MEMORY, CIRCADIAN RHYTHM, AND GOAL-DIRECTED BEHAVIOR IN AN APOE4/APP MOUSE MODEL OF ALZHEIMER'S DISEASE

by

John Graybeal

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Bachelor of Science
Bridgewater College, 2011

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DEDICATION

This thesis is dedicated to all the people who missed me while I worked on this project, especially to my wife, my family, and friends who patiently waited to spend time with me during the many hours I invested in this research. Thank you for being a source of support when it would have been easy to encourage me to take shortcuts instead of pursuing excellence in all the areas of my life.

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I would like to thank the Alzheimer's and Related Diseases Research Award Fund for providing the financial support necessary to complete this project. I would also like to thank Angie Railey for her pioneering work with the ε4/hAPP mouse model and for working with Dr. Flinn to secure funding for the project. I would like to thank my committee, Dr. Flinn, Dr. McKnight, and Dr. Smith for all of their guidance and for teaching me to ask better questions and to think deeper. Finally, I would like to thank all the graduate students and undergraduate students who helped me complete this research: Lorenzo Bozzelli, Lacey Graybeal, Sarah Clark, Caitlin Groeber, Stephen Lippi, Neja Taneja, Nicole Liehr, Mary Curtis, Samira Ahktar, Peter Kakalec, Kevin Schmidt, Katelyn Boggs, and Stefanie Howell.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

MEMORY, CIRCADIAN RHYTHM, AND GOAL-DIRECTED BEHAVIOR IN AN APOE4/APP MOUSE MODEL OF ALZHEIMER'S DISEASE

John Graybeal, MA

George Mason University, 2013

Thesis Director: Dr. Jane Flinn

While previously developed models of Alzheimer's Disease (AD) have led to significant insights into the mechanisms of AD pathology, the majority of these models have a collective limitation. They utilize an early-onset mutation to induce pathology, which is representative of only about five percent of the AD population. We have developed a novel mouse model that builds on the Westaway mouse, which contains a single copy of doubly mutated human amyloid precursor protein (hAPP) gene. In addition to this doubly mutated hAPP gene, our model incorporates the strongest genetic risk factor for late-onset AD, the Apolipoprotein E (ApoE) ε4 allele. This study aimed to establish a behavioral profile for the ε4/hAPP mouse and to demonstrate its ability to model the changes seen in human AD pathology, especially in individuals with the ApoE ε4 allele. This study examined disruption to medial temporal lobe function, disruption to circadian rhythm function, and impairments in goal-directed behaviors, by comparing

them to the traditional hAPP model on which they were based. To this end, the Novel Object Recognition (NOR) and Morris Water Maze (MWM) tests were employed to examine memory deficits. Circadian rhythm disruption was evaluated by tracking wheel-running behavior with Clocklab software (Colbourne Instruments). Nest construction was also evaluated. Behavioral outcomes were then correlated with key markers of inflammation, TNF-α and IL-1β. Differences in MWM and NOR performance were non-significant, except during the twenty-four hour probe trial when ε4/hAPP mice showed a lower average distance from the platform (Gallagher measure). Compared to wild-type (WT) controls, ε4/hAPP mice showed significantly more frequent ($p=.002$) but shorter ($p=.014$) bouts of activity while displaying non-significant differences in average activity levels, indicating disruption to the regular circadian patterns seen in WT controls. Both ε4/hAPP mice and hAPP mice showed impairments in nest-building behavior. Across all statistically different behavioral measures, ε4/hAPP mice outperformed hAPP mice, which were significantly different from WT mice on many measures. This trend was consistent with the pattern of significant differences observed in IL-1β levels. While both transgenic models showed increased inflammation compared to WT controls, ε4/hAPP mice showed less inflammation than hAPP mice. In this study, the ε4 allele provided beneficial effects, which we believe reflects the young age at which the animals were tested.

INTRODUCTION

While the past several decades of research have not resulted in a cure for Alzheimer's disease (AD), significant progress into understanding the disease has been made, particularly in regards to its genetic basis. Knowledge of the genetic underpinnings of AD has led to the creation of various mouse models of AD. These models are extremely beneficial because they allow faster collection of data and provide an appropriate ethical avenue for experimentation. The first transgenic mouse model of AD was the Hsiao mouse. These mice used the Swedish mutation on the human amyloid precursor protein gene (hAPP) in order to develop plaques and began to show behavioral deficits at nine to ten months of age (Hsiao et al., 1996). A subsequent model, the Westaway mouse (CrND8), contains both the Swedish and the Indiana mutations of the hAPP gene and the disease progresses significantly faster in this model. Consequently, Westaway mice begin developing plaques by three months of age and show neuritic pathology at five months of age (Chisti et al., 2001). A number of other transgenic mouse models have been created by utilizing various crosses and combinations of different mutations on the APP gene, the presenilin 1(PS1), and tau genes (Richardson & Burns, 2002).

However, all of these mouse models of AD are best considered analogues to the early onset variety of the disease. Unfortunately, of the 5.4 million Americans living

with AD, only about 200,000 have early onset AD (Alzheimer's Association, 2012). Therefore, it was decided to try to develop a mouse model of AD that is more representative of the vast majority of AD cases (Railey, 2011). The biggest genetic risk factor for late onset AD is a specific isoform of the Apolipoprotein (ApoE) gene. The ApoE gene has three isoforms in humans, the ε2, ε3, and ε4 alleles. The ε2 allele is generally regarded as protective, while the ε4 allele is dramatically overrepresented in patients with AD (Lippa et al., 1997). Humans have two copies of the ApoE gene, receiving one allele from each parent, and different combinations are associated with different risks of AD.

However, as inheriting two copies of the ε4 gene does not guarantee that a person will be diagnosed with late-onset AD, a likely hypothesis is that the ApoE gene interacts with the environment and other genes in a way that results in AD (Moceri et al., 2001). Therefore, in order to better model late-onset AD, our transgenic mouse model includes both the doubly mutated hAPP gene from the Westaway mouse and a copy of human ApoE ε4 isoform that replaces the endogenous mouse ApoE gene. Since endogenous mouse APP does not form amyloid plaques, it is necessary to induce plaque development by inserting a human APP gene in addition to the ApoE ε4 allele. Thus, ApoE ε4 mice were crossed with the Westaway mouse. The purpose of this study was to further explore the ε4/hAPP mouse model, investigating circadian rhythm changes, goal-directed behaviors, and memory impairments in this model of AD.

