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CAN A MORRIS WATER MAZE TEST DISTINGUISH ALZHEIMER'S MICE FROM WT MICE?

A Major Qualifying Project Report

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disease caused by the accumulation of amyloid-beta (Aβ) in the brain. Our laboratory previously established an *in vitro* model for AD by treating cultured human neuronal cells with Aβ, and showed that neuronal survival could be restored by treating the cells with a neurotrophic factor (NTF) mimetic. The purpose of this MQP is to expand the tests *in vivo* using 3X transgenic Alzheimer's mice to analyze AD mouse behavior. This project developed a procedure for performing the Morris water swimming test in-house at WPI, and determined whether this test can distinguish WT from AD behavior at 4 months of age, one month past the age at which they first show brain alterations. The data indicate that at 4 months of age, this AD strain is indistinguishable from WT mice in the Morris test. In preparation for future *in vivo* tests with various NTF peptides, we also compared the activity of a variety of Aβ batches on human neuronal cell morphology using the *in vitro* AD model, and conclude that all Aβ batches induced significant morphological alterations relative to untreated control cultures.

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BACKGROUND

Alzheimer's Disease

Alzheimer's disease (AD) is a neurodegenerative disorder that causes problems in logic, memory, and behavior. As the most common type of dementia, AD can eventually impair an individual's ability to perform the simplest functions such as walking and swallowing and is ultimately fatal (Alzheimer's Association, 2012). Accounting for an estimated 60-80% of dementia cases, it causes healthy brain cells to lose their function and communicate with each other and eventually die. Its prevalence is increasing, with an estimated 115 million expected to be diagnosed worldwide by 2050 (Alzheimer's Association, 2012). AD is the 6th leading cause of death in the United States, and the 5th leading cause of death for people 65 years and older (Alzheimer's Association, 2012). There are no known preventions or cures, and until recently the only definitive method of diagnosis was a post-mortem autopsy.

AD Symptoms

The first clinical symptoms of AD are often memory loss, such as difficulty in remembering names and events, and other cognitive problems, such as trouble with language and decision-making (Alzheimer's Disease Progress Report, 2011). Early symptoms also include apathy and depression. AD patients progress from mild to moderate to severe stages of the disease at different rates. In the late stages, symptoms include impaired judgment, disorientation, confusion, personality and behavior changes, and difficulty speaking, swallowing, and walking.

Histological Characteristics of AD

The hallmark characteristics of AD brains are extracellular senile plaques, that are deposits of the protein fragment amyloid-beta (Aβ), and intra-neuronal neurofibrillary tangles, that are twisted strands of tau protein (Selkoe, 2001). These two cellular features became the very first diagnostic criteria of the disease as they were first visualized by a young and accomplished German doctor named Alois Alzheimer in brain slices of the first diagnosed patient, Auguste Dieter, who had an early onset version of the disease.

Aβ is formed by the cleavage of amyloid precursor protein (APP) by gamma- and beta-secretase enzymes. Intracellular neurofibrillary tangles are formed from hyper-phosphorylated tau proteins that dissociate from microtubules (Kawashima and Ihara, 2002). Although both amyloid plaques and neurofibrillary tangles are present in low amounts in all normal aging individuals, accumulation of these two features can lead to AD (Munoz and Feldman, 2000; Kawashima and Ihara, 2002).

Additionally, in AD patients, synaptic connections between groups of neurons stop functioning and begin to degenerate in large numbers. As networks of neurons lose their connections, break down, and die throughout the brain, affected regions begin to shrink in a process known as brain atrophy (Alzheimer's Disease Progress Report, 2011). Cerebral atrophy is a characteristic of AD (**Figure 1**). However, this loss of both gray and white matter is not enough to diagnose AD because it does not distinguish between normal ageing and the disease (Munoz and Feldman, 2000). Until recently, the only definitive way to diagnose AD involved a post-mortem autopsy to demonstrate the presence of high levels of amyloid plaques and neurofibrillary tangles in the brain (Luchsinger and Mayeux, 2004).

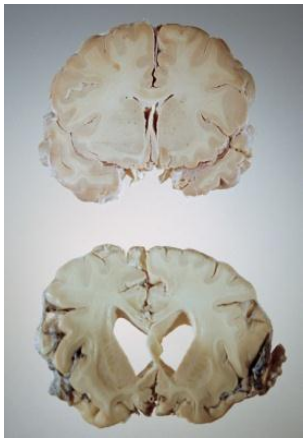

Figure-1: Photograph of a Normal Human Brain (upper) and an AD Brain (lower). Note the appearance of areas of the AD brain with loss of gray and white matter. (Sanders, 2011)

Amyloid plaques, neurofibrillary tangles, synaptic loss, and cell death are the most prominent features of the AD brain. However, scientists are also discovering many other cellular changes that occur in the brain of AD patients. Signs of inflammation in response to cellular injury, brain blood vessel and neuron degeneration in response to neuronal malfunction, and abnormal glial cells have been observed in AD patients (Alzheimer's Disease Progress Report, 2011).

Alzheimer's Cell Biology

The most widely held model for cell death in AD is known as the Amyloid Cascade Hypothesis (**Figure-2**) (Selkoe, 2001; Masters *et al*., 2006). The amyloid cascade hypothesis states that AD begins with the extracellular formation and deposition of neurotoxic Aβ, a 40-42 amino acid peptide derived from the proteolytic cleavage of amyloid precursor protein (APP) by cleavage with beta- and gamma-secretase enzymes (diagram upper left). This peptide forms especially in the regions of the brain responsible for memory and cognition, the pre-frontal cortex and the hippocampus. The toxic effects of Aβ are caused by its interaction with the receptor for advance glycation end products (RAGE) (Yan *et al*., 1996). This interaction causes reactive oxidative stress (ROS) in the brain (diagram center). AD patients also have a higher

RAGE expression in the cortex and hippocampus, which creates a positive feedback loop for increased binding to Aβ (Yan et al., 1996). The ROS causes a variety of cellular problems, including lipid peroxidation and membrane depolarization (diagram right), activation of apoptotic kinases (lower left), and tau hyperphosphorylation (lower center). Although initially the AD research field was divided into two camps, the Aβ camp arguing that Aβ was the cause of the disease, and the Tau camp arguing that Tau causes AD, we now know that Aβ initiates AD and tau is a required downstream event. Mice lacking Aβ but containing tau do not get AD, and transgenic mice containing Aβ and missing tau do not proceed to full AD, so both are necessary (Rapoport *et al.*, 2002; Robertson *et al.*, 2007). Other characteristics induced by Aβ include calcium disruption, mitochondrial dysfunction, metabolic interferences, and protein misfolding, all of which help activate apoptosis. Once initiated, the ROS and neurofibrillary tangles spread to other areas of the brain.

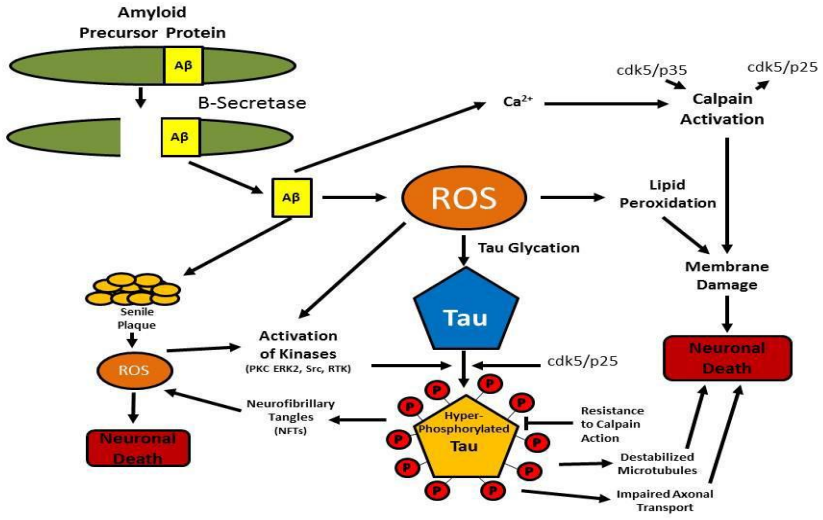

Figure-2: Diagram of the Amyloid Cascade Hypothesis for Alzheimer's Disease. In this hypothesis, the formation of Aβ (yellow, upper left) by cleavage from the amyloid precursor protein (green, upper left) initiates the formation of cellular oxidative stress (ROS, orange). Downstream, tau hyper-phosphorylation (red) leads to the disassembly of axonal microtubules, weakening the synapse. (*Derived from Adams Review; Jackson and Najem, 2012)*

Evidence strongly supporting the Amyloid Cascade Hypothesis was recently obtained from an analysis of the Icelandic genetic database, where a mutation was identified in APP that appears to protect individuals from getting AD (Jonsson et al., 2012). The mutation blocks the production of Aβ, and even aged individuals containing the mutation do not get AD, so this finding supports the hypothesis that Aβ causes AD. Individuals containing the mutation lack Aβ from birth, so questions remain whether lowering Aβ later in life can block the disease. But this significant finding has excited researchers to continue identifying more drugs that affect Aβ production, and has led to several clinical trials for blocking Aβ formation *earlier* in patients (see clinical trials).

