2. P3 is consistently significantly different between young and old participants (Geisler, et al., 1999; Morgan et al., 1999; Murphy et al., 2000; Thesen & Murphy, 2001). Significant differences in P3 latency were due to the older group having longer latencies and decreased amplitudes. While N1 and P2 have been researched more thus far compared to N2 and P3, the latter two components might be a more reliable and stable measure of cognitive changes in older age groups.

**Apolipoprotein**

Another factor that influences OERPs and would increase their utility even further is ApoE ε4 status. When examining multiple risk factors for Alzheimer’s disease such as family history along with ApoE, there are significant differences in N2 and P3 latency (Green & Levey, 1999). Increased N2 and P3 latencies were more pronounced for individuals with
increased risk through both family history and ApoE ε4 status than participants with only one risk factor. This suggests that both family history and ApoE ε4 status contribute to some of the OERP variation independently, but can also combine to have an even stronger effect on the latency of N2 and P3. In non-demented older adults, ApoE ε4 positive status is associated with significantly longer latencies of N1, P2, N2, and P3 (Wetter & Murphy, 2001). The OERP was more sensitive than the traditional olfactory behavioral measures, and had higher specificity than the auditory ERP. Thus when comparing the three types of assessment, OERPs were more likely to detect differences in olfaction than the olfactory behavioral measures and were better at discriminating ApoE ε4 positives from negatives than the auditory ERPs. As ε4 status itself is an AD risk factor and OERPs can detect it, they have the potential to increase risk assessment accuracy much earlier than symptoms of cognitive decline become noticeable. Additional research needs to be conducted to further establish this as a reliable method of measuring likelihood of developing olfactory and cognitive impairment, as well as to determine if early intervention results might be monitored using electrophysiology.

**ADDITIONAL INFLUENCES ON EVENT-RELATED POTENTIALS**

As promising as research has been regarding OERP components measuring AD risk and pathology, there are other characteristics of the participants and paradigms that can influence latency and amplitude and should be taken into account.

**Subject Effects**

Gender consistently affects visual latencies and amplitudes. There are also significant gender effects in olfactory ERP amplitudes. Males typically have longer latencies and lower amplitudes than females across multiple ERP components (Conroy & Polich, 2007; Hoffman & Polich, 1999; Morgan et al., 1997; Steffensen et al., 2008). Also, males tend to experience olfactory decline earlier and at an increased rate compared to females (Evans et al., 1995; Morgan et al., 1997). Similar gender differences also occur in behavioral measures of cognition (Swan et al., 2005). Handedness is also a factor when studying ERPs, as left hand dominant participants tend to have significantly larger amplitudes and significantly shorter latencies, but more commonly for the P3 component than earlier ones (Alexander & Polich,
1997; Hoffman & Polich 1999; Polich & Hoffman, 1998b). Some of this effect may be due to the different distributions of anterior-to-posterior activation patterns between left and right hand dominant participants (Alexander & Polich, 1997).

**ERP Effects**

Increased odor intensity can result in a decrease in OERP latency, and to a lesser extent increase in amplitude (Covington et al., 1999; Tateyama, Hummel, Roscher, Post, & Kobal, 1998). Kobal, Hummel, and Van Toller (1992) found significant differences in ERP latencies and amplitudes when they compared birhinal, right nostril only, and left nostril only administration of the olfactory stimulus. This is possibly due to the lateralization of information across the two hemispheres. Task characteristics such as difficulty can influence ERPs as well (Hagen, Gatherwright, Lopez, & Polich, 2006; Ritter et al., 1982). For example, the presence and shape of P3 subcomponents a and b are influenced by the difficulty of the paradigm, with larger separation in P3a and P3b from oddball paradigms (Conroy & Polich, 2007; Katayama & Polich, 1999; Polich, 2007). A P3 with merged subcomponents can be obtained, measured, and studied in relation to cognition with other paradigms, especially if analyzing the subcomponents is not relevant to the theory (Hoffman & Polich, 1999; Wetter & Murphy, 2003; Wetter, Polich, & Murphy, 2004; Wronka, Kaiser, & Coenen, 2008).

**Topographical Analyses In ERP**

An increasing amount of research is being dedicated to utilizing topographical analyses to assess differences in brain activity. However, there are mathematical challenges in using ERPs as a method of studying topography. This is because for each scalp electrode there is an infinite number of combinations of neurons, or generators, that can be firing at any given pattern to produce a waveform. Currently, there is no mathematical way to solve the problem of infinite generators. However, fMRI research can serve as a comparison of where activity is generated given a broad enough area definition in the ERP data. Such comparisons have been successfully made in visual and auditory oddball stimulus paradigms (Eichele et al., 2005; Minati et al., 2008). While studies such as these have demonstrated a direct link between the topographical distributions found in ERP and fMRI, few studies have examined olfactory topography.
One study of olfactory ERPs and differences in topographical distributions found differences based on the ApoE ε4 allele (Murphy, Solomon, Haase, Wang, & Morgan, 2009). Non-demented older participants were administered an odor memory task by learning odors and later identifying the name of the odor from a list that included non-target words. The ERP topographical distributions were then compared among response patterns and ApoE ε4 status using an Intra-Class Correlation (ICC). ApoE ε4 positive participants had different patterns of ERP amplitude topography compared to their negative counterparts.