ApoE is a chain of 299 amino acids that form a single chain with two functional domains (Weisgraber, 2002). The differences in the three isoforms are due to

the different amino acids found at positions 112 and 158. One known function of ApoE is to bind to and transport lipoproteins. The ApoE ε4 allele is associated with higher levels of cholesterol, an essential component to cell membranes that is known to alter membrane characteristics (Yeagle, 1991). Recent evidence suggests that its role in lipid transportation may protect against the changes in the cell membrane caused by interactions with amyloid (Cecchi et al., 2009). Interestingly, the ApoE ε4 allele is also associated with worse outcomes following head trauma (Weisgraber, 2002). This is important because both AD and traumatic brain injury involve a complex sequence of cellular biochemical processes that involve inflammation.

Inflammation is a characteristic feature of AD where ApoE plays a role. Microglia produce anti-inflammatory reactions in response to amyloid-beta (Aβ) by secreting cytokines that induce complex signaling cascades (Kim, Basak & Holtzman, 2009). Aβ also induces ApoE production which serves to limit neuroinflammation. Furthermore, ApoE facilitates other anti-inflammatory responses. It is possible that the ε4 allele represents a loss of protective function if the inflammation in AD is not well controlled. Multiple studies have shown a greater inflammatory response in mice that contain the human ε4 allele as compared to those with the ε3 allele. The ε4 isoform may produce less functional ApoE, yielding greater inflammation in patients because of a lack of anti-inflammatory effects. However, it remains unclear whether ApoE ε4 leads to harmful function or represents a loss of beneficial function.

Additional mechanisms by which ApoE may play a role in AD pathology include altering the brain's ability to expel AB via the blood brain barrier and increasing neuronal

internalization of AB (Kim, Basak & Holtzman, 2009). After damage to the nervous system, ApoE levels increase and several studies have demonstrated that ApoE may actually facilitate neuronal plasticity. ApoE ε3 has also been shown to promote neurite outgrowth while ApoE ε4 impaired it (Nathan et al., 2002).

ApoE ε4 appears to affect the brain in an age dependent manner. While most of the outcomes associated with the presence of the ε4 allele in AD are negative, research suggests that early in life, ε4 carriers perform better than non- ε4 carriers regarding a variety of cognitive abilities including episodic memory, executive function, and processing speed (Rusted et al., 2013). However, these advantages that occur early in life do not seem to be domain specific. Since our transgenic mouse model is built upon the accelerated pathology development of the Westaway mouse, it is unclear how the ε4 allele will affect AD pathology in our model.

An important goal of this study is to see if our novel model produces behaviors that resemble the clinical population. The complex biochemical events characteristic of AD lead to a variety of behavioral changes. The hallmark symptom of AD is memory impairment which reflects the severe pathology affecting structures in the medial temporal lobe. This damage to the medial temporal lobe should be present in our ε4/hAPP transgenic mouse model and should manifest itself behaviorally in the form of spatial and semantic memory deficits, two types of memory known to be impaired in AD (Minati, Edginton, Bruzzone & Giaccone, 2009). Semantic memory refers to the storing of information that is not dependent on time while episodic memory refers to the storing of information that is dependent on both time and space (Ennaceur, 2010). This study

used the Novel Object Recognition (NOR) test which may have both semantic and episodic components. Mice will preferentially sniff a novel object if they can recognize an alternative from a previous encounter (Antunes & Biala, 2012). Recognition memory may be a blend of both semantic and episodic memory because the mouse must recall the previous event of encountering an object, but must only recall attributes of the object that are not dependent on time or space, such as its color and shape. This study also used the Morris Water Maze (MWM) to evaluate spatial memory, which is a specific type of episodic memory. In this task, mice must use distal visual cues to learn the location of a submerged platform.

Another characteristic change in AD involves impairment of circadian rhythm functioning. Circadian rhythm disruptions are common in patients with AD, affecting approximately twenty-five percent of them (Weldemicahel & Grossberg, 2010). This may be due to decreased functioning of the suprachiasmatic nucleus in AD patients. While the ε4 allele is usually associated with negative outcomes pertaining to AD, within the subpopulation of individuals who already have AD, the ε4 allele seems to be protective against circadian rhythm disruption in humans (Yesavage et al., 2004). As patients progressed through the stages of AD, carriers of the ε4 allele experienced higher scores on an index, that measured waking after sleep onset, longitudinally. Non-ε4 carriers saw the same degradation of sleep quality, but also began to score lower on scores of sleep efficiency. Additionally, they showed weaker circadian patterns compared to the group of ε4 patients as determined by an actigraph.

Another study found that AD patients with two copies of the ε4 allele were less likely to experience sleep disturbances than AD patients with only one copy (Cacabelos et al., 1996). In contrast, however, patients with two copies of the ε4 allele had lower post-mortem cerebral spinal fluid concentrations of melatonin than those who had only one (Liu, Zhou, Heerikhuize, Horman & Swaab, 1999). Since melatonin is essential to circadian rhythm functioning (Weldemicahel & Grossberg, 2010), these results seem contradictory to the other findings, suggesting that the relationships between the ApoE gene, melatonin, and circadian rhythm disruptions is complex. In a previous study, the Tg2576 mouse engaged in wheel running behavior for longer periods of time compared to WT controls, while in constant darkness, perhaps indicating a weakening of the circadian rhythm cycle (Wisor et al., 2005). This study sought to confirm that the presence of ApoE ε4 is protective against circadian rhythm disruption by comparing our ε4/hAPP mice to Westaway's model of AD pathology. Circadian rhythm functioning was measured by tracking wheel-running behavior in circadian rhythm cages over the course of five days.

A third characteristic of patients with AD is emotional changes and a significant portion of AD population experiences depression. More specifically, apathy is subsequently characteristic of AD, which can be operationally defined as a decline in goal-directed behaviors (Eposito et al., 2010). A study of patients with AD using single-photon emission computed tomography and statistical parametric mapping analysis linked apathy to the cingulated gyrus (Migneco et al., 2001). It has also been found that the connections between the hippocampus and the cingulate gyrus are more developed in

individuals with the ε4 allele (Bartres-Faz et al., 2008). It is possible that if these connections are more developed in individuals with the ε4 allele that they will be more resistant to the pathology in AD in this region. This would manifest itself behaviorally as a decreased incidence or expression of apathy in individuals with the ε4 allele. To assess goal-directed behavior, mice were given the opportunity to build nests out of a cotton-square and results were photographed and scored blindly.

This study aimed to establish a behavioral profile for the ε4/hAPP model of AD by investigating damage to the medial temporal lobe, changes in circadian rhythm functioning, and goal-directed behavior to see if our model accurately represents the clinical AD population. To this end, the following hypotheses were tested:

1. Both ε4/hAPP and hAPP mice will show disruption to medial temporal lobe functioning compared to WT controls, as demonstrated by deficits in memory, with ε4/APP mice showing the worst impairments.

