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Disruptive Effects of Aβ Oligomers to the Radial-Arm Maze Performance of Rats

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DISRUPTIVE EFFECTS OF Aβ OLIGOMERS TO THE RADIAL-ARM MAZE PERFORMANCE OF RATS

by

Kineta Lynn Morgan-Paisley

A Dissertation
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
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Department of Psychology
Dr. Alan Poling, Advisor

Western Michigan University
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INTRODUCTION

Alzheimer's Disease

Background Information

Alzheimer's disease (AD) is a neuropsychiatric condition that mainly affects the elderly and with approximately 4.5 million cases in the United States alone, AD is the most prevalent neurodegenerative disorder (Hebert, Scherr, Bienas, Bennet & Evans, 2003). AD is a progressive neurological disorder, and the neurodegeneration and dementia seen in AD is unlike the natural decline in memory function associated with normal aging (Lesné et al., 2006). Dementia is a brain disorder that seriously affects a person's ability to carry out daily activities, and is defined as an acquired decline of mental functions in relation to the patient's previous level of functioning (DSM-IV-TR, 2000). According to the DSM-IV-TR there are as many as 12 different classifications for dementia, and it is a disorder characterized by the development of multiple cognitive deficits (including memory impairment) which are not due to the direct physiological effects of a general medical condition, to the persisting effects of a substance, or to multiple etiologies (p. 147). Dementia of the Alzheimer's type is the most prevalent form of age-dependent dementia affecting parts of the brain thought to control memory, language, and thought (Small, Mok, & Bornstein, 2001).

At the present time the exact cause of AD is unknown, and there is no known cure for this disease (National Institutes of Health [NIH], 2007). Dementia of the Alzheimer's type increases dramatically with age, rising from about 0.6% in males and 0.8% in females at age 65, to 11% in males and 14% in females at age 85, and eventually as high as 36% in males and 41% in females at age 95 (DSM-IV-TR, 2000, p. 156). It should not
be surprising, then, that as the life-expectancy in developed countries continues to increase, so does the incidence of AD and its socioeconomic impact on society as a whole (Mueller et al., 2005). Although a great deal of research has already been conducted in this area, no definitive claims can yet be made regarding the cause of the neurodegeneration associated with AD. If researchers can identify the cause(s) of the some of the earliest symptoms of AD (e.g., memory impairment), then treatments could be developed which directly target those variables in order to slow, stop or reverse the process of neurodegeneration.

**Diagnosis**

According to the *DSM-IV-TR* a thorough clinical examination is necessary to diagnose dementia of the Alzheimer’s type. This diagnosis is made mainly on the basis of symptoms, and several important criteria must be met. Memory impairment, which is a prominent and early symptom of AD, is the first diagnostic criterion required to make a diagnosis of dementia of the Alzheimer’s type. Memory may be formally tested by asking individuals to register, retain, recall and recognize information. The diagnostician may simply ask the individual to repeat a list of words (registration), then ask the person to recall the information after a delay of several minutes (retention and recall), and also to recognize the word from a list of multiple words (recognition). Memory can also be examined with non-human subjects via different means which do not involve language, such as maze navigation assays (*DSM-IV-TR*, 2000, p.157). One such non-human animal assay of memory will be discussed in more detail in a later section.

In addition to the impairments in memory, individuals suffering from dementia of the Alzheimer’s type will also exhibit impairments in other cognitive functions. Mental
impairments may extend to areas such as motor performance, language, judgment, executive functions (i.e., planning, organizing, sequencing, abstracting), visuospatial skills, emotional functions, and personality change. The diagnosis also requires that the severity of symptoms be such that they significantly interfere with the individual’s life. The course of dementia of the Alzheimer’s type is characterized by gradual onset and continuing decline, and adherence to this course is required for the diagnostic criteria to be met (DSM-IV-TR, 2000, p. 157).

Differentiating among the 12 types of dementia listed in the DSM-IV-TR is made by discovering the etiology of the dementia. Researchers are attempting to develop tests which are both sensitive and specific to confirm a diagnosis of dementia of the Alzheimer’s type. No such test currently exists and, therefore, it remains a diagnosis of exclusion. In other words, a diagnosis of dementia of the Alzheimer’s type requires that the 11 other types of dementia must first be ruled out. Specifically, the cognitive deficits must not be due to other central nervous system conditions that cause progressive deficits in memory or cognition (e.g., cerebrovascular damage, Parkinson’s or Huntington’s disease), systemic conditions that are known to cause dementia (e.g., HIV infection, hypothyroidism, or vitamin B₁₂ deficiency), or due to the effects of persistent use of a substance (e.g., alcohol). A diagnosis of dementia of the Alzheimer’s type also requires that the deficits not occur exclusively during the course of delirium, and that the cognitive disturbance is not better accounted for by another Axis I disorder such as Schizophrenia (DSM-IV-TR, 2000, p. 157).
Incidence, Prevalence and Course

Researchers estimate that in the year 2000 about 4.5 millions Americans were living with AD, and in 50 years that number is expected to triple to 13 million (Hebert et al., 2003). The percentage of total persons diagnosed with AD varies from state to state depending on population factors such as age. For example, the percentage of persons with AD in the year 2005 was projected to be as low as 0.9% of the total population of Alaska, and as high as 2.9% of the total population of Florida (Hebert, Scherr, Bienias, & Bennet, 2004). Age is a major risk factor for neurodegenerative diseases in general, and particularly for AD. AD is known to be a prominent disorder of old age because the disease typically begins to manifest after the age of 60 years and continues to increase with age. In fact, about 5% of individuals between the ages of 65 and 74 years have AD, whereas nearly 50% of individuals over the age of 85 years may have the disease. Although younger individuals (i.e., those under the age of 60 years) may also develop AD, it is much less common in this group (NIH, 2007).