Studying OERP topography differences throughout the lifespan would be an interesting addition to the established literature, as it would further elucidate possible mechanisms of group differences in sensory processing due to age and the ApoE ε4.

**SUMMARY**

Olfaction changes throughout the lifespan. There are behavioral and electrophysiological methods of measuring changes in olfaction. As a pronounced decline in olfaction can be indicative of incipient AD, these methods can be implemented to assess risk or to assist in early diagnosis. However, ERPs can be influenced by more than age and AD-related deterioration. The presence of the ApoE ε4 allele, gender, and stimulus quality can also impact the latency and amplitude of an ERP. The goal of this study was to add to the understanding of why ERPs vary in normal aging.

**HYPOTHESES**

A significant difference in behavioral tests was not expected, due to participants being screened for cognitive and olfactory functioning. While there is some decline expected with aging, there is a reduced likelihood of such differences being significant because the screening decreases the likelihood of poor threshold, cognitive deficits, or early stages of dementia contributing to OERP differences. A lack of significant differences or smaller effect sizes among groups in these tests could demonstrate the possible increased ability for OERPs to detect more subtle differences associated with ApoE ε4 status.

OERPs were expected to demonstrate significantly longer latencies as age increases. ApoE ε4 positive participants were expected to have significantly longer OERP latencies throughout the lifespan, however this difference was expected to increase with age.
Visual ERP latencies were expected to significantly increase with age, but not differ between ApoE ε4 status or their interaction.

As the earliest stages of AD affect cognitive processing of olfactory stimuli, it was expected that for the young age group P3 would be the most affected by ApoE ε4 status, however more peaks were expected to contribute to discriminating between ApoE ε4 status for the older age group.

OERP amplitudes were expected to significantly decrease with age, with P3 more affected than the other peaks. It was also expected that the ApoE ε4 positive participants would have significantly lower amplitudes than ApoE ε4 negative participants. Both ε4 positives and negatives were expected to have decreased amplitudes with age.

Visual ERP amplitudes were expected to be significantly different based on age, however there were no expected significant differences based on ApoE ε4 status or their interaction.

Topographical differences based on age and ApoE ε4 allele status were expected. While there was an expected shift in activity in the older adults, the effects of the ApoE ε4 allele might lead to even more of a change in distributions of activity, including changes in younger adults, instead of a general decrease in activity associated with increased age and ε4 allele status.
CHAPTER 2

METHODS

PARTICIPANTS

Sixty male and female participants were tested. They were equally stratified across three different age groups of young (18-28 years old), middle age (45-56 years old), and older adults (65 and older years old). Each group contained an equal number of ApoE ε4 positive and negative individuals. Participants were recruited from the San Diego community. Exclusionary criteria for this study included butanol thresholds below four and a DRS score below 129. Participants were screened for overall nasal health, and any individuals with recent head trauma, severe allergies, upper respiratory infection, or those taking antipsychotic medications were not included.

PROCEDURE

The testing for this study was conducted in two sessions.

First Session

Chemosensory tests included the butanol odor threshold, the San Diego Odor Identification test (SDOIT), the Alcohol Sniff Test (AST), and sucrose taste threshold. The butanol thresholds were obtained using a forced-choice ascending method of limits. Two alternatives were presented in two plastic squeeze bottles with one containing the butanol odor, and the other a blank (water only). The criterion for threshold was five correct responses in a row (Cain, Gent, Catalanotto, & Goodspeed, 1983; Murphy et al., 1990). The SDOIT includes common household odors such as chocolate and peanut butter administered in opaque containers. Odors were identified using an array of pictures provided that included all of the stimuli as well as distractors (Murphy, Anderson, & Markison, 1994). For the AST, an alcohol pad was held 30 cm from the nares and was moved 1 cm closer each time the participant exhaled until detected (Davidson & Murphy, 1997). The sucrose threshold was a forced-choice, two-alternative staircase method (Murphy & Gilmore, 1989; Murphy et al.,
1990). Memory and cognitive functioning tests included the Mini-Mental State Exam (MMSE) (Folstein, Folstein, & McHugh, 1975) and the Dementia Rating Scale (DRS) (Mattis, 1976).

**Second Session**

Event-related potentials were recorded in the second session. The stimuli, stimulus presentation, and data acquisition are described in the following.

**Stimuli**

For the visual task, a blue rectangle appeared on a computer screen for 200 milliseconds. The olfactory stimulus was amyl acetate presented for 200 milliseconds.