2. Both ε4/hAPP and hAPP mice will show impaired circadian rhythm functioning compared to WT controls, although the ε4/APP mice will show remediated deficits.

3. ε4/hAPP and hAPP mice will show impairments in goal-directed behaviors compared to WT controls, as assessed by nest-construction

tasks, although ε4/hAPP animals will show less impairment than hAPP animals.

METHODS

Animals

Eight female mice with two copies of the human ApoE ε4 allele were purchased from Taconic for breeding. Offspring always received one copy of the human ApoE ε4 allele. Seven female wild-type (WT) mice, bred at George Mason University were also used for breeding. All females were bred with CrND8 (Westaway) males obtained from the University of Toronto. CrND8 mice carry a single copy of hAPP gene with both the Swedish and the Indiana mutations because having two copies of the hAPP gene is a fatal mutation in mice. As a result, only fifty-percent of the offspring produced inherited a copy of the hAPP gene. Animals that successfully inherited a copy of each gene comprised the first experimental group of ε4/APP mice, while ApoE ε4 carriers that failed to inherit the hAPP gene were excluded from the study (see Table 1.0).

Likewise, two genetic outcomes from the female WT by male Westaway mouse crossings. Animals that successfully inherited the hAPP gene were used to form the second experimental group. Other offspring of WT females that failed to inherit the hAPP gene were used as WT controls. The Westaway mouse uses the hybrid genetic background C3H/B6, so WT breeders of this strain were used. Genotyping was performed by Transetyx.

Table 1: Possible Genetic Outcomes
The possible genetic outcomes for offspring obtained by crossing mice with two copies of ApoE4 or WT females with male Westaway mice, which contains a single copy of doubly mutated hAPP. There is a fifty-percent chance of offspring inheriting the hAPP gene.

Westaway Mouse bred with:	**ApoE4**	**WT**
hAPP gene (50%)	ε4/hAPP, correct model	hAPP, Westaway mouse
No hAPP gene (50%)	ε4 only, removed from study	WT control

Breeding and Housing

All animals were kept in a temperature and humidity controlled colony. Two rounds of breeding, spaced several months apart, were used to produce the subjects. Litters remained with their mother until they were weaned after post-natal day twenty-one. Offspring were then separated by sex and genotype into cages of two to four animals. Mice were handled two to three times per week up until testing in order to reduce anxiety while they were tested. Food and water were available ad libitum. The colony maintained a 12:12 light/dark cycle and the temperature was kept at 22±2°C. Tek-FRESH bedding (Harlan Laboratories) was used in all cages. Each cage included a plastic igloo and a running wheel. Mice were sacrificed at the completion of behavioral testing, using carbon dioxide as the method of euthanasia followed by decapitation. Mouse brains were then extracted to test for the presence of inflammatory cytokines.

Circadian Rhythm

Materials

The circadian rhythm cages are smaller, 13.5" x 7.5" x 5.4", individual cages that contain a running wheel (Colbourne Instruments). Wheel rotations are tracked and data is collected using Clocklab software (Acimetrics) and analyzed in Matlab (Mathworks). Cages were lined with minimally sufficient 1/8" corncob bedding (Harlan Laboratories). Food and water were provided ad libitum during the circadian rhythm testing.

Procedure

Circadian rhythm testing was conducted within the mouse colony at two months and four months of age. Animals were run in three counterbalanced groups. Each animal was placed in the cage for a period of five days. The term "bout" refers to a period of continuous activity. A bout analysis was performed on the data collected. A bout's onset was defined by a minimum threshold of five counts per minute. A bout of activity was considered finished when the animal's activity fell below this threshold of five counts per minute for more than ten minutes. Due to mechanical problems, data from the two month testing is not reported.

Nest-Construction

Materials

The same circadian rhythm cages were used as stated above. The corncob bedding used in the circadian rhythm cages is not optimally comfortable. Consequently, unimpaired animals will readily convert a 2.25"x 2.25" cotton-square (OMNI BIORESOURCES, Inc.) into a nest in these conditions.

Procedure

Mice were given the opportunity to build a nest while in the circadian rhythm cages. Forty-eight hours prior to the completion of circadian rhythm testing, a cotton-square was inserted into all of the circadian rhythm cages. The contents of the cage were photographed after the animals were removed from the cages. Photographs of nests were scored blindly by an independent grader based on the following scale:

0 = Square is almost fully intact (may have up to four small pieces torn off)

1 = Square is mostly intact, but has five or more small pieces torn off

2 = Square is partially intact, with at least one significant cluster of torn pieces

3 = Square is barely recognizable and the vast majority has been torn apart to form a nest

Novel Object Recognition

Materials

Two 19” x 19” x 18.5” boxes (CleverSys) were used for the Novel Object Recognition test. A camera was mounted on the ceiling above the boxes to record behavior. TopScan software (CleverSys) recorded the trials and tracked the frequency and duration of sniffing behavior for each animal.

General Procedure/Preparation

Novel Object Recognition testing took place when the animals were six months of age and the protocol lasted seven days. On day one, mice were placed with their cagemates into the NOR testing boxes for a period of seven minutes to allow the animals to habituate together. On days two through five, mice were placed in the NOR testing boxes for six minutes for individual habituation. The habituation trials were longer than the testing trials so that mice would not expect to be taken out at the end of the trial. On day six, mice underwent three five-minute trials. The first trial consisted of two identical objects, referred to as the animal's standard object. Fifteen minutes later, the mice were exposed to one copy of its standard object and one novel object. Sixty minutes after the first trial, mice were exposed to their standard object and a second novel object. On day seven, mice were exposed to their standard object and a third novel object twenty-four hours after their first encounter with their standard object for five minutes.

Standard object assignment was counterbalanced across sex and genotype, as was the side of the box that standard objects were placed on. Standard objects always remained on the same side for a given animal.

Differences in the frequency and duration of sniffing behaviors between the standard and novel object for a given trial were evaluated by using discrimination ratios. A discrimination ratio for duration was calculated by dividing the amount of time spent sniffing the novel object by the total amount of time spent sniffing. A discrimination ratio for frequency was calculated by dividing the number of times the mouse sniffed the novel object by the total number of times a mouse sniffed either object. A discrimination

ratio of greater than .5 indicates the mouse spent more time sniffing the novel object. A repeated measures ANOVA by genotype was calculated using the discrimination ratios.