The course and speed of progression of AD varies from person to person, but the disease is neurodegenerative in that it generally begins with mild memory impairments and continues to degenerate until the final stages in which severe brain damage is evident (NIH, 2007). The period of time, before a diagnosis can be made, when those mild memory impairments may be evident is known as the prodromal or preclinical period (Small, Gangon, & Robinson, 2007). There are no qualitative differences between the cognitive deficits preceding a diagnosis of AD and the loss of cognitive abilities that accompanies normal aging, but there are quantitative differences. That is, the degree or severity of prodromal cognitive deficits is greater in persons who go on to receive a
diagnosis of AD than in those whom do not (Craft, Cholerton, & Reger, 2003). Once an individual is diagnosed with AD they tend to live an average of 8-10 years, although individuals may live with the disease for as many as 20 years after receiving a diagnosis. Unfortunately, dementia of the Alzheimer’s type is thought to be an irreversible phenomenon. However, scientists continue to look for ways to delay or prevent the neurodegenerative processes associated with AD (NIH, 2007).

Signs and Symptoms of Alzheimer’s Disease

Cognitive Signs and Symptoms

The cognitive effects of AD involve disruptions to memory, language, thinking and reasoning (Alzheimer’s Association, 2007). Studies suggest that poor cognitive performance, specifically poor memory function, is one of the earliest symptoms of AD and can predict the disease up to 15 years prior to the onset of any clinical dementias (Kawas et al., 2003). The two major forms of memory are classified by their duration and include short-term memory and long-term memory. Short-term memory is rapidly formed and can outlast training for minutes or hours, whereas long-term memory may last from hours to days, weeks, or even years (James, 1890). It is important to note that the earliest symptom of AD is the impairment of short-term working memory (Selkoe, 2002). Over time, both declarative and non-declarative memory will become impaired. Episodic memory, a type of memory involved in remembering verbal or visual materials, may also serve as a good marker for impending dementia (Good, Hale, & Staal, 2007). A deficit in visual memory performance is another early expression of AD, and may also be evident years before a diagnosis is made (Kawas et al., 2003).
In its earliest phase AD produces a remarkably pure impairment of memory, in the absence of any other signs of brain injury. As the disease progresses, however, a variety of other cognitive impairments begin to emerge. Patients with AD begin to lose their ability to encode new information (i.e., form new memories), at first being unable to memorize the trivial, and then later the more important, details of life. Furthermore, one of the first manifestations of this progressive disease, noted primarily in the early stages, is a profound inability to form new memories (Selkoe, 2002). Although individuals have difficulty learning new information, their motor and sensory functions remain otherwise intact (Alzheimer’s Association, 2007). In addition, the individual begins to lose their capacity for reasoning, abstraction, and even language (Selkoe, 2002). Other cognitive domains may also be affected, including executive functioning (i.e., organizational skills) and the ability to respond rapidly to verbal and visual materials (e.g., driving a car, following directions; Small et al., 2007). Furthermore, individuals with dementia of the Alzheimer’s type appear to exhibit an accelerated breakdown in certain inhibitory processes (Spieler, Balota, & Faust, 1996).

Behavioral Signs and Symptoms

The “behavioral” or “psychiatric” symptoms describe a large group of additional symptoms that occur (at least to some degree) in many, but not all, individuals with AD. Personality changes in the form of irritability, anxiety and/or depression are common disturbances which may first be demonstrated in the early stages of AD. Other symptoms that may occur in the later stages of AD include sleep disturbances, agitated behaviors, delusions, and/or hallucinations. Physical or verbal outbursts, general emotional distress, restlessness, pacing, shredding paper, and yelling are all examples of agitated behaviors.
An example of a delusion might be a firmly held belief that a person has a characteristic not actually present (e.g., a great singing voice), while examples of hallucinations could include seeing, hearing or feeling things that are not actually there. It is the behavioral and psychiatric symptoms which are often the most distressing to individuals with AD, their families, and their caregivers. Although other factors might contribute to the occurrence of these symptoms (e.g., medication), the chief underlying cause of the behavioral and psychiatric symptoms of AD is the progressive neurodegeneration associated with the disease itself (Alzheimer's Association, 2007).

According to a report of the Surgeon General, psychosis, depression, and wandering are also common behavioral symptoms of AD (U.S. Department of Health and Human Services, 1999). Other behavioral symptoms discussed in the Surgeon General's report include insomnia; incontinence; catastrophic verbal, emotional, or physical outbursts; sexual disorders; and weight loss. Although these behavioral symptoms are not required for a diagnosis of AD, they do have serious ramifications and potentially detrimental consequences to the quality of life of AD sufferers and their caregivers. This is a major concern because for most individuals the behavioral symptoms will occur at some point during the disease with high frequencies. For example, 30 to 50% of individuals with AD will experience delusions, 10 to 25% will have hallucinations, and 40 to 50% will suffer from symptoms of depression (U.S. Department of Health and Human Services, 1999, Ch. 5, p. 4).

Physiological Signs and Symptoms

In 1906 a German doctor by the name of Dr. Alois Alzheimer noticed areas of abnormal brain tissue while conducting an autopsy on a woman who had died of an
unusual mental illness. Dr. Alzheimer had identified abnormal clumps of brain tissue as well as tangled bundles of fibrous brain tissue, which are now known as amyloid plaques and neurofibrillary tangles (NFTs), respectively (Figure 1). Today, these plaques and tangles are a hallmark of AD and are considered to be a sign of the disease. Furthermore, the progressive loss of short-term working memory is correlated with the accumulation of large amyloid plaques and NFTs in the hippocampus and other areas of the brain involved in memory processing (NIH, 2007).

Figure 1. Amyloid Plaques and Neurofibrillary Tangles (NFTs).