**Olfactometer Apparatus**

Olfactory event related potentials were evoked using stimuli delivered by an olfactometer built combining the designs of Kobal & Hummel (1991) and Lorig, Elmes, Zald, & Pardo (1999) (Morgan et al., 1997; Murphy et al., 1994). The olfactometer intakes the ambient air. One Teflon line is attached to the air compressor at one end and the air passes through a humidifier. The line then diverges to two, with flow meters attached to monitor air pressure. The first line is the secondary clean air line that constantly runs a lower pressure of clean air and goes straight to the manifold. This line overshadows changes in pressure or sound associated with alternating the other air line between clean air and odors. The second line is connected to the solenoid assembly. One valve normally remains open to send clean air to the manifold, but closes when the computer controlling the stimulus timing gives an electrical signal. When the clean air solenoid closes the air is instantaneously redirected through one of the other solenoid valves. The stimulus computer, through sending an electrical signal between one and sixteen bits, triggers the solenoid corresponding to the signal connected to the assembly. The redirected air passes to an odor-impregnated filter attached to the manifold on the back of the testing chair. Each filter has a ball that restricts air flow, preventing odor from leaking into the manifold between stimuli. With adequate air pressure through the filter, the ball is removed from the opening, and the odorous air passes through the manifold directly into the participant’s nose via cannula.
ERP Procedure

Sensory modality was counterbalanced across participants by alternating the order of presentation between olfaction and visual tasks. The directions indicated that the participant needed to press a specific button as soon as he or she detected the stimulus. Stimuli were presented twenty times with an inter-stimulus interval of thirty seconds.

ERP Recording and Processing

The scalp recordings were collected through a dense array of 61 electrodes built into a spandex cap following the 10-20 system (Figure 5). All electrodes had an impedance of 10 Ω or less. Ocular artifacts were recorded with two electrodes placed supra- and subocularly at the left eye, and one electrode at each outer canthus. Electrodes were also placed on both earlobes. The electrodes were connected to a computer running the Neuroscan recording system, that was synchronized with the computer controlling the stimulus presentation.

Figure 5. Diagram of 10-20 electrode placement system. Triangle at the top indicates subject's nose.

During data collection, a filter of DC to 32 Hz was used. After the recording session, the Neuroscan system was used for data processing. Data were re-referenced to the two earlobe electrodes. A band pass filter was applied to the data with a high of 0.1 and a low of 9. This removed frequencies below 0.1 Hz and above 9 Hz. Using a Neuroscan algorithm
calculated from the four ocular electrodes, ocular artifacts were subtracted from the other electrode channels. Continuous recordings were epoched 500 ms before to 1500 ms after the stimulus. Trials were rejected based on artifacts that were still present after filtering, epoching, and baseline correcting the data. There was also an additional restriction that the participant must have responded within a particular latency window appropriate for the task and age group. Accepted trials were averaged for each participant, and peaks were chosen based on these individual averages compared to their group averages. Individual and group averages were obtained for each task.

**Data Analysis**

Demographics were analyzed using a chi square test to assess whether ethnicity, and handedness were significantly different among age groups and ApoE ε4 status. Education was tested using an Analysis of Variance (ANOVA).

Behavioral measures included the MMSE, DRS, SDOIT, AST, Butanol Threshold, and Sucrose Threshold. Within subjects ERP measures included N1, P2, N2, and P3 latencies and amplitudes for both visual and olfactory modalities. Between subject variables included Age Group and ApoE ε4 status.

A series of ANOVAs tested each measure for differences in age group and ApoE ε4 status. Correlated subgroups were tested using a MANOVA. The independent variables were age group and ApoE ε4 status.

Next, a set of repeated measures MANOVAs tested the olfactory and visual ERP latencies for each peak. Within each peak, the three electrode sites analyzed were Fz, Cz, and Pz. The same process was repeated for another set of MANOVAS, substituting amplitude measures for latency.

New variables were computed for the peak to peak amplitudes between the N1 and P2 peaks, then the N1 and P3 peaks for the Fz, Cz, and Pz electrode sites. Separate repeated measures MANOVAs were then conducted on the N1P2 and N1P3 variables, with electrode site as the repeated measure.

A forward stepwise binomial logistic regression was conducted including the variables N1, P2, N2, P3 latencies, and N1, P2, N2, P3 amplitudes averaged over the three electrode sites of Fz, Cz, and Pz to obtain sensitivity and specificity measures.
Topographical distributions of the different groups were examined by comparing the Intra-Class Correlation between each pair of groups substantively.
CHAPTER 3

RESULTS

DEMOGRAPHICS

Ethnicity was significantly different among the study groups, $\chi^2(4, 60)=108.5$, $p<.001$. There was a significant difference of handedness, $\chi^2(2, 60)=78.45$, $p<.001$. Education levels were also significantly different by ApoE $\varepsilon$4 status ($F(1,51)=5.210$, $p=.027$). The average number of years smoked was significant effects of age group ($F(2,53)=9.057$, $p<.001$) and the interaction between age and ApoE status ($F(2,53)=3.482$, $p=.038$). Older participants smoked for significantly more years than younger ($p<.001$) and middle age ($p=.009$) participants. Young and older participants had no significant differences between ApoE status for how many years they smoked, however there was a significant difference for the middle age group ($F(1,18)=7.939$, $p=.011$). When examined as current smoker versus non-current, there was no significant difference, $\chi^2(1, 60)=.067$, $p>.05$. For a demographics summary by group, see Table 1.