Morris Water Maze

Materials

A large pool 48" in diameter was used for this test. Water was made opaque with non-toxic paint. A plexiglass platform 6" in diameter was submerged 8mm below the water's surface. Pool water temperature was kept between 25° and 28°C. Video footage was collected and analyzed using WaterMaze3 (Coulbourne) software.

Procedure

Morris Water Maze testing began several days after the completion of the NOR testing when the animals were approximately six and a half months of age.

Mice were given a maximum of sixty seconds to find the platform and remained on it for ten seconds. Mice that failed to find the platform were guided to it and allowed to remain on it for fifteen seconds. There was an intertrial interval of forty-five seconds and mice were placed under a heat lamp in between trials. Each animal followed the same pattern of start positions, which changed daily.

On day one, mice were placed on the platform for thirty seconds prior to the start of the first trial and then were tested for eight consecutive days. Days one through six contained three trials per day. On days two, four, and six, the third trial was an Atlantis probe trial where the platform was inaccessible and the number of times the animal swam

through the platform's location was recorded. At the conclusion of an Atlantis probe trial, the platform was made accessible and mice were guided to it and remained on the platform for fifteen seconds. On day seven, a single twenty-four hour Atlantis probe trial was conducted. Mice were given a longer period of thirty seconds on the platform at the conclusion of the Atlantis probe trial on day six to prepare for the twenty-four hour probe trial on day seven. Day eight consisted of two trials with a different, visible platform to identify animals with visual deficits. Animals that did not reach the visual platform during either of these trials were excluded from the analysis. Dependent variables collected included latency to find the platform, thigmotaxicity, time in the correct quadrant, average velocity, and the number of platform crossings (during Atlantis Probe trials).

RESULTS

Throughout the experiment, a significantly higher rate of subject mortality was observed than expected. Although both male and female mice were tested, sex differences were not examined due to the low sample size.

Hypothesis 1

NOR

One WT mouse was excluded from the analysis because it never approached or sniffed an object. A RM ANOVA conducted on frequency discrimination ratio showed non-significant differences via genotype [$F(2,29)= .722$, $p=.494$] nor was the genotype*time interaction significant [$F(4,58)= .900$, $p=.470$]. Similarly, a RM ANOVA conducted on discrimination ratio of duration revealed non-significant differences based on genotype alone [$F(2,29)= .847$, $p=.439$] and the interaction between genotype and time was also non-significant [$F(4,58)= .916$, $p=.461$]. The sample sizes for this test by genotype were: ε4/hAPP n= 7; hAPP n=11; WT n = 14.

MWM

Four animals were excluded from the analysis because they failed the visible platform test (WT n=2; HAPP =2). Two other WT mice were excluded because they

showed atypical behavior (climbing on the walls, extreme anxiety). A RM ANOVA by genotype conducted on the daily average latency to find the platform on days one through six was not significant [$F(2,22) = 1.276$, $p=.299$]. Similarly, a RMANOVA by genotype conducted on average distance from the platform on days two through six was not significant [$F(2,22) = 1.495$, $p = .246$]. The sample sizes for this test by genotype were: ε4/hAPP n= 6; hAPP n=8; WT n = 11.

While a RMANOVA conducted on the number of platform crossings during the Atlantis Probe Trials on days two, four, and six was not significant [$F(2,22) = .208$, $p = .814$], a trend was observed using a RMANOVA by genotype on average distance from the platform (cm) during these trials [$F(2,22) = 2.968$, $p = 072$]. On average, ε4/hAPP (M=45.094,SE= 3.346) mice were closer to the platform then hAPP (M= 55.366, SE= 2.898) and WT (M= 53.419,SE=2.472) mice.

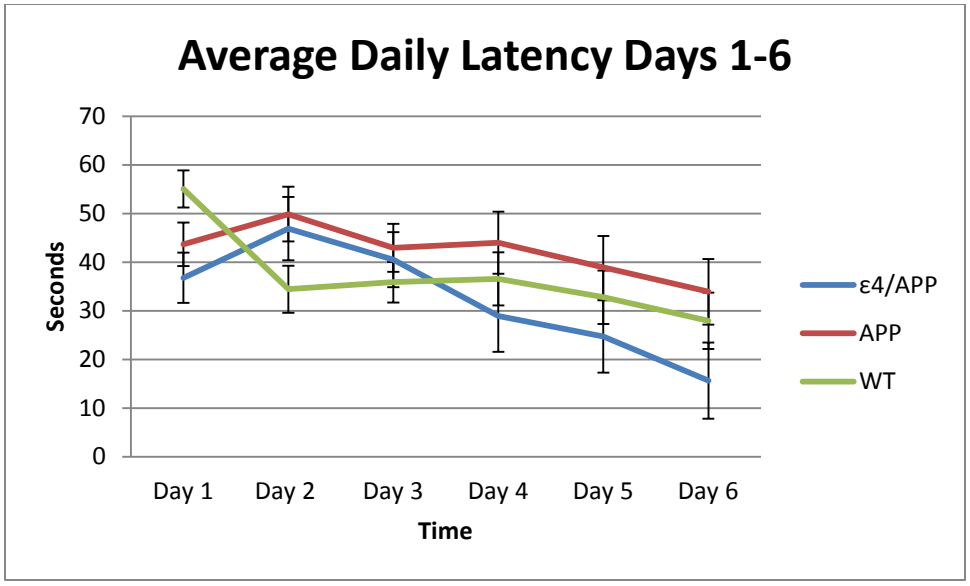

Figure 1: Average Daily Latencies
Differences in daily latency to find the platform were non-significant across days one through six (p=.299).

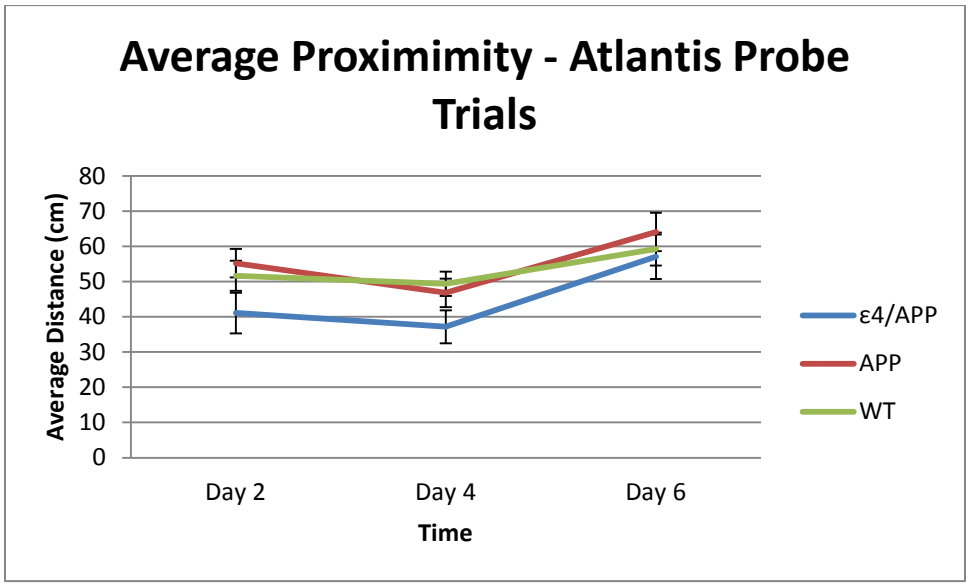

Figure 2: Average Proximity to the Platform During Probe Trials
Differences in the average proximity to find the platform by genotype approached significance (p=.07) such that ε4/hAPP mice swam closer to the platform while searching for it during the Atlantis probe trials on days two, four, and six.