This diagram represents the two hallmarks of Alzheimer’s disease: amyloid plaques and NFTs. On the left is normal or healthy brain tissue, and on the right is the unhealthy Alzheimer’s brain tissue.
**Neurofibrillary Tangles.** NFTs are twisted nerve cell fibers which are the damaged remains of microtubules (Alzheimer's Association, 2005). Microtubules are the support structures which allow the flow of nutrients through the neurons (nerve cells). A key component of these tangled fibers is an abnormal form of the tau protein which, in its healthy state, helps in the assembly of the microtubule structure. Defective tau, however, appears to actually block the actions of the healthy tau protein. Essentially, when the defective tau protein fails to support the microtubule structure it inhibits the supportive actions of the healthy tau proteins, thereby preventing the flow of the nutrients through the neurons. The damaged remains of the microtubules then begin to accumulate forming NFTs (Simon, 2003). NFTs are the most commonly found intraneuronal inclusion in the brains of patients with neurodegenerative diseases (SantaCruz et al., 2005).

**Beta-Amyloid.** The second significant feature discovered in the brains of AD individuals is commonly referred to as either beta-amyloid (β-amyloid) or amyloid beta (Aβ). Beta-amyloid is an insoluble protein that can accumulate forming sticky patches. These patches may be called one of several names (i.e., oligomers, fibrils, beta sheets, or plaques) depending on how much Aβ has accumulated. Regardless, these sticky patches of Aβ are often found surrounded by the debris of dying nerve cells in the brains of AD victims (Simon, 2003). When nerve cells die and connections between nerve cells are disrupted in areas of the brain that are crucial to memory and other mental abilities, impairments in these abilities are assured (NIH, 2007). The β-amyloid peptide is a 38 to 43 amino-acids long sequence derived from the large transmembrane β-amyloid precursor protein (APP). The most common forms are 40 and 42 amino acid residues long and are termed Aβ40 and Aβ42, respectively (Glenner & Wong, 1984). It has been
suggested that the accumulation of Aβ42 plays a causative role in the characteristic neurodegeneration associated with AD (Sweeney, Luedtke, McDonald, & Overmier, 1997). The distinction between the different forms of Aβ (i.e., Aβ40 vs. Aβ42; oligomers, fibrils, sheets and plaques) will be covered in greater detail in a later section.

In addition to nerve cell death, amyloid plaques are relevant to some other identified brain characteristics associated with AD. For instance, higher levels of Aβ are associated with reduced levels of the neurotransmitter acetylcholine. Neurotransmitters are chemical messengers in the brain which carry messages between nerve cells. Acetylcholine is part of the cholinergic system, which is a system involved in learning and memory. Thus, AD may impair thinking and memory by disrupting these messages. Aβ may also disrupt channels that carry sodium, potassium and calcium. These elements act as ions in the brain, producing electric charges that must fire regularly in order for signals to pass from one nerve cell to another (Simon, 2003). If the channels that carry these ions are disrupted or damaged, such an imbalance could interfere with nerve function and signal transmission (Alzheimer’s Association, 2008).

Aβ is also relevant to a process called oxidation, which may be responsible for some of the neurodegeneration associated with AD (Simon, 2003). When Aβ breaks down it releases chemicals called oxygen-free radicals which, once released, bind to other molecules in a process called oxidation. Although the oxidation process is a normal one, when oxidants are over-produced they can cause severe damage in cells and body tissue. When the body’s immune factors attempt to repair the injuries caused by oxidation yet another problem may arise as a result of the body’s inflammatory response. The inflammatory response occurs when the immune system responds to a threat but, in the
process, actually damages the body's own cells (NIH, 2007). A full account of oxidation and the inflammatory response is beyond the scope of the present analysis, but it is important to point out that these processes do provide further evidence for the potential importance of Aβ in the development of AD.

Models, Theories, and Hypotheses of Alzheimer's Disease

Before moving on to the various hypotheses regarding Aβ, it is first necessary to understand the relationship between Aβ and its precursor protein. Amyloid-β is derived from the amyloid precursor protein (APP). To form Aβ, APP is first cleaved by β-secretase, and then is subsequently cleaved by γ-secretase (Figure 2). Depending on the point of cleavage of γ-secretase, any of three different principle forms of Aβ will be produced. These three forms of Aβ compromise 38, 40, or 42 amino acids and, therefore, the three forms are called Aβ38, Aβ40, and Aβ42, respectively. Aβ40 is the most abundant peptide, however, Aβ42 is the longest form and is far more likely to oligomerize and form amyloid fibrils (Alzheimer's Association, 2006).

The reason that Aβ42 is a prime suspect in AD is because it is chemically “stickier” than other fragments produced when APP is cut, which causes it to accumulate. It accumulates by stages into microscopic amyloid plaques that are considered one of the hallmarks of the Alzheimer brain. The pieces first form small clusters called oligomers, then chains of clusters called fibrils, then “mats” of fibrils called beta sheets. The final stage is plaques, which contain clumps of beta sheets and other substances (Alzheimer’s Association, 2006). The normal process of Aβ production goes awry when there is either an over-production of all Aβ or an increased proportion of the 42 amino acid form, both of which may cause early-onset AD (Walsh & Selkoe, 2007).
**Figure 2.** The 2-Step Process of Aβ Formation from the Amyloid Precursor Protein.

Step 1) APP is cleaved at the β-site by β-secretase forming C99.
Step 2) C99 is then cleaved at the γ-site by γ-secretase to finally produce the 39 to 42 amino acids long Aβ peptide.
The “Myelin Model”

Bartzokis, Lu and Mintz (2007) hypothesize that myelin breakdown in vulnerable late-myelinating regions releases oligodendrocyte- and myelin-associated iron that promotes Aβ oligomerization, its associated toxicity, and the deposition of oligomerized Aβ and iron in neuritic plaques observed in AD. Multiple risk factors such as age, certain genetic predisposition, and increasing Aβ and iron levels, result in thinner myelin sheaths which are differentially lost with age in a pattern of bilaterally progressive myelin breakdown. This process is thought to underlie the progressive, bilaterally symmetrical spread of the pathognomonic lesions of AD (i.e., neuritic plaques and NFTs). The myelin model is of interest to this analysis because of its emphasis on Aβ oligomers.