BEHAVIORAL TESTS

Behavioral measures included the MMSE, DRS, SDOIT, AST, Butanol Threshold, and Sucrose Threshold. Between subject variables included Age Group and ApoE $\varepsilon$4 status. While all participants were thoroughly screened for high olfactory and cognitive functioning, there were still significant differences among groups (Table 2). Test scores that differed significantly based on age group included the MMSE ($F(2,43)=3.415$, $p=.044$), odor threshold ($F(2,43)=8.383$, $p=.001$), AST ($F(2,43)=4.944$, $p=.013$), and DRS ($F(2,43)=5.099$, $p=.011$). Post hoc tests revealed that for the MMSE, the young group had significantly better scores than the middle age group ($p=.019$). For the olfactory threshold, older participants had significantly poorer scores than the younger ($p=.001$) and middle age groups ($p=.003$). The middle age group had significantly better AST scores than the older ($p=.004$). For the DRS, the middle age group had significantly poorer scores than the young ($p=.008$) and older groups ($p=.020$). Taste thresholds were significantly poorer for ApoE $\varepsilon$4 negatives than
Table 1. Demographics

<table>
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<th>Young Negative</th>
<th>Young Positive</th>
<th>Middle Negative</th>
<th>Middle Positive</th>
<th>Older Negative</th>
<th>Older Positive</th>
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<td>51</td>
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<td>69</td>
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<td>Education (mean years)</td>
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<td>14</td>
<td>15</td>
<td>17</td>
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Table 2. Mean Behavioral Measures

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<th>Middle Negative</th>
<th>Middle Positive</th>
<th>Older Negative</th>
<th>Older Positive</th>
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<td>MMSE</td>
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<td>28</td>
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<tr>
<td>Butanol Threshold</td>
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<td>6</td>
<td>8</td>
<td>6</td>
<td>5</td>
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<tr>
<td>Taste Threshold</td>
<td>0.006</td>
<td>0.002</td>
<td>0.015</td>
<td>0.002</td>
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<td>SDOIT (corrected)</td>
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<td>6</td>
<td>5</td>
<td>5</td>
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<td>4</td>
</tr>
<tr>
<td>AST</td>
<td>23.37</td>
<td>25.02</td>
<td>27.8</td>
<td>26.83</td>
<td>22.04</td>
<td>18.61</td>
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<td>DRS</td>
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<td>141</td>
<td>136</td>
<td>139</td>
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</table>

positives (F(1,43)=6.241, p=.017). The behavioral test that was significantly different for the interaction between age and ApoE ε4 status was the odor threshold (F(2,43)=4.097, p=.025). ApoE ε4 negative participants had a significant effect of age (F(2,27)=5.028, p=.014). The young group had significantly better olfactory thresholds than the middle age (p=.014) and older groups (p=.008). For ApoE ε4 positive participants also had a significant effect of age.
(F(2,27)=10.561, p<.001). The older age group had significantly poorer olfactory thresholds than the young (p<.001) and middle age groups (p=.001).

**Olfactory Latency**

For each peak, the three electrode sites analyzed were Fz, Cz, and Pz. The Greenhouse-Geisser correction was used as the repeated measures omnibus test statistic.

For the olfactory task N1 peak latency, there was not a significant effect of electrode site, all p's >.05. For the P2 peak, there was also not a significant effect of electrode site, all p's >.05. Only the N2 peak had a significant interaction of electrode site and age group, F(2.79, 75.49)=4.108, p=.011, partial $\eta^2 = .132$, all other p's >.05. Post hoc tests revealed there were no significant differences among electrode sites within each age group (all p's>.05).

However, there were significant effects of age within Fz, F(2,57)=8.35, p=.001, Cz, F(2,57)=9.34, p<.001, and Pz, F(2,57)=11.095, p<.001.

The N1, P2, and N2 peaks all had significant effects of age, ApoE ε4 status, and their interaction, and P3 had significant differences in age and ApoE ε4 status (Table 3). For the olfactory task N1 peak latency, there were significant main effects of age group, F(2,54)=15.018, p<.001, partial $\eta^2 = .357$, and ApoE ε4 status F(1,54)=52.976, p<.001, partial $\eta^2 =.495$. There was also a significant interaction between the two F(2,54)=18.779, p<.001, partial $\eta^2 =.410$. For the P2 peak, there were significant main effects of age group, F(2,54)=12.797, p<.001, partial $\eta^2 = .322$, and ApoE ε4 status F(1,54)=26.772, p<.001, partial $\eta^2 =.331$. There was also a significant interaction between the two F(2,54)=5.709, p=.006, partial $\eta^2 = .175$. The N2 peak had significant main effects of age group, F(2,54)=14.419, p<.001, partial $\eta^2 = .348$, and ApoE ε4 status F(1,54)=21.344, p<.001, partial $\eta^2 =.283$. There was also a significant interaction between the two F(2,54)=4.896, p=.011, partial $\eta^2 = .154$. For the P3 peak, there were significant main effects of age group, F(2,54)=27.609, p<.001, partial $\eta^2 = .506$, and ApoE ε4 status F(1,54)=65.007, p<.001, partial $\eta^2 = .546$. However, there was only a marginally significant interaction between the two F(2,54)=3.107, p=.053, partial $\eta^2 =.103$. The post hoc tests for P3 showed that comparing age groups, the older age group had significantly longer latencies than the young (p<.001) and middle (p<.001) groups. Furthermore, the middle age group had significantly
Table 3. Olfactory Latency Omnibus Between Effects