Current AD Treatments

Several drugs have received FDA approval for treating AD, but these treatments only temporarily relieve the AD symptoms, they do not cure or treat the underlying neurodegeneration. The five currently approved drugs for treating AD are: *Donepezil* (Aricept), *Galantamine* (Razadyne), *Rivastigmine* (Exelon), *Tacrine* (Cognex), and *Memantine* (Namenda). The first four are acetycholinesterase (AChE) inhibitors, while Namenda is an NMDA (N-methyl-D-aspartate) modulator that decreases excitotoxic glutamate. The AChE inhibitors block the enzyme that breaks down acetylcholine (a neurotransmitter that helps in memory formation) thereby increasing the levels of acetylcholine in the synapse, but the benefits are temporary (Adams, 2013). These drugs only help AD patients perform daily activities, and may temporarily aid symptoms related to logic, memory, and speaking (Alzheimer's Disease Progress Report, 2011).

Future AD treatments in clinical trials (discussed below) are being researched attempt to slow the disease and inhibit AD progression. Because Aβ plays a central role in AD, current strategies aim to block toxic Aβ formation, remove existing Aβ, or reverse Aβ-induced neurodegeneration (Adams, 2013). Other lesser-known research areas devoted to AD treatment strategies include treating tau tangles, targeting apolipoprotein E gene (APOE), using anti-inflammatory drugs, and using drug combinations.

Due to the common acceptance of the amyloid cascade hypothesis, a large amount of research is devoted to Aβ modulation. Altering toxic Aβ can be achieved through inhibition of beta or gamma secretase proteins in the brain, or Aβ itself can be modulated to prevent its aggregation into the toxic oligomers that strongly contribute to the neurodegeneration. Merck has developed a gamma-secretase inhibitor that went into clinical trials (see below), while Purdue University has patented a beta-secretase inhibitor. Elan Pharmaceuticals is working on developing an AD vaccine that removes existing Aβ plaques. Additionally, David Adams laboratory focuses on using neurotrophic factors for regenerating the brain following neurodegeneration (Adams, 2013). Unfortunately, the search for a true disease-modifying AD treatment continues, and the world remains relatively far away from having a commercial treatment.

AD Clinical Trials

Although Aβ has been shown to initiate AD, clinical trials to block its formation by blocking gamma-secretase (Merck), or by removing existing Aβ (Elan Pharmaceuticals, Pfizer's bapineuzumab, Genentech's crenezumab, Eli Lily's solanezumab) failed in Phase III clinical testing. Scientists who argue against the amyloid cascade hypothesis claim these failures prove

that Aβ is not a cause of AD, or the patient's cognition would have improved. But proponents of the cascade hypothesis point out that the treatments were only initiated very late in the patient's prognosis, perhaps far too late for Aβ removal to do any good. Thus, three upcoming clinical trials, expected to begin this year, will attempt to prevent dementia *much earlier* in AD patients by treating early-onset individuals known to be at risk for the disease decades before they develop symptoms (Miller, 2012). Families with an inherited form of AD will be targeted in their 30's instead of their 70's. One trial is affiliated with the Dominantly Inherited Alzheimer Network (DIAN), another trial is led by the Alzheimer's Prevention Initiative (API), and the third is termed the Anti-Amyloid Treatment of Asymptomatic Alzheimer's (A4) (Miller, 2012).

The DIAN trial will contain 240 participants who have mutations in any of the three genes linked to early-onset AD: PSEN1, PSEN2, or APP. The first stage of the trial will test three anti-amyloid treatments, the exact drugs have yet to be determined, which will target Aβ by either slowing its production or clearing it from the brain (Miller, 2012). The initial phase will assay several biomarkers to identify the most promising drug candidate for a follow-up phase to examine cognitive effects (Miller, 2012). Recent work by DIAN researchers show that Aβ concentrations in cerebrospinal fluid dip up to 25 years before the onset of AD symptoms (as Aβ accumulates in the brain it appears to be removed from the CSF). Brain scans show amyloid accumulation and atrophy at least 15 years prior to the onset of symptoms (Miller, 2012). Therefore, researchers will monitor these levels and other biomarkers during the first stage of the trial, which is scheduled to last 2 years (Miller, 2012).

Similarly, the API trial will be conducted in Colombia, South America, where 300 members of a very large Colombian early-onset family will participate. 100 members are known to be carriers of the mutated PSEN1 gene (Miller, 2012). The API trial will test the drug

crenezumab, an anti-amyloid antibody developed by Genentech. Crenezumab was chosen as the candidate drug because animal studies suggest it effectively clears several different forms of Aβ. In addition, crenezumab was designed to avoid the harmful inflammatory side effects of other anti-amyloid antibody treatments, such as swelling caused by cross-reactivity of the Aβ antibody with APP, and micro-hemorrhages in the brain caused by leaky blood vessels (Miller, 2012). So far, no evidence of these side effects have been observed in Genentech's clinical trials in mild to moderate AD patients. The drug will be used to measure changes in several cognitive tests, and API researchers will collect biomarkers and brain scans to measure Aβ accumulation and brain atrophy in real time (see below) (Miller, 2012). The API trial is designed to last about 5 years.

Unlike the API and DIAN trials, trial A4 will take a different approach to prevent the far more common non-genetic form of AD (Miller, 2012). This trial will include participants who are 70 and older and who test positive on a scan for Aβ accumulation in the brain (Miller, 2012). 1500 healthy seniors will be used, of which 500 test positive for Aβ on the scans (Miller, 2012). 500 of the Aβ-positive participants and 500 of the Aβ-negative elderly controls will take part in a 3-year trial that will track changes in cognition (Miller, 2012). The other 500 Aβ-negative participants will partake in a parallel study of aging and cognition (Miller, 2012). The API team has yet to select the drug candidate, but is watching closely the bapineuzumab trials and phase III clinical trials of solanezumab, an anti-amyloid antibody developed by Eli Lilly (Miller, 2012).

AD Diagnostics

The long held method for determining that a patient has AD is autopsy of the brain at time of death to search for plaques and tangles. Prior to death and autopsy, a suspected patient was said to have Alzheimer-type pathology by looking through the patient's medical history, physical exams, memory tests, and other mental assessments. But recent research has developed

methods for detecting Aβ in living patients, early and accurately. Biomarker tests and the use of magnetic resonance imaging (MRI) scans for early AD diagnosis are two growing areas of research generating promising results that can someday be used in general medical practice.

Biomarker tests measure a compound in the body that accurately indicates the presence or absence of disease or the chance of developing the disease. Two major categories of biochemical AD markers are cerebrospinal fluid (CSF) and plasma markers. Studies report several CSF biomarkers showing consistent elevations in AD, as well as predicting the conversion of normal to cognitive impairment. Combinations of CSF markers $A\beta_{42}$ and tau have proven particularly promising as potential diagnostic and prognostic biomarkers with accurate sensitivity and specificity (Teunissen *et al.,* 2002; Hampel *et al.,* 2008). Plasma markers, on the other hand, have provided mixed results. Most are unable to distinguish AD from controls, or predict progression from MCI to AD. But some proteomic studies of plasma and serum are promising (Teunissen *et al.,* 2002; Hampel *et al.,* 2008).

Along with biochemical markers, neuroimaging techniques are increasingly used to detect brain changes associated with AD, and have potential use as markers of disease progression, monitors of therapeutic effects, and predictors of future dementia prior to symptoms. These imaging techniques include computed tomography (CT), magnetic resonance imaging (MRI), functional MRI (fMRI), positron emission tomography (PET), and single photon emission computed tomography (SPECT) (Mentis, 2000; Zhang *et al.,* 2008; Vermuri *et al.,* 2010; Prvulovic *et al.,* 2011). Each imaging technique appears to provide predictive value, and the appropriate sensitivity and specificity. However, most imaging techniques are not ready for widespread use. They are laborious and time-consuming, and are only used for clinical studies and as an end point for treatment effects. PET has been shown to give an excellent distinction

between controls and AD patients, and a combination of FDG-PET and PIB-PET shows promise for distinguishing MCI and AD (Ramanan et al., 2013). However, other imaging markers need further development for accuracy in the early diagnosis of AD.

Alzheimer's Mouse Models

Other than higher primates, animals do not normally get AD, so models had to be devised to test treatments. The original Alzheimer's mouse model was created by Games *et al*. in 1995. This PDAPP model used the PDGF promoter to express a variant of human amyloid precursor protein found in an Indiana early-onset AD pedigree (Murrell et al., 1991). The variant caused an increased expression of Aβ in the cortex, corpus callosum, and hippocampus at 5-14 times the amounts in wild type mice (Games *et al*., 1995). The mice show age related memory loss, synaptic loss, and reduction in size of the hippocampus, corpus callosum, and fornix, comparable to AD patients (Kobayashi and Chen, 2005). The fact that young mice show an increase in Aβ but no hippocampal reduction supports the amyloid cascade hypothesis as Aβ being the initiator.