Genetic Models

The major known genetic risk factor for AD is the apolipoprotein E-4 (ApoE-e4), an epsilon4 allele associated with lowered parietal, temporal, and posterior cingulate cerebral glucose metabolism in patients with a clinical diagnosis of AD (Small et al., 2000). However, the exact genetic markers for the disease differ depending on whether it is early- or late-onset AD. In early-onset AD of the familial form, the disease appears to be caused by mutations on chromosomes 21, 14, and 1 and is transmitted in autosomal dominant mode. Each of these mutations appears to result in an overproduction of the protein found in neuritic plaques (i.e., β-amyloid). The early-onset familial form of the disease accounts for less than 5% of AD cases, which is a relatively small proportion of total cases (Cummings, Vinters, Cole, & Khachaturian, 1998). However, according to Mohs, Breitner, Silverman and Davis (1985) if individuals with a family history of AD are followed into their 80s and 90s approximately 50% will develop the disorder.
themselves (i.e., late-onset AD). The results of twin studies suggest that heredity is involved in about 60% of all cases of AD, and this percentage includes both the early- and late-onset varieties (Bergem, Engedal, & Kringlen, 1997).

Late-onset AD is the more common form of the disorder, and begins after 60 years of age. Genetic studies have identified an association between late-onset AD and the presence of the ApoE-e4 allele on chromosome 19 (Bookheimer et al., 2000). In addition to being the chief genetic risk factor for AD, ApoE-e4 is also the most common cause of dementia late in life. Functional MRI scanning comparing subjects with this genetic marker to normal subjects have indicated that patterns of activation differ between these two groups (Bookheimer et al., 2000). Specifically, it appears that individuals with this genetically predisposing allele show increased signal intensity (i.e., greater activation) in brain regions necessary for tasks requiring memory including the left hippocampal, parietal and prefrontal regions. This increased brain activation is hypothesized to be the result of compensatory processing, that is, greater brain activation is required to meet the task demands when compared to healthy controls. In other words, the individuals with the ApoE-e4 allele have to work harder to perform the same memory tasks. Regardless, the presence of the ApoE-e4 allele on chromosome 19 has been shown to increase the risk for developing AD because this allele increases the deposition of β-amyloid. Once again, the critical variable appears to be the over-production and accumulation of β-amyloid.

*Amyloid Hypotheses*

Amyloids are insoluble fibrous protein aggregations sharing specific structural traits. The abnormal accumulation of amyloids in organs can lead to amyloidosis, which
may play a role in certain diseases including AD. Aβ is a small self-aggregating peptide produced at low levels by normal brain metabolism. Researchers suggest that in AD, self-aggregation of Aβ becomes rampant and is manifested as the amyloid fibrils of senile plaques. These fibrils are known to kill neurons in culture, which suggests that the fibrils might initiate the neurodegeneration associated with AD (Klein, Grant & Finch, 2001). More recently, however, it has been determined that the Aβ fibrils are not the only form of Aβ relevant to AD. Research suggests that the smaller, pre-fibrillar assemblies of Aβ oligomers are also deleterious (Walsh & Selkoe, 2007).

Although several hypotheses regarding the cause of AD are currently under investigation, one which has garnered a great deal of evidence is the amyloid cascade hypothesis (Hardy & Higgen, 1992). This hypothesis contends that AD is the result of an accumulation of senile plaques consisting mainly of β-amyloid peptides. According to this hypothesis, the memory loss seen in AD occurs due to neuron death caused by fibrillar Aβ. Research based on this hypothesis, however, has failed to demonstrate a convincing correlation between dementia and amyloid plaque burden. To address this issue a newer hypothesis has emerged which suggests that early memory loss is considered a synapse failure caused by soluble Aβ oligomers (Lacor et al., 2004). Such oligomers are believed to block long-term potentiation, and they are strikingly elevated in AD brain tissue and transgenic mouse AD models.

A hypothesis proposed by Matsuoka and colleagues (2003) compares the body’s immune system to a sink, which traps Aβ and depletes it from the central nervous system. Under this hypothesis, the neurodegeneration associated with AD (i.e., dead or dying neurons, activated microglial cells, and reactive astrocytes) is thought to be the result of
the interaction between the body's immune system and Aβ aggregation. This hypothesis rests on the assumption that Aβ aggregation is the causal event in AD pathology, because amyloid deposits of Aβ found in the limbic and association cortices are surrounded by signs of neurodegeneration (Mrak & Griffin, 2001).

Although the exact etiology of AD is still unknown, the ever-accumulating knowledge about the histopathology of AD is allowing for the development of drugs aimed at modifying the neurodegenerative process of this devastating disease (Mueller et al., 2005). One leading hypothesis has proposed that these senile plaques, which are formed by aberrant processing of APP, are an important step in synaptic, neuronal, and cognitive deterioration (Hardy, 2006). Although the evidence for the role of Aβ in AD is both suggestive and encouraging, it is by no means exact or conclusive. What is known is that the neuropathological features of AD include amyloid plaques, NFTs, and selective neuronal loss. The impetus for these features and their exact causal role in the symptomology of AD, however, requires a great deal more research.

Researchers have examined mice with APP mutations and have found that they develop impairments in hippocampus-dependent memory tasks (Janus, Phinney, Chishti, & Westaway, 2001). Such findings are consistent with the amyloid hypothesis. Neurobiological theories contend that the hippocampus carries out functions relevant to spatial information and episodic memory (Smith & Mizumori, 2006). Although an all-encompassing account of the function of the hippocampus and the various types of memory is beyond the scope of the present analysis, it is worth describing spatial and episodic memory. In 2005 Cleary and colleagues reported that naturally produced Aβ oligomers from 7PA2 cells, but not Aβ monomers or non-Aβ CM, produced cognitive
impairments in rats tested under an alternating lever cyclic ratio (ALCR) procedure. Holscher, Gengler, Gault, Harriot and Mallot (2007) reported reversible effects when they found that three daily ICV injections of soluble Aβ(25-35) produced short- and long-term memory impairment in rats tested in a radial arm maze on post-injection days 12-20, but not on post-injection days 3-11 or 20-28. The difference in the time-course of effects with soluble Aβ (25-35) and with 7PA2 is noteworthy and merits investigation with respect to mechanism.