<table>
<thead>
<tr>
<th>Factor</th>
<th>N1</th>
<th>P2</th>
<th>N2</th>
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<td>&lt;.001</td>
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<td></td>
<td>.357</td>
<td>.322</td>
<td>.348</td>
<td>.506</td>
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<tr>
<td>Apoe e4 Status</td>
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<td>&lt;.001</td>
<td>&lt;.001</td>
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<td></td>
<td>.495</td>
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<tr>
<td></td>
<td>.410</td>
<td>.175</td>
<td>.154</td>
<td>.103</td>
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</tbody>
</table>

P and partial eta² values shown.

longer P3 latencies than the young (p=.04). Participants positive for the ApoE ε4 allele had significantly longer P3 latencies than those negative for the allele.

In order to further understand the significant interactions, ApoE ε4 status was examined for each level of age group, then age groups were compared within each level of ApoE ε4 status (Table 4). Electrode site was not significant for any of the following analyses. Within the young participants, N1, P2, and N2 had no significant effects of ApoE ε4 status. However, there was a significant effect for P3 F(1,18)=8.032, p=.011, partial η²=.309. Middle aged participants had no significant ApoE ε4 effect for P2. The other three peaks were significant: N1, F(1,18)=7.176, p=.015, partial η²=.285, N2, F(1,18)=5.034, p=.038, partial η²=.219, P3, F(1,18)=15.929, p=.001, partial η²=.469. For older participants, N1 (F(1,18)=122.425, p<.001, partial η²=.872), P2 (F(1,18)=34.192, p<.001, partial η²=.655), N2 (F(1,18)=29.044, p<.001, partial η²=.617), and P3 (F(1,18)=77.05, p<.001, partial η²=.811) were significant.

The simple effects of age group at each level of ApoE ε4 status were then examined. Electrode site was not significant in any of these analyses as well. For participants negative for the ApoE ε4 allele, only P3 had a significant effect of age, F(2,27)=5.396, p=.011, partial η²=.286. Participants positive for the ApoE ε4 allele had significant effects of age group for all 4 peaks: N1 (F(2,27)=24.005, p<.001, partial η²=.640), P2 (F(2,27)=14.813, p<.001, partial η²=.523), N2 (F(2,27)=17.498, p<.001, partial η²=.564), and P3 (F(2,27)=29.152, p<.001, partial η²=.683) were significant. Young versus middle age latency pairwise comparisons were significant for the N1 (p=.018) and N2 (p=.031) peaks. Young versus
Table 4. Olfactory Latency Simple Effects

<table>
<thead>
<tr>
<th>Factor</th>
<th>N1</th>
<th>P2</th>
<th>N2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young(^a)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>.011</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.309</td>
</tr>
<tr>
<td>Middle(^a)</td>
<td>.015</td>
<td>-</td>
<td>.038</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>.285</td>
<td>.219</td>
<td>.469</td>
<td></td>
</tr>
<tr>
<td>Older(^a)</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>.872</td>
<td>.655</td>
<td>.617</td>
<td>.811</td>
</tr>
<tr>
<td>Negative(^b)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>.011</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.286</td>
</tr>
<tr>
<td>Positive(^b)</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>.640</td>
<td>.523</td>
<td>.564</td>
<td>.683</td>
</tr>
</tbody>
</table>

\(^a\)Simple Effect of Apo e4 Status  
\(^b\)Simple Effect of Age Group

older and middle age versus older pairwise comparisons were significant across all four peaks (all p's ≤ .001).

**VISUAL LATENCY**

Visual latencies had no significant effects of electrode site for any of the four peaks. There was a significant effect of age for the N1, F(2,54)=5.386, p=.007, partial \( \eta^2 = .166 \), P2, F(2,54)=14.711, p<.001, partial \( \eta^2 = .353 \), N2, F(2,54)=18.326, p<.001, partial \( \eta^2 = .404 \), and P3 peaks, F(2,54)=17.394, p<.001, partial \( \eta^2 = .392 \). There were no effects of ApoE or the interaction between the two. Post hoc tests revealed significant differences among the young age group compared to the other two for most peaks (Table 5).

Table 5. Visual Latency Post Hoc Tests

<table>
<thead>
<tr>
<th>Peak</th>
<th>Young-Middle</th>
<th>Young-Older</th>
<th>Middle-Older</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>-</td>
<td>0.002</td>
<td>-</td>
</tr>
<tr>
<td>P2</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>-</td>
</tr>
<tr>
<td>N2</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>-</td>
</tr>
<tr>
<td>P3</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>-</td>
</tr>
</tbody>
</table>
SENSITIVITY AND SPECIFICITY

In order to better understand the strength of the ApoE status effect, forward stepwise binomial logistic regression analyses were performed using the olfactory N1, P2, N2, P3 latencies and amplitudes averaged over the three electrode sites of Fz, Cz, and Pz.