Since the original 1995 model, other models have been developed. A second Aβ-producing mouse model was created by over-expressing the Swedish double mutant form of human APP695 (Hsiao et al., 1996). These transgenic mice, called the Tg2576 mouse, are similar to the PDAPP mice except they do not exhibit the synaptic loss or reduction in hippocampal size. Many other transgenic mouse models also have been created, including multiple gene transgenic mice, the first of which was developed to express mutated presenilins and human APP (Holcomb et. al 1998). Presenilins are enzymes containing gamma-secretase activity, and are required for the accumulation of $A\beta_{1-42}$ (Brunkan and Goate, 2005)

The transgenic mice used in this MQP (JAX stock #034843 or #007027) are 3X transgenic for the human APP Swedish mutation K670N/M671L, the Dutch mutation E693Q, and the Iowa mutation D694N, each of which is found in early-onset pedigrees and results in the increased production of Aβ. The APP expression is under the control of the mouse thymus cell antigen-1 *Thy1* promoter to ensure expression in the cortex, hippocampus, and corpus callosum. The mice are commercially available from the Jackson Laboratory, and were originally engineered by William Van Nostrand's lab at Stony Brook University (Davis et al., 2004) in a C57 background strain. The AD strain shows Aβ plaque deposition beginning at 3 months of age in the subiculum, hippocampus, and cortex. After 6 months of age, the plaques have spread to the olfactory bulb and thalamic region. By 12 months of age, the plaques are found throughout the entire forebrain. Due to the hardy nature of this strain, they can be bred as homozygotes, eliminating the need for genotyping the offspring. The wild type C57 mice used as controls in this MQP represent the same background as the AD, and are one of the most commonly used inbred strains in biological research.

AD Mouse Model Behavior

Most of the successful AD mouse models show behavioral changes. The behavioral alterations mimic those in AD patients, and can be used to monitor the efficacy of potential AD therapies in the model. Only a few AD mouse behavioral studies have been performed to date (Moran *et al*., 1995; Nalbantoglu *et al*., 1997; Chen *et al*., 2000; Boutajangout *et al*., 2010; Cramer *et al*., 2012). Because Aβ production in the hippocampus alters memory, most of the behavioral tests measure hippocampal-dependent spatial-based learning and memory, as is altered in AD. Most of the tests showed conflicting results, including the rotarod test, general

activity test, body temperature test, elevated maze test, and the novel objective recognition test. However, some of the previous studies agree that the Morris water test is capable of distinguishing WT from AD mice.

The Morris water maze is a sensitive test used to examine memory in rodents (Morris, 1984). Based on the few studies done so far with AD mice, this test appears to be the most reliable for distinguishing AD from WT mice. It is particularly sensitive for hippocampal function, one of the earliest and most affected brain regions in AD (Bryan et al., 2009). To perform this test, in the training phase, the mouse is gently placed in a circular tank of room temperature water and is timed as it searches to find a marked platform. In the subsequent phase, the mice are timed as they locate a hidden platform, whose position has been moved relative to the training phase. Several trials are performed daily, and the mice usually learn to find the platform faster and with less distance traveled. Modern versions of the test use a camera and computer software to track latency time, distance traveled, and time spent in each quadrant. Using marked, unmarked, and probe trials (with no platform) the mouse's ability to retrieve and retain learned information can be tested.

Past studies using the Morris test have shown that AD mice show age-related deficits in spatial learning (Moran *et al.,* 1995). In the Moran 1995 study, no statistically significant difference was found between 6-12 month old WT and AD mice in the initial visible platform training phase, but in the 2nd and 3rd no platform probe trials, the WT mice searched the correct training quadrant significantly more than the AD mice. This demonstrates that the AD mice have deficits in memory affecting their ability to remember the location of the platform. While all mice in general improve their performance over several days training, WT mice improve faster (Moran *et al*., 1995; Nalbantoglu *et al.*, 1997).

David Adams Laboratory Overview

The Adams laboratory at WPI focuses on the use of neurotrophic factors (NTFs) as a potential treatment for AD. NTFs function during normal development to help nerve cells survive and divide when the nervous system is initially forming. So, it is hypothesized that the use of such factors may facilitate neuronal survival during AD. However, full-length NTFs do not efficiently cross the blood-brain barrier (BBB) when injected intravenously, intra-peritoneally, or when taken orally, so our lab's approach is to use short peptide fragments that mimic the action of full-length NTFs.

Our laboratory specifically focuses on the neurotrophic factor ependymin (EPN). EPN has been previously shown to be up-regulated in goldfish brains after performing a series of training and learning events (Benowitz & Shashoua, 1997). EPN has multiple functions, including facilitating optic nerve elongation and hippocampal long-term memory formation. After being characterized in mice, monkeys, and humans (Adams & Shashoua, 1994; Adams *et al.*, 1996, Apostolopoulos *et al.* 2001), EPN became a major interest in our lab as a possible treatment for neurodegenerative disorders. Our laboratory has shown that goldfish EPN peptide mimetics CMX-8933 and CMX-9236 induce neuroprotection both *in vitro* (Adams *et al.*, 2003) and *in vivo* (Sashoua *et al.*, 2003; 2004), and can upregulate anti-oxidative enzymes SOD-1, catalase, and glutathione peroxidase (Shashoua *et al.*, 2003; 2004). We also showed that treating human neuroblastoma cells *in vitro* with Aβ followed by goldfish EPN mimetics partially restores cell viability (Stovall, 2006), lowers caspase activation (Kapoor, 2007), decreases tau hyper-phosphorylation and TUNEL staining for apoptosis (Ronayne, 2008), and lowers cathepsin-D activity (Donahue and Lobdell, 2011). Recently, our laboratory showed that treatment of cultured human SHSY neuroblastoma cells *in vitro* with a short *human* ependymin

(hEPN) neurotrophic factor mimetic increases neuronal cell survival and increases cellular levels of anti-oxidative superoxide dismutase (SOD-1) (Jackson and Najem, 2012).

PROJECT PURPOSE

Although our lab has shown that neurotrophic factor mimetics can restore neuronal survival against an Aβ challenge *in vitro*, we have not yet shown whether the NTF treatment improves mouse behavior in an AD model. The purpose of this MQP is to develop the procedure for performing the Morris water swimming test in-house at WPI, and determine whether this test can distinguish the behavior of WT from 3X transgenic AD mice at 4 months of age, an age that is one month past the published age at which they first begin to show cognitive decline. In addition, an *in vitro* model for AD, in which cultured human SHSY neurons are treated with a variety of Aβ batches, will be used to compare the changes in cell morphology, as part of the initial phase of identifying potential NTF peptides to test *in vivo*.

METHODS

AD Mouse Model

Homozygous 3X transgenic AD mice were purchased from The Jackson Laboratory (strain #034843-JAX; stock number 007027). This strain is a triple transgenic, containing three early-onset mutations in the human transgene. The mutations lead to high levels of neurotoxic Aβ production when that peptide is cleaved inappropriately from the APP protein. AD female mice were bred to AD male mice in-house at WPI to produce AD offspring for testing. The WT mice were also purchased from The Jackson Laboratory (stock number 000664). This strain has the same C57 genetic background as the selected AD mice. WT males were bred to WT females in-house at WPI to produce WT offspring for comparison to age-matched AD mice. All pups were aged to 4 months to allow the cognitive decline to initiate, while leaving them young enough to swim well. 3 WT males, 2 WT females, 3 AD males, and 2 AD females were used in this study.

Morris Water Swimming Test

All tests were performed during the animal's light cycle phase of the day. Food and water were provided in the animal's cages throughout the entire experiment ad libitum. The water tank consists of a circular pool (120 cm diameter x 76 cm height) filled with water. The goal platform for the mice to escape onto was a 10 cm x 10 cm square made of Plexiglas. The water was kept at room temperature during all testing, and when changed was allowed to warm overnight to room temperature. The pool was divided geographically into four quadrants, with four equidistant mouse release points on the peripheral edge of each quadrant. Various cues were placed on the walls outside the maze, and all trials were video recorded from above the pool for

later analysis. The software records the time required to locate the platform and the total distance travelled.

Animals were monitored at all times they were in the pool. Any test in which a mouse became submerged was immediately terminated by removing the mouse from the pool. All mice were placed for 45 seconds into a clean cage with dry towels between each trial. The pool water was drained and cleaned after every 3-4 days of testing. Three types of tests will be performed:

	Test-1 Visible Platform Testing	Test-2 Unmarked Platform Testing	Test-3 Probe Trial
Testing Day:	**1 2 3**	**4 5 6 7 8**	**9**

Test-1: Visible Platform (Cued) Testing

On days 1-3, visible platform tests will be performed. In this portion of the testing, the platform was positioned halfway between the pool wall and the pool center in one of the four quadrants, and positioned flush with the water surface (visible). **Fixed Platform Location:** The platform remained in the *same* position throughout this portion of the tests. **Release Points:** the starting points of each of four trials per day (see below) varied; the starting points consisted of *each* of the four possible release points (on the edges of each tank quadrant) in random order (two of the start points are relatively close to the platform, and two are slightly further from the platform). Using all four release points ensured that no mouse was released from the same point on the same training day (which would give it an unfair advantage). Using different release points forced each mouse to use visual cues to locate the platform. **Four trials** were conducted per day for three days. The trial was started by the handler placing the mouse gently into the water from the start site, and the trial ended by either the mouse climbing onto the platform or by

a 90 second timeout if the mouse could not locate the platform. In the case of the timeout, the mouse was gently placed onto the platform. All mice were left on the platform for 15 seconds to help teach them the location of the platform, prior to being towel dried and placed in a clean holding cage for 45 seconds between trials.