*Experimental Memory Assays and Alzheimer’s Disease*

Experimental assays of memory are especially important to the study of AD since a wide variety of memory disruptions have been implicated in this disease. Before moving on to some of the experimental assays of memory used to study AD, it is important to first understand a bit about the different classifications of memory as they pertain to AD. Memory is divided into two main categories based on duration which include short-term memory and long-term memory (James, 1890). Short-term memory is further divided into sensory memory and working memory, and working memory is further divided into visual memory and executive memory (Baddeley, 2003). The type of memory used for the temporary maintenance and storage of information is commonly referred to as short-term working memory, whereas long-term memory refers to a more permanent storage of information and can be further divided into declarative memory and procedural memory (James, 1890). Declarative memory refers to facts, events or relationships and is further divided into semantic memory and episodic or spatial memory (Tulving & Markowitsch, 1998). Unfortunately, memory is not discussed in a consistent manner across research studies, which can make understanding the distinction between
various memory assays difficult. Figure 3 is a visual representation of the subdivisions of memory and all of the aforementioned types of memory relevant to animal models of AD are highlighted in yellow (i.e., short-term, working, visual, executive, long-term, declarative, and spatial). This figure should become useful as some of the experimental animal models of AD are presented.

Memory

![Memory Diagram]

*The types of memory relevant to animal models of AD are highlighted in yellow.*

*Figure 3. Visual Representation of the Subdivisions of Memory*
The ALCR Schedule

The ALCR schedule of food reinforcement is a very sensitive behavioral assay for examining the effects of a variety of psychoactive drugs (O’Hare et al., 1996; Weldon, O’Hare, Kuskowski, Cleary, & March, 1996) and insoluble forms of Aβ (Richardson et al., 2002) on the behavior of non-human subjects. The ALCR procedure is a type of schedule-controlled behavior requiring subjects to move from one lever to the other each time a specific response requirement is met and it enables the simultaneous measurement of anticipatory ratio tracking (post-reinforcement pause durations), preservations (lever-switching errors), and non-specific peripheral drug effects (running response rates). This modified version of the cyclic-ratio schedule was originally designed to examine the effects of atropine sulfate on reference memory (Weldon et al., 1996).

In 2005 Cleary and colleagues used the ALCR assay to examine the memory of rats and demonstrated disruptive effects to the memory of complex learned behavior when a solution of 7PA2 CM containing Aβ dimers and trimers (but not monomers) was injected into the lateral ventricles. Although this procedure is remarkably sensitive, it only examines disruptions to aspects of executive memory. Thus, it is unclear from the aforementioned ALCR study whether or not the 7PA2 CM solution would have produced disruptive effects to other aspects of memory commonly seen in AD sufferers, such as aspects of long-term memory like spatial (i.e., episodic) memory.

The Morris Water Maze

The Morris water maze was developed by the neuroscientist Richard Morris to examine spatial memory in rodents (1982). This maze consists of a large round tub of opaque water, usually made white with powdered milk, so that the animal cannot see
through the water to locate one of the two small hidden platforms placed 1-2 cm under the surface (Figure 4). The pool is usually 4 to 6 feet in diameter and 2 feet deep. To examine spatial learning, external visual cues, such as colored shapes, may be placed around the maze to help guide behavior. There is also a side wall above the water to prevent the rat from being distracted by laboratory activity. When released, the rat swims around trying to find a platform. The researcher can record various measures like the amount of time spent in a particular area of the pool, latency to reach platform, and total distance swam. If the rat does not find a platform within 1 or 2 minutes, it is rescued. The Morris water maze can measure various aspects of short- and long-term memory, both of which are implicated in memory impairments associated with AD.

Figure 4. The Morris Water Maze.

The maze consists of a large round tub filled with opaque water. The animal begins on the starting platform and this platform is released which forces the animal to swim in order to locate the end platform.
In 2006 Lesné and colleagues used the Morris water maze to examine the cognitive performance of rats who had received injections into the lateral ventricles of a soluble Aβ complex. The researchers had identified a specific complex of soluble Aβ oligomer called Aβ* (abeta star). This complex was shown to be negatively correlated with cognitive performance in the APP over-expressing Tg2576 mouse model of AD. The injections disrupted performance in the Morris water maze, suggesting a disruption to spatial reference memory (Lesné et al., 2006). In 2007 Huang, Liang, Chen, Chen and Hsieh-Li used the Morris water maze to examine the effects of intra-hippocampal injections of Aβ1-40 on the performance of hyperglycemic mice. These researchers also found that the injections disrupted spatial learning and memory (Huang et al., 2007).

*The Radial-Arm Maze*

Maze preparations, in general, are particularly well suited for the study of spatial memory in laboratory animals, and understanding such behavior can advance our understanding of cognitive processes (Olton, 1979). The radial-arm maze (RAM) has the advantage of allowing the researcher to arrange the procedure so that both working and spatial reference memory may be assessed separately (Wenk, 2007). The RAM is one of the most commonly used mazes and is useful for analyzing cognitive processes of rodents, especially Sprague-Dawley rats (Boast, Walsh, & Bartolomeo, 2000). The RAM was originally designed by Olton Samuelson in 1976 to examine spatial learning and memory in the rat, but has since been used to examine the cognitive processes of a variety of other species including mice (Bane, 1997), gerbils (Maurer, Storch, LaForge, & Boast, 1995) and humans (Aadland, Beatty, & Maki, 1985). The RAM can be used to examine working memory, a type of memory in which representations of previously
experienced events or episodes are temporarily maintained, as well as spatial reference memory, a type of memory that contributes to the performance of well-learned responses in the presence of an appropriate discriminative stimulus (Boast, Walsh, & Bartolomeo, 2000). Thus, the RAM can be used to examine aspects of both short- and long-term memory. Since its development, the RAM has proven to be sensitive in detecting the cognitive effects of a variety of toxicants and drugs of abuse (Levin, 1988).