24-Hour Probe

A one-way ANOVA was conducted on the average proximity to the platform (cm) revealed significant differences across genotype [F(2,22) = 4.578, p =.022]. The bonferroni post-hoc test revealed on average ε4/hAPP mice (M=45.74, SD=15.4) were closer to the platform in their search attempts compared to both hAPP (M=65.47, SD=12.9, p=.033) and WT mice (M=63.43, SD=12.1, p=.044).

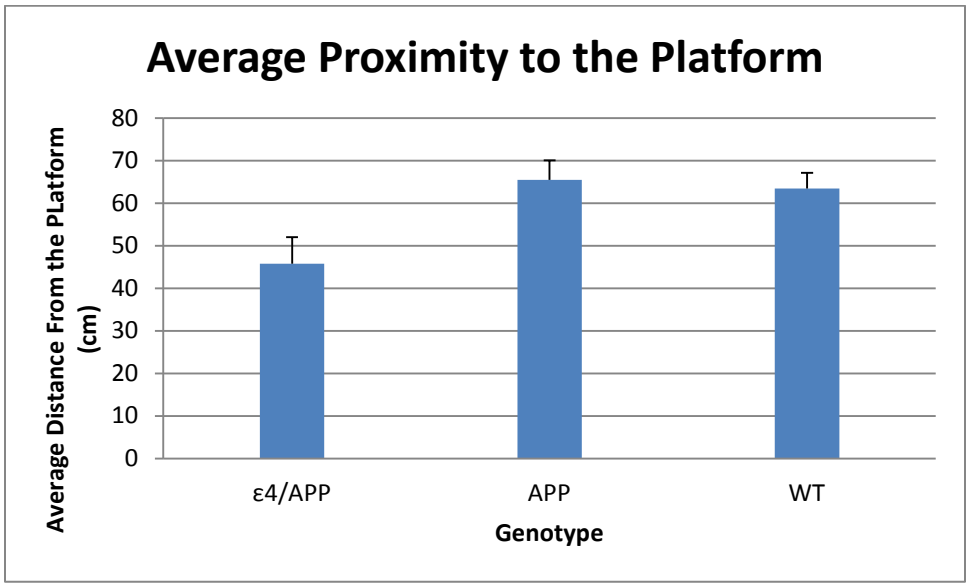

Figure 3: Average Proximity During the Twenty-Four Hour Probe Trial
Differences in the average proximity to find the platform by genotype during the twenty-four hour probe trial were significant (p=.03) such that ε4/hAPP swam significantly closer to the platform than both WT and hAPP mice.

Hypothesis 2

Circadian Rhythm

A one-way ANOVA revealed significant differences in the average bout length by genotype [$F(2,37) = 11.53$, $p < .001$]. The bonferroni post-hoc test revealed that WT ($M=52.17$, $SD=20.82$) mice ran for longer periods of time then the ε4/hAPP ($M=28.48$, $SD=27.10$, $p=.014$) and hAPP mice ($M=15.45$, $SD=10.26$, $p<.001$). The sample sizes by genotype were as follows: ε4/hAPP n= 12; hAPP n=12; WT n = 16.

A one-way ANOVA conducted on the average number of bouts per day by genotype failed the homogeneity of variance tests, so a robust test of the equality of means was used instead. Welch's test of equality of means revealed significant differences in the average number of bouts per day by genotype [$F(2,19.1) = 8.548$, $p =.002$]. The Games-Howell post-hoc test revealed ε4/hAPP ($M=13.92$, $SD=4.46$) mice showed a significantly greater frequency of bouts per day compared to WT controls ($M=7.92$, $SD=2.48$, $p=.002$) but showed only a trend compared to hAPP mice ($M=9.42$,$SD=5.35$,$p=.088$).

Interestingly, a third one-way ANOVA conducted on average activity levels again demonstrated significant differences based on genotype [$F(2,37) = 6.90$, $p = .003$]. In this case, WT mice ($M=10.29$, $SD=4.26$) showed significantly higher activity levels compared to hAPP mice ($M=4.04$, $SD=3.60$, $p=.002$), but not compared to ε4/hAPP mice ($M=7.75$, $SD=5.25$, $p=.417$).

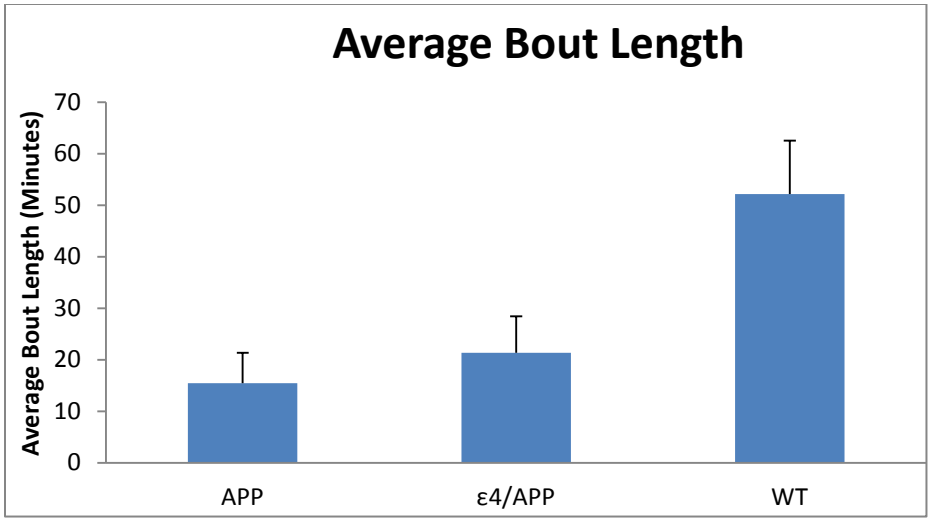

Figure 4: Average Bout Length
Average bout length. Significant differences were observed in the average bout length per group (p<.001). WT mice ran for longer periods of time then both ε4/APP (p<.001) and APP mice (p<.001).