Test-1 is a relatively simple swimming task to locate a visible platform, and any problems observed here would suggest impairment of motor function or a decrease of simple cognitive processing.

Test-2: Unmarked Platform Testing

On days 4-8, unmarked platform testing was performed. This tested their ability to locate a submerged platform, which requires a functional hippocampus for efficient performance. Five days of testing were conducted with four acquisition trials per day. **Platform Location:** The platform was located 0.5 cm underwater and remained in the *same* quadrant of the pool for all testing. **Release Points:** As before, the mice were released from each of the four possible release points on the edge of the tank in random order. After reaching the platform, mice were allowed to remain on the platform for 15 seconds. If the mice failed to reach the platform within 90 seconds, they were gently placed on the platform for 15 seconds. As before, between trials, the mice were placed in a clean cage with dry towels for 45 seconds.

Test-3: Probe Trial

On day-9, one probe trial was conducted the day after the last unmarked platform trial. For the probe trial, the platform was completely removed from the tank, and the animal's search behavior was recorded. The amount of time spent swimming in each quadrant was monitored for

90 seconds as an index of memory retention. High functioning mice should spend the most amount of time in the quadrant previously containing the platform during the unmarked tests. Mice will either be timed out at 90 seconds, or will be immediately removed if they cannot swim.

Notes on Mouse Safety During Behavioral Tests:

1. Water Temperature: The water used for swimming was room temperature. The temperature was tightly controlled inside the Vivarium where the tank was placed.

2. Experiment Termination: If any mouse could not keep his nose above water, it was immediately retrieved from the water, and not tested further in any experiments.

3. Time-Out Period: The maximal swimming time was 90 seconds for any trial. If a mouse could not locate the platform in 90 seconds, it was placed on the platform and left there for 15 seconds (to help it remember the location). Published protocols use a maximum of 1-2 minutes for the time-out. We chose 90 seconds (towards the upper limit) to help display a difference between WT and AD mice.

4. Rest Period Between Trials: All mice were rested 45 seconds between trials in a clean dry cage with toweling. Published protocols vary widely for the rest period, from 30 seconds to 20 minutes. We chose 45 seconds to allow a brief rest, as we wish to push the mice here to help distinguish WT from AD mice.

5. Water Cleaning: The pool was drained and cleaned after every 3-4 days of testing.

Morris Water Maze Computer Program

The Morris Water Maze system was purchased from Coulbourn Instruments, and was run in accordance with their manual with a few adjustments. The WPI system was set up in the Vivarium on the second floor of WPI's Life Sciences and Bioengineering Center. Since the system and experiment was new to WPI, troubleshooting had to be done to adjust the system to the facility. To summarize and clarify the instructions for running the maze, a small tutorial can be found below as well as a screenshot of the programs analysis functions (**Figure-3**).

Using the WaterMaze Software

1. Log on to computer

- Username: staff
- Password: staff123

2. Make sure camera is plugged in
3. Test camera in STCamSW
4. Open WaterMaze software and open an existing project or make a new project
    - To open an existing experiment: Project → Open then select the project file you would like to open
    - To create a new project: Project → New
        - Adjust the number of animals in your new project by using the arrows next to the # of Animals box on the screen
        - Adjust the number of days in your new project by using the arrows next to the # of Days box on the screen
        - Adjust the number of trials per day by using the arrows next to Trials box for each day (NOT the # of Sets/Day box)
    - Save your new project: Project → Save As – then type in a file name for your project and press OK
5. Check pool and platform settings
    - For pool go to: Settings → Pool
        - Here you can adjust the pool diameter and move the pool outline to match the camera image
        - You can adjust the pool size/location by clicking and dragging the left and right edge of the outline. Use this to match up pool outline to the water in the camera image.
    - For platform go to: Design → Platforms
        - Here you can adjust the size and location of the platform
        - To adjust platform **size** use the arrows next to the platform size box on the screen
        - To adjust platform **location** click in the center of the platform and drag it to the desired location
6. Run a trial
    - Switch to Run Mode: Mode → Run – this will bring up the trial run screen
    - Select a trial
        - To run the next trial in an experiment click Run Next
        - To run a particular trial select the trial in the trial list and then click Run Selected
    - The pool image will come up – ensure that the pool outline and platform are properly located
    - When you are ready to start the trial press Reference
    - When the trial has ended press SAVE and the software will automatically bring you back to the trial run screen

7. Analysis
    - There are two different analysis screens – the Export Analysis Screen and the View Analysis Screen
    - The Export Analysis Screen can be found by pressing Analysis → Export from the trial run screen
        - In the Export Analysis screen you can export an Excel data file that has the project data for each animal, day, and trial.
            1. To create a file with data from all the trials from all the animals in the project you first need to select all of the animals and all of the trials. Do do this click Select →All Animals – All Trials
            2. To create a file with data from all the trials from only a selected animal in the project click Select → Selected Animal – All Trials
            3. To export the Excel file click Export Analysis → To File and enter a name for the Excel file. Click OK to save the file.
            4. To view this Excel file of the exported project data click the Windows Start button → My Documents → the file you just created (it will appear as "file name".csv)
        - In the Export Analysis Screen you can also view the density plots for each trial
            1. Select a trial in the project by clicking on it in the trials list
            2. Click Density Plots → View to view the density plot for that trial
        - To exit the Export Analysis Screen click File → Exit – this will bring you back to the trial run screen
    - The View Analysis Screen can be found by clicking Analysis → View from the trial run screen
        - In the View Analysis Screen you can see images of the paths taken in each trial
            1. View path images for each trial by clicking on the trial in the trials list
        - You can also view path videos from the View Analysis Screen
            1. View path videos for each trial by double-clicking on the trial in the trials list.
            2. This will bring up a separate window where you can watch a video of the trial overlaid with the path
            3. To exit the video screen click File → Save & Exit
        - To exit the View Analysis Screen click File → Exit – this will bring you back to the trial run screen

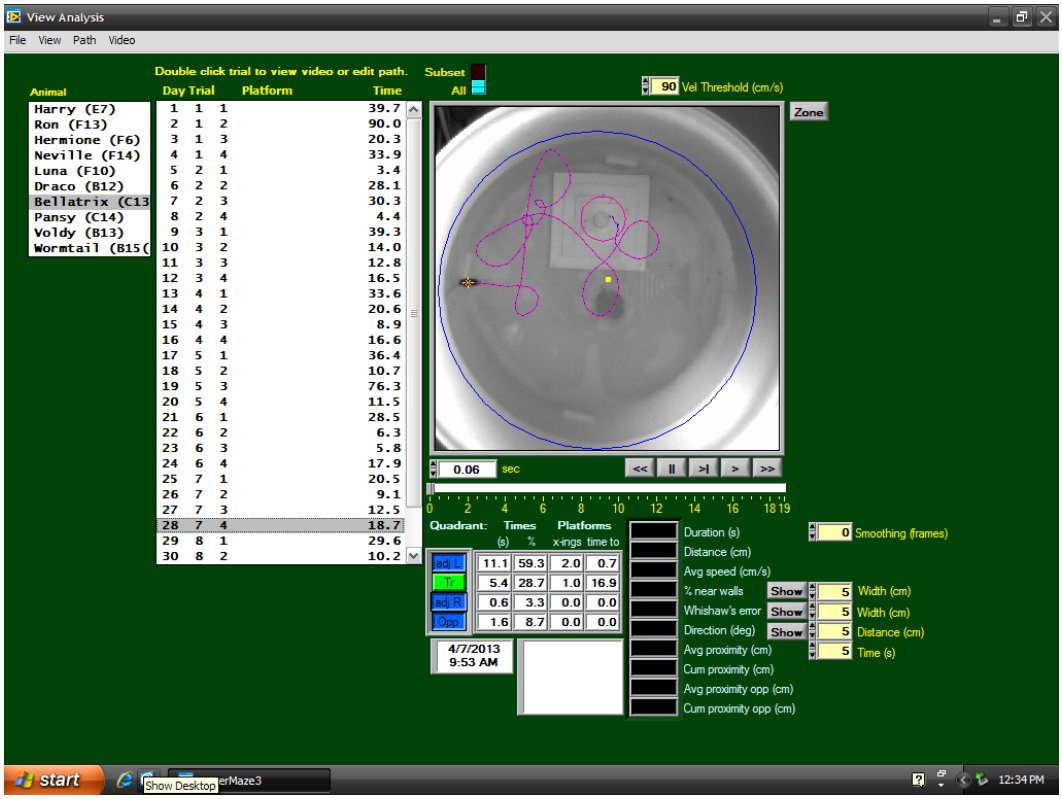

Figure-3: Morris Water Maze Analysis Screenshot.