The RAM (Figure 5) is usually constructed from clear plexiglass, with visual cues at various locations throughout the maze. The apparatus generally consists of 8 arms extending from a central chamber, however, this type of maze has been constructed with as many as 48 arms (Cole & Chappell-Stephenson, 2003). In the normal RAM preparation, the animal is placed in the center of the maze and allowed to enter each baited arm to obtain a small piece of food (usually cereal). The center of the platform can be sectioned off in order to isolate the animal from going down any or all of the arms. This partitioning-off of the maze arms can be done manually or may be automated, and such experimental preparations are often used for RAM procedures involving delays. Training generally involves habituation to the RAM (which may or may not include shaping). Open-arm training occurs next, followed by blocked-arm training (if blocks are to be used). If a delay is required, the delay is implemented after blocked-arm training, and finally experimental testing can begin. Training criterion may be a set number of trials, or may be related to subject performance (e.g. less than or equal to 2 errors per session for 3 consecutive days). Subjects are usually food deprived prior to training and experimental sessions to increase motivation to perform. To minimize odor cues mazes should be wiped down with an ethanol solution between test sessions (Boast et al., 2000).
Figure 5. The Eight-Armed Radial Arm Maze (RAM).

Visual depiction of the eight-armed RAM with the subject in the starting position facing arm 1.
The RAM is a spatial analogue to operant experimental procedures and, as with other operant-type experimental preparations, there are a variety of different RAM procedures that can be used to fit the needs of the research situation. In a match-to-sample (MTS) free-choice arrangement all arms will be open, but only some arms will contain food. The same arms will always be baited throughout the study, and this arrangement is designed to test long-term memory. The simplest arrangement is the non-match-to-sample (NMTS) free-choice procedure in which an animal is free to select any arm, initial entries are rewarded with food, and no delay imposed. By imposing a delay researchers can examine short-term working memory, and during these delay periods animals are either confined to the central chamber or removed from the maze entirely. In a delayed-match-to-sample (DMTS) free-choice version of the RAM some arms are baited and some are not. A delay is imposed and, following the delay, the subjects must visit the arms which were previously reinforced during pre-delay (Young, Stevens, Converse, & Mair, 1996). In a delayed non-match-to-sample (DNMTS) free-choice arrangement the animal freely visits a subset of the total arms (e.g. 4 of the 8 arms). Next, a delay is imposed and then, following the delay, the animal is allowed to visit all of the arms but only those arms which were not visited during pre-delay now contain food (Porter & Mair, 1997). In contrast, in the DNMTS forced-choice procedure the subject visits a subset of the arms which are open and contain food, and the remaining arms are blocked. A delay is then imposed and, following this delay, all eight arms are open but only those previously blocked arms now contain food.

The present study uses this type of DNMTS forced-choice arrangement, however, a wide variety of RAM arrangements have been used in the past to examine aspects of
memory and cognitive performance. Animal assays, such as the RAM, provide researchers with a means to study the effects of certain variables which may otherwise be unethical or impractical using human preparations. These studies may even be helpful in determining the roles which certain stimuli play in the development of neurodegenerative diseases like AD. One of the most prominent symptoms of AD is the progressive loss of short-term working memory, and this is correlated with the accumulation of large amyloid plaques and neurofibrillary tangles in the hippocampus and other areas of the brain important to memory (Selkoe, 1991). Although such discoveries are exciting, it is not possible to experimentally manipulate such variables using human subjects, but non-human experimental preparations can be used. Furthermore, the RAM has proven to be an effective means to examine a variety of stimuli as they relate to memory and performance in non-human animal subjects.

There are many examples of research which demonstrate the utility of the RAM procedure as a tool to examine the effects of different variables on the memory of laboratory animals, with the intention of telling us something about human conditions like brain injury or neurodegenerative diseases. For example, a study by Li, Kim, Ichikawa and Meltzer (2003) sought to determine the effects of repeated administration of phencyclidine (PCP) on working/spatial memory performance in rodents (both rats and mice) using an eight-armed RAM. PCP is a drug which has been reported to produce psychosis in normal volunteers and exacerbate psychosis in schizophrenic individuals. Therefore, an animal model of working memory impairment paralleling the deficits found in schizophrenia would be valuable in studying the pathophysiology of schizophrenia. Although this study failed to demonstrate that repeated administration of PCP to rodents
produces enduring memory impairment, the experimental arrangement used to conduct their research is of interest to the present analysis.

Li and colleagues first shaped the rodents for 5 days by placing them in the center of the platform and allowing them to explore, and then gradually restricting the pellet location to closer and closer to the food cups at the end of each arm. After shaping was complete they then began the eight-arm baited RAM training. The rodent would be placed at the center of the RAM and allowed to access all 8 baits. A 10-second delay was introduced after each choice, during which time the rodent would be confined to center platform. After seven days of training this delay was increased to 60-seconds, and on the 16th to 18th trial it was further increased to a 120-second delay. To be included in the PCP test phases that followed training, all rodents had to meet a pre-defined performance criterion of one or less re-entries into an already visited arm per session, and fewer than two total errors for three consecutive sessions.

When a RAM memory assay seeks to examine memory by implementing a delay, the delay need not occur following each response, and the animal need not be restrained within the maze itself during the delay period. For example, Chrobak, Hanin, Lorens and Napier (1995) used an experimental RAM preparation with rats with a delay procedure very different from that arranged by Li et al. (2003). Following a 5-day habituation period, and 25 days of open-arm RAM training, the researchers began the DNMTS forced-choice RAM procedure in which a delay was imposed during the 4th and 5th choices. Initially during this phase, the rat was placed inside an eight-armed RAM that had 4 of the 8 arms blocked off. After the rat had visited the fourth available arm, the rat was removed from the maze and returned to their home cage during the 1-hour delay
period. During this time the researchers rotated the maze between 90 and 180 degrees. Then, following the delay, the rat was placed back into the maze that now had all arms open, but only those arm that were previously blocked contained food.