An omnibus test that combined all age groups had an overall classification rate for P3 latency of 78.3% (Sensitivity=83.3%, Specificity=73.3%). For the young age group, P3 was the most predictive of ApoE ε4 status with an overall classification rate of 75% (Sensitivity=70%, Specificity=80%). The middle age group also had P3 resulting in an overall classification rate of 80% (Sensitivity=80%, Specificity=80%). In the older age group N1 latency was the most predictive, with an overall classification rate of 100% (Sensitivity=100%, Specificity=100%). Therefore the peak that best discriminated the ApoE ε4 positives from ε4 negatives was the P3 cognitive component for the young and middle aged adults, however for the older adults it was the sensory N1 peak.

OLFACTORY AMPLITUDE

Olfactory ERP amplitudes for the three groups did not significantly differ by electrode site for any peaks. There were also no between-subjects effects for the N1 and P2 peaks.

However, for N2 there was a significant interaction of age and ApoE status, F(2,54)=3.49, p=.03, partial η² =.11. The simple effects within this interaction were then tested. There was no significant difference in N2 amplitude as a function of ApoE ε4 status for any of the age groups when examined individually (all p's>.05). ApoE ε4 negative participants had no significant differences in amplitude among age groups. There were significant olfactory N2 amplitude differences in the ApoE ε4 positive participants among age groups, F(2,27)=4.55, p=.02, partial η² =.25. Post hoc tests of the age variable demonstrated significant differences between the young and middle age groups (p=.03), young and older age groups (p=.008), however there were no significant differences in amplitude between the middle age and older groups (p>.05).

There was also a significant effect of age for the P3 peak, F(2,54)=9.37, p<.001, partial η² =.26. Post hoc tests demonstrated a significant difference between the young and
middle age groups (p=.002), young and older groups (p<.001), however there was no significant difference between the middle age and older groups (p>.05).

N1P2 had a significant interaction between electrode site and ApoE ε4 status, F(2,103)=3.55, p=.03, partial η² = .06. There was also a significant effect of the interaction between age group and ApoE ε4 status, F(2, 54)=5, p=.01, partial η² = .16. Post hoc tests found no significant differences for the electrode by ApoE ε4 status effect. The simple effects within the age by ApoE ε4 status interaction were then explored. There were no significant differences among age groups when the ApoE ε4 negatives and positives were examined separately (all p's>.05). The young and older age groups had no significant differences between ApoE ε4 status (all p's >.05). However, in the middle age group N1P2 peak to peak amplitudes were significantly larger in participants negative for the ApoE ε4 allele compared to positives, F(1, 18)=6.84, p=.01, partial η² = .28.

N1P3 had a significant effect of age group, F(2, 54)=7.71, p=.001, partial η² = .22. The post hoc tests demonstrated a significantly greater amplitude difference between the young and middle age groups (p<.002), young and older age groups (p=.001), but not between the middle and older age groups (p>.05).

**Visual Amplitude**

Visual ERP amplitudes showed no significant differences for the P2 peak, and no significant interactions for the N2 peak. For the N1 peak, there was a significant interaction between electrode and age, F(2,74)=2.89, p=.04, partial η² = .09, and a significant interaction between age group and ApoE ε4 status, F(2,54)=3.46, p=.03, partial η² = .11. For the interaction between electrode and age, there was only a significant difference of age for the Fz electrode site, F(2,57)=3.85, p=.02, where the young group had significantly larger N1 amplitudes than the middle age group (p=.01) and older age group (p=.02). For the interaction between age group and ApoE ε4 status, the only significant simple effect was a significant difference in visual amplitudes by age group within the ApoE ε4 negative participants, F(2,27)=4.13, p=.02, partial η² = .23. Post hoc tests showed the only significant difference in age group to be between the young and middle age groups (p<.01). N2 had a significant effect of electrode site, F(1, 90)=8.42, p=.001, partial η² = .13. Fz had significantly larger amplitudes than Cz and Pz. The P3 peak had a significant effect of electrode, F(1,
96)=6.71, p=.003, partial $\eta^2 = .11$, age, F(2,54)=3.34, p=.04, partial $\eta^2 = .11$. Post hoc analyses demonstrated a significant difference between young and middle age groups (p=.01), but not between any other group combinations (all p's >.05).

New variables were also computed for the visual peak to peak amplitudes between the N1 and P2 peaks, then the N1 and P3 peaks for the Fz, Cz, and Pz electrode sites. Separate repeated measures MANOVAs were then conducted on the visual N1P2 and N1P3 variables, with electrode site as the repeated measure.

N1P2 had a significant effect of the interaction between electrode and age group, F(2, 54)=5, p=.01, partial $\eta^2 = .16$. However, all post hoc tests resulted in non-significance.

N1P3 had a significant effect of electrode, F(2,103)=6.11, p=.004, partial $\eta^2 = .10$, age group, F(2, 54)=7.71, p=.001, partial $\eta^2 = .22$. Post hoc tests for age demonstrated a significant difference between the young and middle age groups (p<.001), young and older age groups (p=.001), but not between the middle and older age groups (p>.05).