The complete manual of the software can be found on the company's website:

http://www.coulbourn.com/v/vspfiles/assets/manuals/ACT-200%20WaterMaze%20Manual.pdf

Human SH-SY5Y Cell Culture

Human SH-SY5Y neuroblastoma cells (commonly termed SHSY) were obtained from liquid nitrogen stocks previously obtained from ATCC and expanded in our laboratory.

The culture medium was prepared in a 500 mL bottle by adding together 500 mL DMEM-F-12 (ATCC), 50 mL of FBS (Hyclone) (to give a final concentration of 10%), and 0.275 mL of 10 mg/mL gentamycin (Gibco) (to make give a final concentration of 5 µg/mL). The mixture was filter sterilized using a 0.2 µm filter and stored at 4°C.

SHSY cells were thawed from liquid nitrogen storage and placed in a 37°C water bath for 1-2 minutes until completely thawed. The vial was wiped down with reagent alcohol, and the cells were carefully re-suspended and transferred to a T-25 flask containing 4 mL of pre-warmed culture medium. The flask was placed in a 37°C + 5% CO_2 humidified incubator. After 24 hours of incubation, the spent medium was siphoned and replaced with fresh, pre-warmed medium.

The cell cultures were fed every 3-4 days until the flasks achieved approximately 80% confluency or above. To feed the cell cultures, the old medium was aspirated and replaced with 4 mL of fresh, pre-warmed medium. Once the cells reached a confluency of 80% or above, the cells were split 1:2 (never greater than this) into new flasks. To split the cultures, the old medium was aspirated and replaced with 8 mL pre-warmed medium and the flask was scraped to release the cells from the floor of the flask. The cells were then re-suspended and 4 mL of the suspension was pipetted into each of two new flasks.

Splitting the cells greater than 1:2 was found to decrease the cell density low enough to enter a lag phase of growth. Flasks that were at low density were pooled together and replaced with fresh complete medium. Additionally, splitting the cells for a long time can cause the cells to attach in clumps and in several layers, making it difficult to get an accurate count of cells. In order to break apart clumps, 1 mL of trypsin was added to the cell flask, placed in the 37°C incubator for 2-3 minutes, and then 5 mL of complete medium was added to neutralize the trypsin. The cells were plated overnight, and the medium was replaced the following day. Using trypsin might send the cells into a slightly longer lag phase of growth.

When a T-25 flask was 90% confluent, the cells were frozen down by siphoning off the spent medium and replacing it with 1 mL of pre-warmed freezing medium (Gibco). The cells were scraped and re-suspended, then pipetted into a cryovial, and stored overnight at -80°C in a

Styrofoam® rack for insulation to slow the freezing process. After 12-24 hours, the cells were moved to liquid nitrogen storage.

For plating experiments, T-25 flasks that were 80% or above confluent (3 plates) were split and plated onto six T-25s. For Aβ treatment conditions, four different 1.0 mM Aβ batches dissolved in 1 mM Sodium Bicarbonate were introduced to the flasks at a final concentration of 20 μM (80 μL of 1 mM stock per 4 mL medium). The Aβ-treated flasks were cultured up to 72 hours, at which point cells were subjected to morphology cell counts. Untreated, control flasks generally reached approximately 80% confluency after 72 hours.

Cell Morphology Counts

Twelve representative regions of each experimental flask were imaged at 72 hours post-plating by a Leica inverted microscope and camera attachment at 10x magnification. Images were used to count unconnected neurons (solo stellate cells), dying cells (solo non-stellate cells), and total cells per viewing field. Counts from all twelve representative regions of each flask were averaged.

RESULTS

The purpose of this project was to establish a procedure for performing the Morris water swimming test in-house at WPI, and to determine whether this test can distinguish between WT and 3X transgenic AD mice at 4 months of age, an age that allows the cognitive decline to initiate while leaving them young enough to swim well. Additionally, cultured human SHSY neuronal cell cultures were tested with a variety of Aβ peptide batches as an initial step towards helping identify NTF candidates for testing *in vivo*.

In Vitro Alzheimer's Model Testing With Several Aβ Batches

Our laboratory previously demonstrated that adding Aβ to human SHSY neuroblastoma cells in culture is an effective *in vitro* model of AD. The Aβ increases neuronal cell death (Stovall, 2006), increases caspase-3 activation levels similar to AD patients (Kapoor, 2007), increases tau hyper-phosphorylation as in AD (Ronanyne, 2008), increases apoptotic TUNEL staining (Ronayne, 2008), and increases cathepsin-D activity levels (Donahue and Lobdell, 2011). When simultaneously adding an NTF mimetic with the Aβ, all these effects decreased. This *in vitro* system was used in this project to test a variety of Aβ batches, as a prelude for identifying NTF peptides for testing *in vivo.*

To compare the activity of a variety of Aβ batches on cell morphology, an *in vitro* assay was set up using an SHSY human neuronal cell line. At the time of flask splitting, four flasks received 20 μM of one of the four Aβ batches, while the fifth flask was left untreated as a control. The five flasks were imaged after 72 hours using a Leica inverted microscope

microscope and camera attachment. The images were divided into twelve regions to count the total number of cells, number of unconnected neurons, and the number of dying cells (**Figure-7**). All four Aβ batches showed the desired effect of a significant ($p < 0.001$) decrease in total human neuronal cell numbers, and a significant increase ($p < 0.001$) in unconnected neurons in two of the Aβ treatments and a slight increase ($p < 0.05$) in the other two. The dying cell data was inconclusive for this experiment.

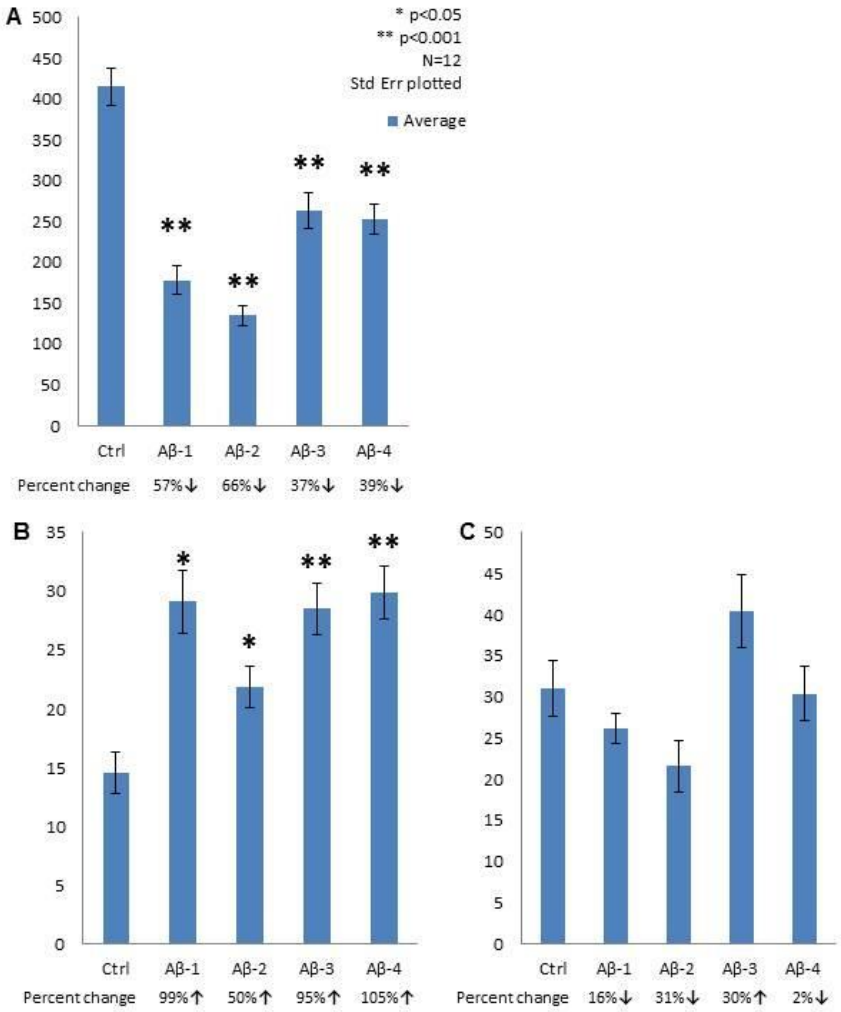

Figure-7: All Four Aβ Batches Tested Strongly Affect Human Neuronal Cells. Cell morphology determinations after 72 hr treatment with Aβ. **A**) Average (N=12) of total raw cell counts. **B**) Average (N=12) of unconnected neurons (solo stellate) raw cell counts. **C**) Average (N=12) of dying cells (solo non-stellate) raw cell counts. Each histobar represents the average of twelve representative regions of one experimental flask. * $p<0.05$. ** $p<0.001$.