Using this experimental preparation, the researchers collected data on four dependent measures: (1) the number of correct choices during the first four post-delay choices; (2) retroactive errors, or the number of post-delay choices that were entries into pre-delay chosen arms (those which had been open during pre-delay); (3) proactive errors, or repeated entries into post-delay arms (i.e. a rat enters an arm which was blocked during pre-delay, more than once during post-delay); and (4) the latency of choices during post-delay. The combination of arms which would be blocked during the pre-delay sessions varied quasi-randomly among a fixed set of 20 combinations. In this study the researchers used a within-subjects experimental design to determine the effects of aging, thus, each rat was tested at 6, 12 and 18 months of age (Chrobak et al., 1995). The general research strategy that they used appears to be useful for examining the effects of drugs, aging and neurodegeneration on short and long-term memory, and was used in the present study.

*Alzheimer's Research using the RAM.* The RAM has also been used to demonstrate memory impairments induced by Aβ25-35 (Holscher et al., 2007; Stepanichev, Moiseeva, Lazareva, Onufriev, & Gulyaeva, 2003; Stepanichev, Zdobnova, Zarubenko, Lazareva, & Gulyaeva, 2006). Holscher and colleagues (2007) used an eight-armed RAM to examine the effects of intracerebroventricular (ICV) injections of Aβ25-35 on both short-term working and long-term memory. A control group received similar injections which did not contain the active compound. Control groups are often used to
ensure that the deficits in memory are due to the compound of interest and not simply the result of the stressful injection process, since stress has been shown to interfere with memory in rats (Baker & Kim, 2002). The researchers used a MTS free-choice arrangement in which 3 of the 8 arms were always baited, and no delay was imposed. An error in working memory was said to occur if a rat visited an arm during the same session which was baited, but had already been visited during that session. Visits to arms that had never been baited were considered long-term memory errors. The researchers found that the injections produced significant impairments in both short-term working memory and long-term memory, but these effects were reversible (Holscher et al., 2007).

Stepanichev and colleagues (2003) used an eight-armed RAM to examine the effects of stereotaxic injections of Aβ25-35 on spatial and working memory in rats using a NMTS free-choice arrangement. Under this arrangement all eight arms in the maze are open and baited. A rat is placed into the maze and allowed to visit all eight arms, with no delay imposed. After the response is well-learned the subjects are divided into two groups. The control group received a single vehicle injection and the experimental group received an injection of the Aβ25-35 compound. The rats were again tested 60 days after the compound or vehicle solution was administered, and there were no differences in the two groups. From these results the researchers concluded that Aβ25-35 had no effect on memory (Stepanichev et al., 2003).

In 2006 Stepanichev and colleagues used a MTS free-choice RAM preparation to once again test the effects of one injection of the Aβ25-35 compound on the long-term and short-term working memory of rats. Unlike the previous experiment, only five of the eight arms were baited. The sequence of which five arms would be baited differed for
each individual rat, but was held constant throughout the study. Unlike the previous experiment, the rats did not learn the task until 4 weeks (recovery period) and 2 days (food-deprivation period) after the each rat had already had the surgery and received the single injection of either vehicle or Aβ25-35. The experimental group showed statistically significant differences with regards to errors when compared to the control group. Furthermore, when the animals were sacrificed following the experiment, the researchers found that the number of errors made on testing days negatively correlated with the number of nerve cells in the hippocampal field CA1. These results suggest that impairments in learning and memory can be induced by a single administration of Aβ25-35, and that these impairments are associated with neurodegenerative changes in the hippocampal field CA1 (Stepahnichev et al., 2006).

The RAM procedure has also been used with rats to demonstrate memory impairments induced by exogenous Aβ1-40 (Hashimoto et al., 2002; Hashimoto et al., 2005; Sweeney et al., 1997). Sweeney and colleagues (1997) examined the effects of bilateral intrahippocampal injections of Aβ1-40 on rat performance in a RAM foraging task. The researchers used a DNMTS free-choice arrangement in which Aβ1-40 was administered immediately before the test session began, and a 30-minute delay was imposed between the 4th and 5th choices. The researchers chose to give the injections immediately prior to the beginning of each test session to ensure that the performance decrements were actually the result of the Aβ1-40 compound itself, and not simply the result of the injection or the stress of the injection process. Interestingly, the injections had no effect on pre-delay choices, but had a significant effect on post-delay choices.
The results of this study suggest that Aβ1-40 does have acute effects on short-term memory and may play a significant role in some memory deficits seen in AD.

In 2002 Hashimoto and colleagues used an avoidance learning task to determine the effects of docosahexaenoic acid (DHA), a major n-fatty acid, on the memory-impairing effects of cerebral ventricle infusions of Aβ1-40 peptide. As expected, the infusions did produce impairments in learning and memory. The researchers determined that DHA had protective and restorative effects (Hashimoto et al., 2002). In 2005 Hashimoto and colleagues examined these same variables using a MTS free-choice arrangement in an eight-armed RAM and found similar results. Specifically, the researchers found that ventricle infusions of the Aβ1-40 peptide produced impairments to both short-term working memory and long-term (reference) memory, and that DHA once again had protective and restorative effects (Hashimoto et al., 2005).