**TOPOGRAPHY**

In order to further explore the differences in amplitudes, topographical distributions were compared using the Intra-Class Correlation function in Neuroscan. All 64 electrodes were compared between ApoE $\varepsilon$4 positives and negatives within each age group to measure the degree of similarity among the waveforms. The time window specified for the olfactory task was 400-1500ms for all age groups, and 0-1500ms for the visual task. While all pairs of waveforms were highly correlated, the patterns of what areas were correlated were different depending on the task. For the visual task, the young ApoE $\varepsilon$4 positives and negatives had a correlation of 0.755, the middle age was 0.668, and the older was 0.774. For the olfactory task however, the correlations were 0.708 for the young, 0.296 for the middle age, and 0.740 for the older. Correlations were also also high within ApoE $\varepsilon$4 allele groups when each pair of age groups were compared (Tables 6 and 7).
### Table 6. Olfactory Intra-Class Correlations

<table>
<thead>
<tr>
<th></th>
<th>Young ε4 Positive</th>
<th>Middle ε4 Positive</th>
<th>Middle ε4 Negative</th>
<th>Older ε4 Positive</th>
<th>Older ε4 Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young ε4 Negative</td>
<td>0.708</td>
<td>-</td>
<td>-</td>
<td>0.647</td>
<td>-</td>
</tr>
<tr>
<td>Young ε4 Positive</td>
<td>-</td>
<td>0.515</td>
<td>-</td>
<td>-</td>
<td>0.557</td>
</tr>
<tr>
<td>Middle ε4 Negative</td>
<td>-</td>
<td>0.296</td>
<td>0.592</td>
<td>-</td>
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</tr>
<tr>
<td>Middle ε4 Positive</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.440</td>
</tr>
<tr>
<td>Older ε4 Negative</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.74</td>
</tr>
</tbody>
</table>

### Table 7. Visual Intra-Class Correlations

<table>
<thead>
<tr>
<th></th>
<th>Young ε4 Positive</th>
<th>Middle ε4 Positive</th>
<th>Middle ε4 Negative</th>
<th>Older ε4 Positive</th>
<th>Older ε4 Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young ε4 Negative</td>
<td>0.755</td>
<td>0.714</td>
<td>-</td>
<td>0.702</td>
<td>-</td>
</tr>
<tr>
<td>Young ε4 Positive</td>
<td>-</td>
<td>-</td>
<td>0.891</td>
<td>-</td>
<td>0.770</td>
</tr>
<tr>
<td>Middle ε4 Negative</td>
<td>-</td>
<td>-</td>
<td>0.668</td>
<td>0.687</td>
<td>-</td>
</tr>
<tr>
<td>Middle ε4 Positive</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.744</td>
</tr>
<tr>
<td>Older ε4 Negative</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.77</td>
</tr>
</tbody>
</table>
CHAPTER 4

DISCUSSION

BEHAVIORAL TESTS

While there were significant differences in the behavioral measures based on age, all scores were all in the range of normal olfactory and cognitive function and as such were not clinically significant. The lack of significant differences in behavioral measures except for the olfactory threshold across ApoE ε4 status and the interaction between the two variables was expected. Significant differences in olfactory measures among ApoE ε4 groups and age are less frequent in cross-sectional studies, however longitudinal studies have been shown to be more reliable (Small et al., 2000).

OLFACTORY LATENCY

Olfactory latencies increased with age. The increase in latency was much more pronounced for participants positive for the ApoE ε4 allele (Figure 6). These results are in agreement with findings from previous research of olfactory and cognitive processing. In non-demented elderly participants, those positive for ApoE ε4 took significantly longer to acquire a conditioned eye-blink response to an olfactory stimulus than ApoE ε4 negatives. This effect was not demonstrated when the conditioned stimulus was auditory (Moore et al., 2005). The differences in young, middle age, and older adults were of particular interest, as each age group was impacted by the ApoE ε4 allele. While age has been a significant factor for all four peaks in previous studies, many of these studies compared the differences between young and older participants only (Geisler, Morgan, Covington, & Murphy, 1999; Morgan et al., 1997; Morgan et al., 1999; Thesen & Murphy, 2001). Other studies have found age differences while including middle aged participants, however unless broken into enough groups to treat age as a continuous variable (Murphy et al., 2000), these studies only found differences between the young and older or young and middle age groups (Evans et al., 1995; Hummel, Barz, Pauli, & Kobal, 1998). The finding of olfactory latencies being significantly different for the middle age group is a novel contribution to
Figure 6. Differences in ERP latency due to age and ApoE ε4 status.
understanding olfactory processing and age. Comparisons of ApoE ε4 status have even more limited age groups examined (Green & Levey, 1999; Murphy et al., 2009; Wetter & Murphy, 2001). Thus the current results of consistent increased latencies with age and an effect of ApoE ε4 status within all three age groups gives a more complete picture of the amount of olfactory processing that changes throughout the lifespan due to age and the ApoE ε4 allele.

**Visual Latency**

For the visual detection task, the N1, P2, N2, and P3 peak latencies significantly increased with age (Figure 6), but had no significant differences based on ApoE ε4 status or the interaction. While visual processing is least effected by age when compared to other modalities, it still demonstrates a significant increase in P3 latencies across studies (Polich, 1997a; Polich, 1997b; Polich & Hoffman, 1998a).