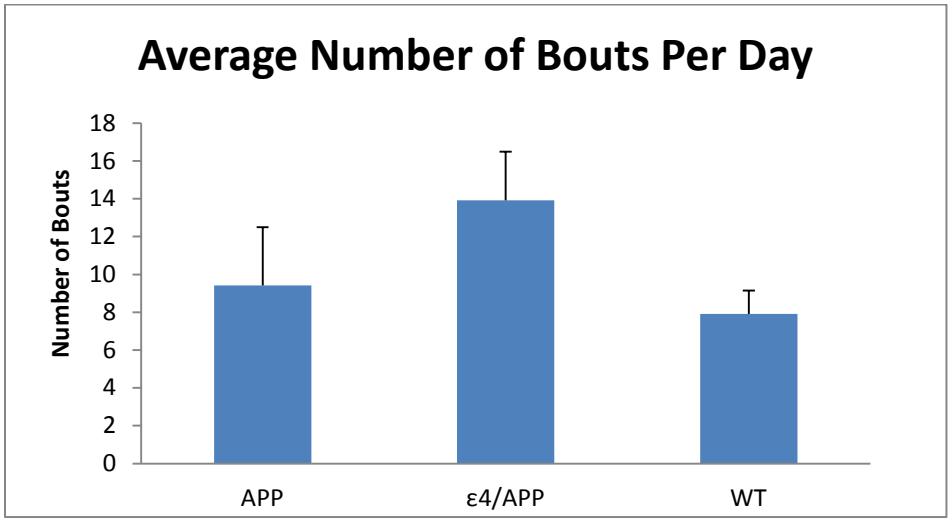

Figure 5: Average Number of Activity Bouts Per Day.
Significant differences were observed in the average number of bouts per group, p=.002. ε4/hAPP mice showed more frequent bursts of activity compared to WT controls (p=.002) and hAPP

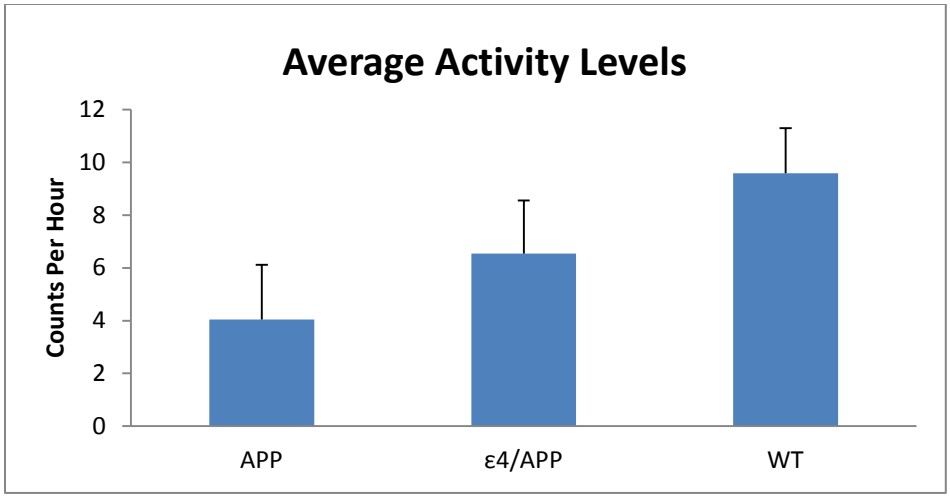

Figure 6: Average Activity Levels at Four Months of Age.
Significant differences were observed in the average daily activity, p=.003. hAPP mice differed significantly from WT controls (p=.002), but ε4/hAPP and WT mice did not significantly differ (p=.417).

Hypothesis 3

Nest Construction

A one-way ANOVA revealed significant differences based on genotype but failed Levene's test for homogeneity of variance (p=.023) and was replaced by a robust test of equality of means. Welch's test demonstrated significant differences based on genotype [F(2,2.674) = 137.249, p<.001]. The Games-Howell post-hoc test revealed significant differences between WT mice and both ε4/hAPP mice (p<.001) and hAPP mice (p<.001). Differences between ε4/hAPP mice and hAPP mice were not significant (p=.545). The sample sizes for this test by genotype were: ε4/hAPP n= 12; hAPP n=10; WT n = 12.

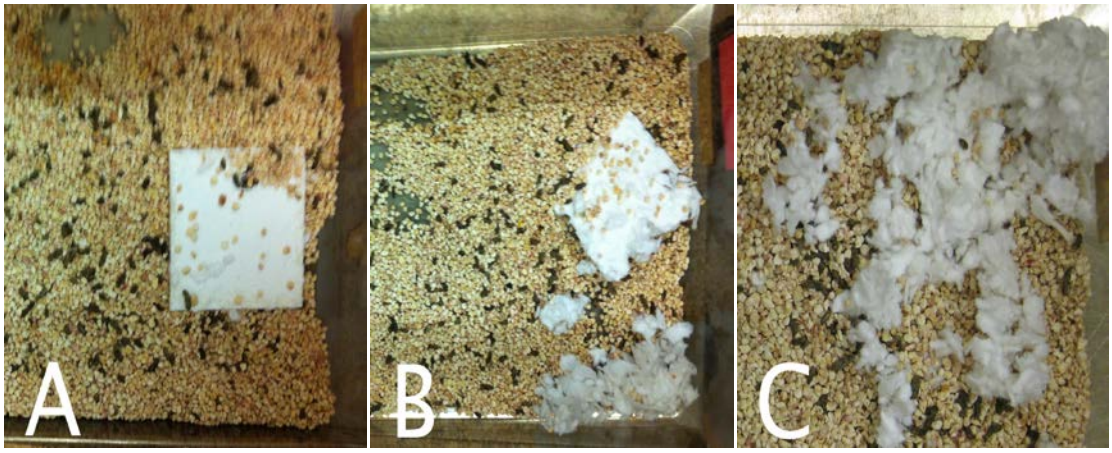

Figure 7: Photographs of the Nest-Construction Task.
Mice were able to complete the nest-construction task to varying degrees based on genotype. The pictures above are randomly selected photographs of a mouse from each genotype. **A. h**APP mouse, score of 0. **B. ε4**/hAPP mouse, score of 2. **C.** WT mouse, score of 3.