Morris Water Maze Test of Mouse Cognitive Performance

As described in the Methods section, the swimming behaviors of 5 AD mice and 5 WT mice were observed using a camera placed above a water tank. In the first three days of testing (cued test), the platform was raised to the water surface and marked with black tape. The visible cue was removed and the platform slightly submerged during the next five days of testing (unmarked or non-cued test). Then on the last day of testing (probe test), the platform was completely removed from the tank. The time it took for each animal to locate the platform, the length of swimming, and the time spent in each hemisphere were recorded. **Figure-4** shows example swimming paths recorded by the WaterMaze program.

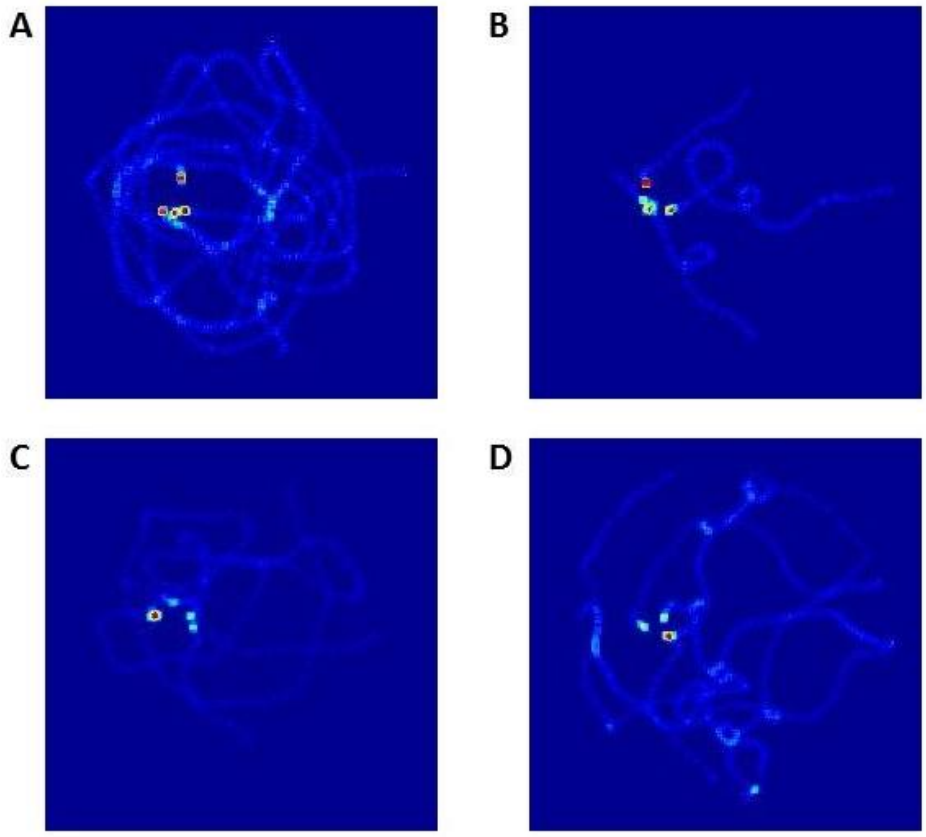

Figure-4: Selected Mouse Swimming Density Graphs. Shown are typical swimming graphs for WT mice (A, B) and AD mice (C, D) for unmarked trial day-1 (A, C) and day-5 (B, D). Note the reduced swimming path for WT mice after 5 days of training (B).

In phase-1 cued tests using a *visible* platform, all mice improved their time to locate the platform over the 3 days of training (**Figure-5A)**. Surprisingly, the WT mice (blue curve) appeared to take slightly longer to locate the platform than AD mice (red curve). Statistical differences between WT and AD mice were observed on Day 2 and Day 3 ($p < 0.05$ single asterisks). The mice slightly increased their time spent in the target hemisphere, with AD mice performing slightly better than WT mice (**Figure-5B)**. After 5 days of testing with the unmarked platform, all mice showed a slight improvement in time to platform (**Figure-5C)**. No statistical difference was observed between WT and AD mice. WT mice increased their time spent in the target hemisphere containing the unmarked platform, while AD mice spend a decreased amount of time in the target hemisphere (**Figure 5D**).

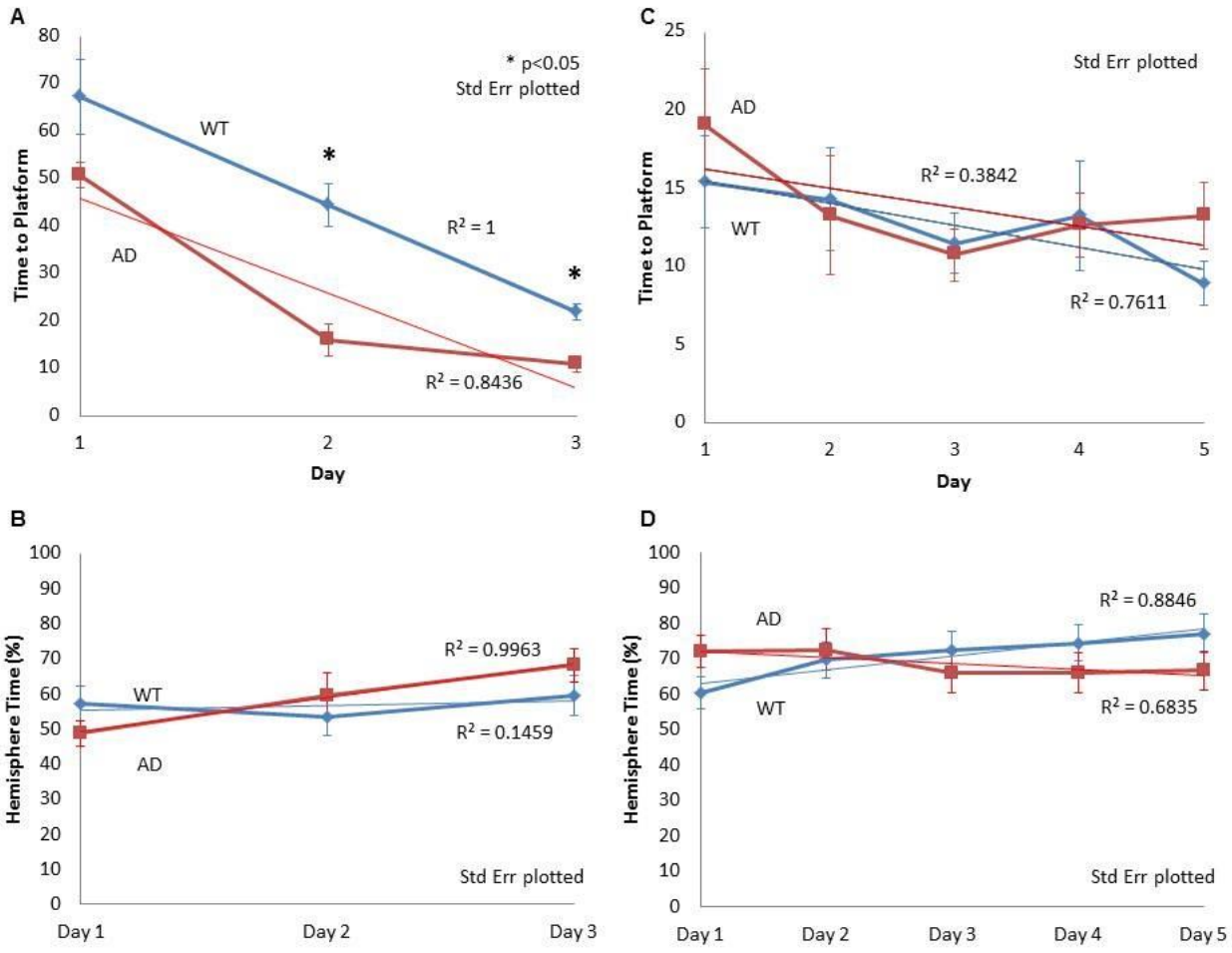

Figure 5: AD Mice At 4 Months of Age Are Indistinguishable from WT Mice in a Morris Water Maze test. WT and AD mice were tested over a three day period using a *marked* platform (**A and B**) and then over a five-day period using an *unmarked* platform (**C and D**). **A**) Average time to locate marked platform over three days. **B**) Average percentage of time spent in the target hemisphere containing the marked platform. **C**) Average time to locate the unmarked platform. **D**) Average percentage of time spent in the target hemisphere containing the unmarked platform.

On day-9 of the tests, the platform was removed (probe test) and the amount of time the mice spent in the target hemisphere (where the platform used to reside) was tracked (**Figure-6**). All mice spent more time in the correct location, with WT mice (blue) and AD mice spending approximately equal time in the correct area (**Figure-6**).