**Sensitivity and Specificity**

Similar to previous studies, different ERP components were statistically different in latency depending on age (Evans et al., 1995; Hummel et al., 1998; Morgan et al., 1997; Morgan et al., 1999; Murphy et al., 1994; Murphy et al., 2000; Thesen & Murphy, 2001). P3 latency had an overall classification for the young age group rate of 75% (Sensitivity=70%, Specificity=80%), and for the middle age group of 80% (Sensitivity=80%, Specificity=80%). In the older age group N1 latency was only significant predictor with an overall classification rate of 100% (Sensitivity=100%, Specificity=100%).

This further demonstrates the sensitivity of the P3 in the young and middle age years to the presence of the ApoE ε4 allele, and could be the result of cognitive differences being more detectable and the sensory differences being less affected in the young and middle age groups. This was different from the older participants, where the most sensitive peak was by far N1, which is a sensory component, suggesting that there is more peripheral damage in the older adults.

**Olfactory Amplitude**

In contrast with previous studies, the N1 and P2 peak amplitudes of the current study showed no significant differences (Hummel et al., 1998; Morgan et al., 1997; Murphy et al., 1994). However there were significant reductions in N2 and P3 amplitudes due to age
(Figures 7 and 8). This was expected as an indicator of normal olfactory decline (Geisler, Covington, & Murphy, 1999; Morgan et al., 1997; Morgan et al., 1999; Murphy et al., 2000). There were also significant differences when the peak to peak amplitudes were examined. More specifically, the peak to peak amplitudes between N1 and P2 were significantly longer with the middle age ApoE ε4 negative participants compared to the positives, suggesting olfactory cognitive processing deficits in the middle age ApoE ε4 positive group. The peak to peak amplitude between N1 and P3 was significantly larger in the younger than middle age and older age groups. These amplitude measures have been used in previous studies, and have at times been significant even when not all of the individual peak amplitudes are significantly different (Hummel et al., 1998; Murphy et al., 1994; Thesen & Murphy, 2001). However, these studies focused on age, and did not include ApoE ε4 allele status as a factor.

**Visual Amplitude**

Visual amplitudes were significantly different based on age, depending on the electrode site for the N1 and P3 peaks (Figures 7 and 9). The young age group had significantly larger amplitudes than the middle age group. This is consistent with previous research that demonstrated significantly decreased visual P3 amplitudes with age (Polich, 1997a; Polich, 1997b; Polich & Hoffman, 1998a).

**Topography**

The Intra-Class Correlations between ApoE ε4 positives and negatives were very strong for the visual task, however they differed based on age group for the olfactory task. There was also a change in the patterns of olfactory correlations for the middle age and older age groups (Figures 10 and 11). This pattern is similar to Murphy et al. (2009), despite the differences in tasks. In conjunction with the amplitude results, this supports the theory that age and the ApoE ε4 allele do not necessarily affect cognition by an overall reduction of brain activity as measured by ERPs; but instead a difference in the patterns of brain areas affected, resulting in less efficient processing (Murphy et al., 2003).
Figure 7. Differences in ERP amplitude due to age and ApoE ε4 status.
Figure 8. Changes in olfactory amplitudes.

Figure 9. Changes in visual amplitudes.
SUMMARY

This study has demonstrated that the detrimental effect of the ApoE ε4 allele can be measured by olfactory event-related potential latencies and amplitudes across the lifespan. Such differences are even visible in young and middle aged participants. This was independent from the effect of age and their interaction. These results are even stronger when the fit between this study and previous literature examining visual ERPs are taken into account. This study has also demonstrated that P3 is the peak most likely to detect these differences in the young and middle aged groups. For older participants, there was potential cognitive compensation resulting in cognitive components not discriminating between ApoE ε4 positives and negatives, but the N1 sensory component. Overall, these olfactory deficits were not the result of a lack of brain activation, but changes in the pattern of brain activation based on age and ApoE ε4 status as measured by ERPs processing the olfactory stimuli.
A possible limitation of this study would be the middle age group not being representative of their population. Of the behavioral measures implemented in the screening process, the middle aged ApoE ε4 negatives had the lowest scores on many of the tests (Table 2), when the lowest scores would be expected to be in the older age group. However, the middle age ApoE ε4 positive participants demonstrated a more expected pattern of behavioral measures. Also, the results of the behavioral measures were not as strong as the olfactory ERPs in this study, which found differences that may be more accurate than examining behavioral measures alone. Also, as statistical tests comparing all of the age by ApoE ε4 were not conducted for the topographical analyses, comparisons should be made with caution. The graphs produced are consistent with the amplitude analyses, but should not be used as a stand-alone analysis of changes in topographical ERP distributions.

Future research can go in numerous directions from the present study. One would be to test other late stages of olfactory processing and how participants positive for the ApoE ε4 allele differ from negatives. There should also be continued examination of the changes in topographical distribution of the OERP and how it changes across the lifespan depending on the ApoE ε4 allele with a greater emphasis on different methods of comparing ERP topographies. As it is still a new field, this would have to be examined in visual as well as olfactory modalities in order to provide a reference of the stability of the analysis. Also, now that young and middle age participants have been shown to have delayed OERP latencies resulting from the ApoE ε4 allele, younger children could be studied to see if a possible mechanism of the allele's impact begins with a particular stage of brain development.
REFERENCES