Inflammation

As a concurrent part of this study, measures of key inflammatory markers in the brain tissue of subjects were quantified using RT-PCR. The two inflammatory cytokines measured were TNFα and IL-1β. While the full results of the inflammation analysis are beyond the scope of this thesis, these inflammatory scores were correlated with behavioral measures. The inflammatory scores represent a ratio of inflammation compared to pooled WT control groups, so the natural log transformation was used in the correlation analysis. The six WT mice included in the correlation were assigned a value of 1.0. IL-1β was significantly correlated with average bout length [$r(16)=-.590$, $p=.013$], the average number of counts per bout [$r(16)=-.573$, $p=.020$], the average peak rate during a bout [$r(16)=-.509$, $p=.031$] and the average number of counts per bout-minute

[r(16)=-.647, p = .004]. TNFα did not correlate with behavioral scores. The sample sizes for this test by genotype were: ε4/hAPP n= 5; hAPP n= 7; WT n = 6.

DISCUSSION

The purpose of this study was to establish a behavioral profile for the ε4/hAPP mouse. This study's first hypothesis was that both ε4/hAPP and hAPP mice would show disruption to medial temporal lobe functioning compared to WT controls, with ε4/hAPP mice showing the worst impairments. This hypothesis was not supported by the data due in part to the fact that the WT mice did not learn as expected. While the study found the expected memory deficits in the hAPP mouse model, there was no evidence of worse functioning in ε4/hAPP mice. The results of the NOR task failed to show any substantial differences between any of the mouse models. Similarly, results from the MWM failed to show significant differences between genotypes across most measures. When significant differences were observed, they were in an unexpected trend, showing that the ε4/hAPP mice performed better than the hAPP mice and WT controls. One possible explanation for this is that the ε4/hAPP mice were tested at too young an age and the ε4 allele is still acting in an advantageous manner regarding some cognitive functions at six months of age. In humans, the ε4 allele, while a risk factor later on, appears to confer cognitive advantages in terms of a wide variety of cognitive functions (Rusted et. al, 2013). The Westaway model of APP induced pathology is accelerated substantially compared to other mouse models and it is possible that the ε4 allele was still acting in an advantageous fashion, despite the early induction of APP pathology. If the ε4/hAPP mice

were tested at a later age or the ε4 allele was inserted into a different mouse model, such as the Tg2576, that took longer to develop plaques, perhaps the expected ε4 related deficits in temporal lobe function would have been observed.

One of the limitations of this study is the WT mice did not perform as well as expected and were outperformed by the ε4/hAPP mouse model. It is unclear as to whether our results suggest that the ε4/hAPP mice showed enhanced cognition compared to the WT mice, which seems unlikely given how poor the WT mice did, or whether some other factor affected WT performance. The results of the MWM were compromised by a major environmental event that occurred on the second day of testing. Strong fumes from a nearby construction site entered the building and the testing facility, inducing memory loss in at least one person in the building. This occurred in the middle of testing, so not all of the animals were exposed to the same concentration of fumes on the second day of testing. The WT mice showed appropriate learning on day two but failed to show substantial improvement after this point in time. The fumes were strong enough and remained in the building for a long enough period of time to raise concern about whether the neurological functioning of the animals was normal for the remainder of the test.

This study's ability to draw firm conclusions was further limited by the unexpectedly high mortality rate of transgenic animals. Sample sizes were much smaller than anticipated when the animals were six months of age. Due to some mechanical problems in the colony, humidity levels dropped to unacceptable levels at one point

during the study. This has been linked to subject mortality in these mice previously in our lab (Railey, 2011).

However, the ε4 allele function is associated with improved cognition early in life in humans and these alternative explanations are not mutually exclusive to real benefits conferred by the ε4 allele. A very similar mouse model, the ε4/APP-Yac mouse model, over expresses human APP and contains the ε4 allele (Moreau, 2013). Young ε4/APP-Yac mice have recently been shown to outperform ε3/APP-Yac mice in the Morris Water Maze. The ε3 allele is the most common allele in humans and serves as a reference point for establishing the effects of the ApoE gene's alternate isoforms, thus indicating beneficial function of the ε4 allele compared to a baseline in Moreau's study.

Our second hypothesis was both ε4/hAPP and hAPP mice would show impaired circadian rhythm functioning compared to WT controls, although the ε4/hAPP mice would show remediated deficits. This hypothesis found stronger support in the data as both transgenic mouse models displayed impaired performance in measures of circadian rhythm functioning compared to WT controls. Since hAPP mice showed decreases in both bout length and average activity levels, it appears that they engage in the least wheel-running behavior. While this may be reflective of disruption to circadian rhythm function, it also probably reflects the behavioral deficits present in the strain. The hAPP mice were observed becoming locked in pathological circling patterns that probably impaired their ability to engage in this test properly. Others have also observed increased stereotypical behaviors in the CrND8 mouse model (Ambrèe et al., 2006). Based on laboratory observations, circling behavior appeared to predominantly affect only hAPP

mice. The observation that ε4/hAPP mice do not seem to be impaired by circling behaviors, as supported by the non-significant differences in overall activity levels between ε4/hAPP and WT mice, provides direction for future studies. Investigating the relationship between IL-1β and circling behaviors might be a beneficial avenue of future research.

While the overall activity levels between the ε4/hAPP mice and the WT controls did not differ significantly, these strains do significantly differ in terms of both the number of bouts and average bout length, such that ε4/hAPP mice engage in more bouts that are noticeably shorter. This provides strong evidence of circadian rhythm disruption because the WT mice engage in several, long bouts of wheel-running behavior in a regular and cyclic pattern. This cyclic pattern appears weaker in the ε4/hAPP mice such that they run for a statistically equivalent amount of time over shorter, more frequent bouts of activity. Other researchers have found circadian disruption in Tg2576, 3xTgAD, APPxPS1, and APP23 mice (Wisor et al., 2005; Sterniczuk, Dyck, Laferla & Antle, 2010; Duncan et al., 2012; Vloeberghs et al., 2004), but this is the first study that we are aware of that shows disruption to circadian rhythm in an ε4/hAPP model. While this study lacks statistically significant differences between the ε4/hAPP and hAPP mice, the ε4/hAPP mice showed less disruption then the hAPP mice which corresponds to the reduced deficits observed in humans (Yesavage et al., 2004).