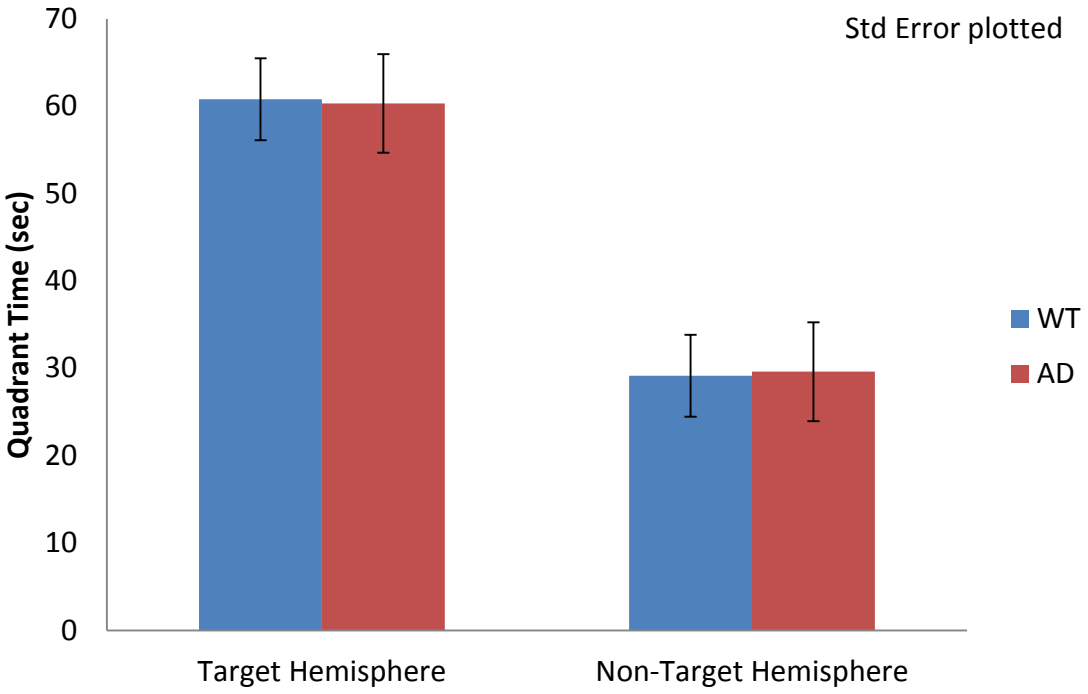

Figure-6: Probe Test. WT mice (blue) and AD mice (red) spend about equal amounts of time in the target hemisphere (previously containing the platform) during the probe trials.

DISCUSSION

This project extended our laboratory's previous *in vitro* and *in vivo* AD experiments by setting up the Morris water maze test in-house at WPI. Previous research in our lab showed that treating human neuroblastoma cells *in vitro* with Aβ followed by *goldfish* EPN mimetics partially restores cell viability (Stovall, 2006), lowers caspase activation (Kapoor, 2007), decreases tau hyper-phosphorylation and TUNEL staining for apoptosis (Ronayne, 2008), and lowers cathepsin-D activity (Donahue and Lobdell, 2011). Recently, treatment of cultured human SHSY cells with a short *human* ependymin (hEPN) neurotrophic factor mimetic increased neuronal cell survival and increased cellular levels of anti-oxidative superoxide dismutase (SOD-1) (Jackson and Najem, 2012) following Aβ treatment. This MQP extended these studies by setting up a mouse behavioral test in-house at WPI that will eventually be used to determine whether NTF mimetics can improve mouse behavior.

In Vitro Alzheimer's Model

Our lab's *in vitro* neuronal cell morphology assay was used to test four different Aβ batches. After 72 hours of treatment, each batch showed significant ($p<0.001$) cell morphological alterations relative to control cultures, indicating the Aβ is chemically active. It would have been interesting to monitor the changes in cell morphology at each day of treatment, but the 72 hr time point was used by our lab in the past. The next step would be to use the Aβ samples in the *in vitro* model to test the activity of a variety of NTF mimetics, and then test the most active NTF in the *in vivo* model.

Morris Water Maze Test

Previously, several researchers have attempted to use a variety of behavioral assays to distinguish WT and AD mice, including using the Y-maze, Rotarod, Elevated Plus-Maze test, and others. However, several of the tests show minimal differences between AD and WT mouse behavior, suggesting the assays cannot easily differentiate the two groups. However, one study in particular used transgenic female mice homozygous for human β-APP and showed the Morris water maze test to be effective in distinguishing AD and WT mice (Moran *et al.*, 1995).

In our tests, the data indicated that at 4 months of age, this strain of AD mice show a behavior that is generally indistinguishable from WT mice. The marked platform trials showed a significant difference that AD animals perform slightly better than WT animals. On all three days of marked trials, the AD mice located the platform faster, although the R^2 slope of the WT mice is higher than that of the AD indicating a steeper learning slope. Several theories could explain this data. This may reflect the low sample number of mice tested, their young age in which hippocampal degeneration is not yet significant, or we may need a different AD mouse strain. In a study conducted by Garcia-Osta and Alberini (2009), researchers showed Aβ-42 in low concentrations when injected into rats appears to mediate memory formation and enhancement. This indicates that elevated levels of Aβ may *increase* brain function initially before the higher levels begin to cause neurodegeration. Also, since AD mice can show erratic behavior it could be that they are swimming faster covering a greater area over the given time.

In the unmarked platform trials, there was no statistical difference between WT and AD mice. It was hypothesized that WT mice would locate the platform more quickly due to the cognitive deficit of AD mice. Again the R^2 value was higher for the WT mice indicating a steeper learning slope.

In the probe trials, where the platform was completely removed from the tank, both AD and WT mice spent more time searching for the platform in the correct target hemisphere (where the platform used to reside), and the WT spent about the same time in the target hemisphere as the WT mice.

Using the WaterMaze system from Coulbourn Instruments required troubleshooting several problems. When originally setting up the system, it we learned that is important to perform several steps not found in the manual. Lighting in the room is an important factor in getting accurate measurements. Glare from lights on the water will interfere with the tracking software and prevent the tracking of the test animal. By covering overhead lights and adjusting the light settings in the camera software, this problem can be solved. Also, if problems still occur, adjusting the setting on the removable camera lens can remedy it.

Setting up an experiment in the Coulbourn software requires centering the platform in the quadrant of interest. In the platform setup menu, the camera view of the tank will be visible as well as an adjustable setting for the water maze and four lines dividing the tank. These are not the dividers for the quadrants but rather these indicate the diagonals for the quadrants. When orienting the platform, these lines indicate the center of the quadrants and are helpful for accurate placement.

When running a trial, timing is the hardest factor to compensate for. The reference frame should be taken with no obstacles in the frame of the camera. Any movement or change in the image (such as the experimenter's shadow) will disrupt the tracking software. Once in the reference frame, but before the start button is pressed, the experimenter should prepare to place the mouse in the appropriate drop site, and have some way to dry the mouse after the trial. As quickly as possible the experimenter should gently place the mouse in the tank and move out of

frame before the video begins to record the animal's swimming path. To allow time for the experimenter to get out of frame, the mouse can be gently placed in the tank facing the tank wall. Once the experimenter is clear, the start button should be pressed and the trial can be run as the protocol prescribes.

Future experiments include aging the AD mice to 6 months, using a larger sample size, possibly trying a different AD genetic strain, and trying a different behavioral test, such as the Novel Object Recognition test.

BIBLIOGRAPHY

Adams D, Shashoua V (1994) Cloning and sequencing the genes encoding goldfish and carp ependymin. *Gene,* 141: 237-241.

Adams D, Kiyokawa M, Getman M, Shashoua V (1996) Genes encoding giant danio and golden shiner ependymin. *Neurochemical Research,* 21: 377-384.

Adams DS, Hasson B, Boyer-Boiteau A, El-Khishin A, Shashoua VE (2003) A Peptide Fragment of Ependymin Neurotrophic Factor uses Protein Kinase C and the Mitogen-Activated Protein Kinase Pathway to Activate c-Jun N-Terminal Kinase and a Functional AP-1 Containing c-Jun and c-Fos Proteins in Mouse NB2a Cells. *Journal of Neuroscience Research,* 72: 405-416.

Adams DS (2013) Alzheimer's Disease: Treatment Approaches with Neurotrophic Factors. WPI Department of Biology and Biotechnology.

Alzheimer's Association (2012) Alzheimer's disease facts and figures. *Alzheimer's and Dementia: The Journal of the Alzheimer's Association*. March 2012; 8: 131–168.

Apostolopoulos J, Sparrow RL, MclEod JL, Collier FM, Darcy PK, Slater HR, Ngu C, Gregorio-King CC, Kirkland MA (2001) Identification and characterization of a novel family of mammalian ependymin-related proteins (MERPs) in hematopoietic, nonhematopoietic, and malignant tissues. *DNA Cell Biology,* 20: 625-635.

Benowitz LI, Shashoua VE (1997) Localization of a brain protein metabolically linked with behavioral plasticity in the goldfish. *Brain Research,* 11(2): 227-242.

Boutajangout A, Quartermain D, Sigurdsson EM (2010) Immunotherapy Targeting Pathological Tau Prevents Cognitive Decline in a New Tangle Mouse Model. *Journal of Neuroscience,* 30(49): 16559–16566.

Brunkan AL, Goate AM (2005) Presenilin function and gamma-secretase activity. *Journal of Neurochemistry*, 93: 769–792.