This study's third hypothesis was ε4/hAPP and hAPP mice would show impairments in goal-directed behaviors compared to WT controls, although ε4/hAPP animals would show less impairment than hAPP animals. Partial support for this

hypothesis was found in that WT mice were the only strain able to consistently complete the nest construction task. However, while ε4/hAPP mice did perform better on this task than the hAPP mice, differences between these two groups were not statistically significant. The lack of significance between ε4/hAPP mice and hAPP mice may be at least partially due to a lack of sensitivity in the scoring scale employed. A better method of categorizing nest-building behavior would be useful for future studies. However, this behavioral test shows great promise because it was able to easily distinguish WT from transgenic mice and it is very inexpensive to implement. Others have shown impaired nesting abilities in a variety of other mouse models not utilized in this study, including the APP/PS1, 3xTgAD, Tg2576 strains (Filali & Ladonde, 2009; Torres-Lista & Giménez-Llort, 2013; Wesson & Wilson, 2011). However, this is the first study that we are aware of that examines mice with the ApoE ε4 allele in terms of nest construction and hints that it may reduce such deficits in an AD mouse model.

By analyzing data collected after the animals in this study were sacrificed, a separate analysis used RT-PCR to quantify the levels of IL-1β, an inflammatory cytokine (Unpublished data). Significant differences were observed between genotypes, such that hAPP mice showed the highest levels of inflammation, while ε4/hAPP mice also exceeded levels of the inflammatory cytokine exhibited by WT controls (See Appendix). This corresponds to the pattern of impairments observed in this analysis regarding disruption to circadian rhythm and nest-construction. WT's showed no deficits in nest construction and had low IL-1β scores, ε4/hAPP mice showed intermediate impairment in nest construction and had intermediate IL-1β scores, and hAPP mice showed the worst

impairments with the highest inflammation. A significant correlation between IL-1β and circadian behavioral scores was then observed. As IL-1β levels increased, mice exhibited more circadian disruption across genotypes, manifested as changes in bout length, peak running rates during a bout, and the average number of counts per minute during a bout.

In conclusion, while we expected to see clear memory-related deficits in ε4/hAPP mice that overshadowed impairment in hAPP mice, we only found support for the hypotheses that predicted that the ε4 allele would be beneficial. This may be due to the accelerated nature of our model and ε4/hAPP mice may need to be tested after six months of age before the memory-related deficits normally associated with ε4 allele will become visible. Increases in IL-1β correlated with circadian rhythm disruption. Inflammation, circadian rhythm changes, and disruption to goal-directed behaviors were most extreme in the hAPP mice and least extreme in the WT control group. It appears that in our mouse model, at a relatively young age, the ε4 allele is partially protective against AD pathology that affects circadian rhythm and goal directed behaviors.

APPENDIX

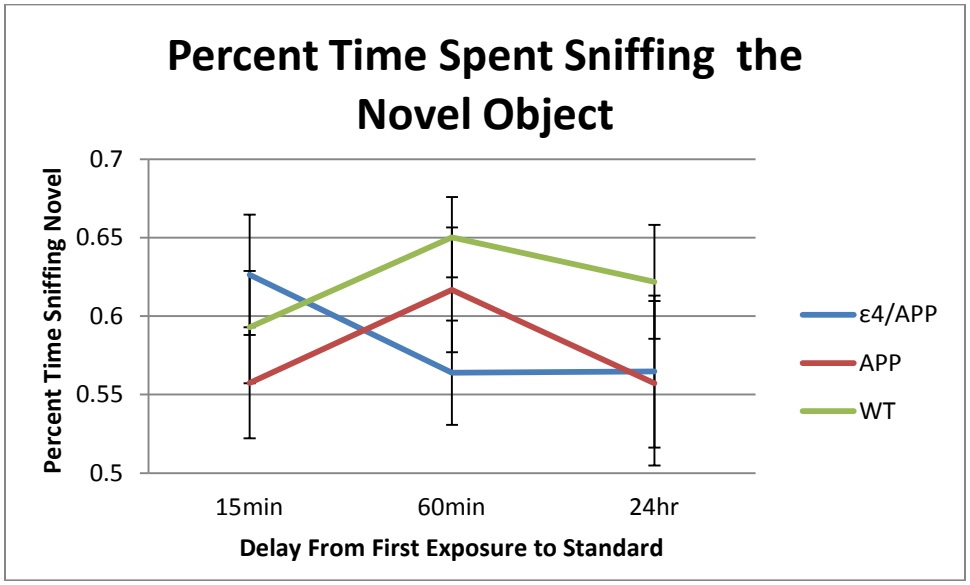

Figure 8: Percent Time Spent Sniffing the Novel Object
Differences by genotype in the duration of time spent sniffing the novel object compared to total time spent sniffing were non-significant (p=.439).

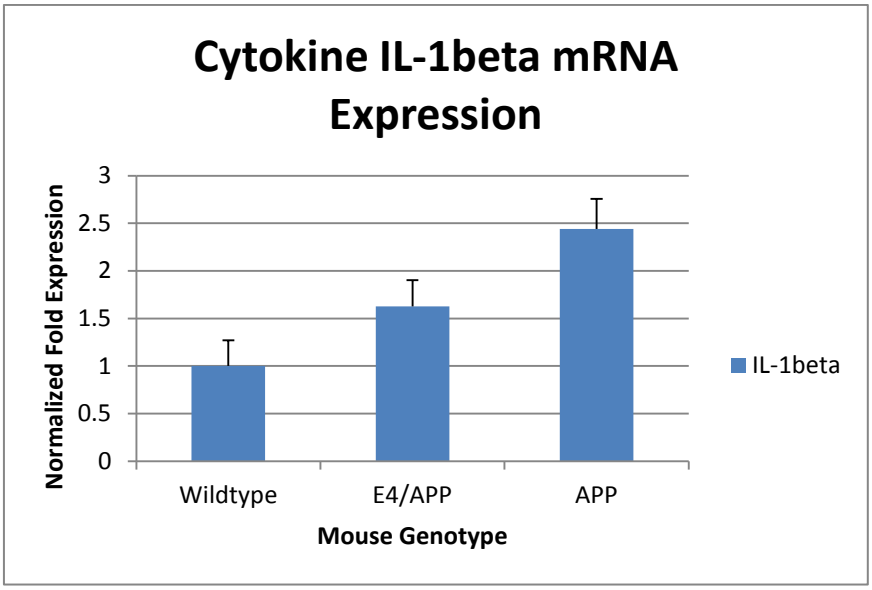

Figure 9: Inflammation by Genotype
A separate analysis of the data from this study used RT-PCR to quantify the levels of IL-1β in the brain tissue of the subjects. A Kruskal – Wallis test (see Table 1) showed that there were significant differences between the groups in the levels of IL-1β ($p<.05$). APP and WT mice showed significantly different levels of IL-1β ($p<.05$) and a trend was observed between hAPP/E4 and WT ($p<.1$)

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BIOGRAPHY

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