Bryan KJ, Lee H, Perry G, et al. (2009) Transgenic Mouse Models of Alzheimer's Disease: Behavioral Testing and Considerations. In: Buccafusco JJ, editor. Methods of Behavior Analysis in Neuroscience. 2nd edition. Boca Raton (FL): CRC Press; 2009. Chapter 1. Available from: http://www.ncbi.nlm.nih.gov/books/NBK5231/

Chen G, Chen KS, Knox J, Inglis J, Bernard A, Martin SJ, Justice A, McConlogue L, Games D, Freedman SB, Morris Richard GM (2000) A learning deficit related to age and β-amyloid plaques in a mouse model of Alzheimer's disease. *Nature,* 408: 975-979.

Cramer PE, Cirrito JR, Wesson DW, Lee CY, Karlo JC, Zinn AE, Casali BT, Restivo JL, Goebel WD, James MJ, Brunden KR, Wilson DA, Landreth GE (2012) ApoE-directed therapeutics rapidly clear β-amyloid and reverse deficits in AD mouse models. *Science,* 335(6075): 1503-1506.

Davis J, Xu F, Deane R, Romanov G, Previti ML, Zeigler K, Zlokovic BV, Va Nostrand WE (2004) Early-onset and robust cerebral microvascular accumulation of amyloid-beta protein in transgenic mice expressing low levels of a vasculotrophic Dutch/Iowa mutant form of amyloid beta-protein precursor. *Journal of Biological Chemistry*, 279: 20296-20306.

Donahue M, Lobdell G (2011) Role of SOD and Cathepsin-D in Alzheimer's Disease Aβ Cascade Models. WPI Major Qualifying Project, May, 2011.

Games D, Adams D, Alessandrini R, et al. (1995) Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. *Nature*, 373: 523–527.

Garcia-Osta A, Alberini CM (2009) Amyloid beta mediates memory formation. *Learning and Memory,* 4: 267-272.

Hampel H, Bürger K, Teipel SJ, Bokde ALW, Zetterberg H, Blennow K (2008) Core candidate neurochemical and imaging biomarkers of Alzheimer's disease. *Alzheimer's & Dementia,* 4: 38-48.

Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G (1996) Correlative memory deficits, Ab elevation, and amyloid plaques in transgenic mice. *Science*, 274: 99-102.

Holcomb L, Gordon MN, McGowan E, et al. (1998) Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nature Medicine,* 4: 97–100.

Jackson T and Najem M (2012) Human Ependymin Neurotrophic Factor Mimetic Increases Alzheimer's Mice Cognitive Performance in a Morris Water-Maze Test. WPI Major Qualifying Project, May, 2012.

Jonsson T, Atwal J, Steinberg S, Snaedal J, Jonsson P, Bjornsson S, Stefansson H, Sulem P, Gudbjartsson D, Maloney J, Hoyte K, Gustafson A, Liu Y, Lu Y, Bhangale T, Graham R, Huttenlocher J, Bjornsdottir G, Andreassen O, Jönsson E, Palotie A, Behrens T, Magnusson O, Kong A, Thorsteinsdottir U, Watts R, Stefansson K (2012) A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature,* 488: 96-99.

Kapoor V (2007) Mechanism of reversal of Alzheimer's disease Aβ-induced neuronal degeneration in cultured human SHSY cells using a neurotrophic ependymin mimetic. WPI Master's Thesis, July, 2007.

Kawashima M and Ihara Y (2002) Alzheimer's Disease: β-Amyloid Protein and Tau. *Journal of Neuroscience Research,* 70: 392-401.

Kobayashi DT, Chen KS (2005) Behavioral phenotypes of amyloid-based genetically modified mouse models of Alzheimer's disease. *Genes, Brain, and Behavior*, 4: 173–196.

Luchsinger J and Mayeux R (2004) Dietary factors and Alzheimer's disease. *The Lancet Neurology,* 3: 579-587.

Masters CL, Cappai R, Barnham KJ, Villemagne VL (2006) Molecular mechanisms for Alzheimer's disease: implications for neuroimaging and therapeutics. *Journal of Neurochemistry,* 97(6): 1700-1725. http://www.ncbi.nlm.nih.gov/pubmed/16805778

Mentis MJ (2000) Positron emission tomography and single photon emission computed tomography in Alzheimer's disease. *Neurologist,* 6: 28-43.

Miller G (2012) Stopping Alzheimer's Before It Starts. *Science,* 337: 790-792.

Moran PM, Higgins LS, Cordell B, Moser PC (1995) Age-related learning deficits in transgenic mice expressing the 751-amino acid isoform of human 18-amyloid precursor protein. *Proceedings of the National Academy of Science, USA,* 92: 5341-5345.

Morris R (1984) Development of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods,* 11(1): 47-60.

Munoz D and Feldman H (2000) Causes of Alzheimer's disease. *Canadian Medical Association,* 162: 65-72.

Murrell J, Farlow M, Ghetti B, Benson MD (1991) A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease. *Science,* 254: 97-99.

Nalbantoglu J, Tirado-Santiago G, Lahsaini A, Poirier J, Goncalves O, Verge G, Momoli F, Welner SA, Massicotte G, Julien JP, Shapiro ML (1997) Impaired learning and LTP in mice expressing the carboxy terminus of the Alzheimer amyloid precursor protein. *Nature*, 387: 500–505.

Prvulovic D, Bokde ALW, Faltraco F, Hampel H (2011) Functional magnetic resonance imaging as a dynamic candidate biomarker in Alzheimer's disease. *Progress in Neurobiology,* 95: 557-569.

Ramanan VK, Risacher SL, Nho K, Kim S, Swaminathan S, Shen L, Foroud TM, Hakonarson H, Huentelman MJ, Aisen PS, Petersen RC, Green RC, Jack CR, Koeppe RA, Jagust WJ, Weiner MW, Saykin AJ (2013) APOE and BCHE as modulators of cerebral amyloid deposition: a florbetapir PET genome-wide association study. *Molecular Psychiatry,* doi:10.1038/mp.2013.19

Rapoport M, Dawson HN, Binder LI, Vitek MP, Ferreira A (2002) Tau is essential to β-amyloid-induced neurotoxicity. *Proceedings of the National Academy of Sciences, USA*, 99: 6364-6369.

Rawal D (2010) Use of a neurotrophic factor mimetic to block amyloid toxicity in Alzheimer's disease models. WPI Thesis, 2010.

Robertson ED, Scearce-Levie K, Palop JJ, Yan F, Cheng IH, Wu T, Gerstein H, Yu GQ, and Mucke L (2007) Reducing endogenous tau ameliorates amyloid β-induced deficits in an Alzheimer's disease mouse model. *Science*, 316: 750-754.

Ronayne R (2008) Human ependymin-1 neurotrophic factor mimetic reduces tau phosphorylation and cellular apoptosis *in vitro* and *in vivo* in Alzheimer's disease models. WPI Master's Thesis, September, 2008.

Sanders L (2011) Memories Can't Wait: Researchers rethink the role of amyloid in causing Alzheimer's. *ScienceNews*, 179(6): 24.

Selkoe DJ (2001) Alzheimer's disease: genes, proteins, and therapy. *Physiological Reviews*, 81: 741–766.

Shashoua VE, Adams D, Boyer-Boiteau A (2001) CMX-8933, a peptide fragment of the glycoprotein ependymin, promotes activation of AP-1 transcription factor in mouse neuroblastoma and rat cortical cell cultures. *Neuroscience Letters,* 312:103-107.

Shashoua VE, Adams DS, Boyer-Boiteau A, Cornell-Bell A, Li F, Fisher M (2003) Neuroprotective effects of a new synthetic peptide, CMX-9236, in *in vitro* and *in vivo* models of cerebral ischemia. *Brain Research*, 963: 214-223.

Shashoua VE, Adams DS, Volodina NV, Li H (2004) New synthetic peptides can enhance gene expression of key antioxidant defense enzymes *in vitro* and *in vivo*. *Brain Research*, 1024: 34-43.

Stovall K (2006) Partial restoration of cell survival by a human ependymin mimetic in an *in vitro* Alzheimer's disease model. WPI Master's Thesis, August, 2006.

Teunissen CE, de Vente J, Steinbusch HW, De Bruijn C (2002) Biochemical markers related to Alzheimer's dementia in serum and cerebrospinal fluid. *Neurobiology of Aging*, 23(4): 485-508.

Vermuri P, Jack Jr CR (2010) Role of Structural MRI in Alzheimer's disease. *Alzheimer's Research & Therapy*, 2: 23.

Yan SD, Chen X., Fu J, Chen M, Zhu H, Roher A, Slattery T, Zhao L, Nagazhima M, Morser J, Mighelo A, Nawroth P, Stern D, Schmidt AM (1996) RAGE and amyloid-β peptide neurotoxicity in Alzheimer's disease. *Nature*, 382: 685-691.

Zhang Y, Londos E, Minthon L, Wattmo C, Liu H, Aspelin P, Wahlund LO (2008) Usefulness of computed tomography linear measurements in diagnosing Alzheimer's disease. *Acta Raidiologia*, 49: 91-97.