In 1997 Sweeney and colleagues examined the effects of bilateral intrahippocampal injections of βA4 on the performance of rats in a DNMTS free-choice RAM foraging task, with a delay imposed following the 4th choice. The primary core of the amyloid plaques seen in AD individuals is the βA4 peptide. Previous research examining the effects of βA4 on performance in a classic RAM had given injections 30 minutes before the test sessions concluding that, although the compound had no effect on this short-term memory task, it did interfere with long-term retention (McDonald et al., 1994). Sweeney and colleagues felt that in the previous study, not only was the drug administered too long before the test session, but also that the delay between the 4th and 5th choices was too long (i.e., effects of βA4 injections may have worn off). This discrepancy, they hypothesized, might account for the absence of an effect.
To rectify this, Sweeney and colleagues administered βA4 immediately before each test session, and imposed only a brief 30-second delay between the 4th and 5th choices on the RAM free-choice task in rats that had been food-deprived to 90% of their free-feeding weight. During the procedure all eight arms were baited, injections were delivered immediately before each test session, and rats were placed into the maze facing a different arm each session. After the 4th choice a 30-second delay was imposed during which time the rat was taken out of the maze and placed in a carrier for 30 seconds. Then the rat was then returned to the central platform and entries into unvisited arms were considered correct choices, whereas, entries into already visited arms were considered errors. The rat must travel greater than or equal to the length of 53 cm down any one arm for and entry/choice to be recorded. The session was terminated when either: (a) all 8 food pellets had been consumed, (b) 10 choices had been made, or (c) 5 minutes had elapsed, whichever occurred first. Injections were delivered into the hippocampus, through a cannula in the skull. Vehicle injections were also given to ensure that performance-effects were not due to simply giving the injection.

Results of this study indicate that βA4 injections did increase errors immediately following the delay (i.e. for choice # 5), which suggests that there is an effect on short-term memory. Although the researchers concluded that βA4 does have acute effects on memory-based performance, these effects may be short-lived. In fact, the injections themselves may have made the rats more susceptible to interference, because performance on choice 6 was comparable to that of choice 4 suggesting that the feedback provided from choice 5 (correct or incorrect) was enough to reinstate the animal’s proficient performance. This suggests that some memory of the choices 1-4 must have
been available, which would not be the case had the impairment been one of learning and retention. The detrimental effects of βA4 were both acute and transient but not, therefore, due to an aggregative neurodegenerative processes (Sweeney et al., 1997).

The earliest features of AD include impaired memory for recent events, followed by deficits in spatial learning and memory (Gottfries, 1995; Kálmán, Maglóczky & Janka, 1995). When compared to healthy volunteers, patients with AD will show a delay-dependent reduction in choice accuracy under a visual DMTS task and this is thought to be indicative of impaired short-term working memory (Ballard & McAllister, 1999). Of all of the aforementioned procedures, the delayed non-match-to-sample (DNMTS) procedure is the best experimental RAM preparation to examine both short-term working and long-term (i.e., spatial) memory. Under this procedure rodents are first allowed to visit a subset of the arms, are then removed or prevented from responding for some period of time (delay), and are then allowed to visit the remaining arms. The present study used a DNMTS forced-choice procedure in which a delay was imposed between the 4th and 5th choices to examine the effects of Aβ oligomers on memory.

Rationale for the Present Study

Post mortem neuropathology of AD sufferers reveals the presence neuritic plaques, which are mainly composed of large extracellular aggregates of Aβ, as well as the presence of NFTs. Accordingly, most AD research conducted in recent years has focused on these two types of proteinaceous brain inclusions. Specifically, research has tended to examine amyloid plaques and the synthesis, cleavage, and deposition of Aβ. The genetic mutations associated with inherited AD involve genes linked to APP and Aβ synthesis (Walsh & Selkoe, 2007). Data from transgeneic mouse models of AD have
shown that memory loss is poorly correlated with the presence of NFTs, and evidence suggests that NFT formation follows amyloid deposition (SantaCruz et al., 2005). Furthermore, total plaque burden does not correlate with the progression of cognitive decline associated with AD, casting further doubt on the importance of these large extracellular plaques in the development of the disease (Braak & Braak, 1998).

The early memory loss associated with AD may be better explained by the presence of small soluble forms of Aβ, than by the presence of NFTs or large aggregated amyloid plaques (Walsh & Selkoe, 2007). The concept of small soluble forms of Aβ was advanced in 1995 by Oda and colleagues. Other researchers went on to demonstrate that these small soluble forms of Aβ could be formed synthetically (Lambert et al., 1998; Klein et al., 2001). In 2002 Walsh and colleagues demonstrated that Chinese hamster ovary cells (CHO) transfected with a familial AD-linked human mutant APP (valine to phenylalanine at position 717, near the γ-secretase site) over-express Aβ oligomers in the culture medium. Conditioned media (CM) from these cells are called 7PA2 cells and these have been shown to disrupt rat hippocampal long-term potentiation (LTP), which is a cellular process often used to model short-term memory formation (Walsh et al., 2002).

Although researchers have demonstrated a correlational relationship between poor memory/cognitive performance and levels of oligomeric Aβ in the brain, very few true experiments have examined the cognitive effects of Aβ oligomers. A true experiment requires an experimental procedure (i.e., behavioral assay) which has proven efficacy for examining the target response in the population of interest. The ALCR procedure is one example of such an experimental assay. Developed by Weldon and colleagues in 1996, the ALCR procedure has proven to be a remarkably sensitive experimental assay which
can be used to examine the effects of Aβ oligomers on the reference memory of rodents (Weldon et al., 1996). Although several studies (O’Hare et al., 1999; Richardson et al., 2002; Townsend et al., 2006) have used this procedure to demonstrate the disruptive effects of Aβ oligomers on the reference memory of rodents, the remarkable sensitivity of the ALCR procedure may hinder the generalizability of these findings to other types of memory, aside from reference memory. Furthermore, although the ALCR procedure is a good test of executive functioning, there are far better experimental assays which are proven to measure various other types of memory relevant to AD, and the RAM is one such example.

Due to the limitations of the ALCR procedure, there is a need for studies which use other well-established experimental assays proven to measure aspects memory and cognitive performance in order to examine the effects of Aβ oligomers on different types of behaviors associated with AD dysfunction. An advantage of the RAM is that it is a highly adaptable preparation which can be altered to fit the needs of the research situation. In addition, the RAM is well-established as an experimental assay designed to examine various types of memory in rodents, especially spatial and short-term working memory. Furthermore, the RAM can be used to assess both working and reference memory separately, and delays can be imposed so that researchers may examine both short-term and long-term memory. The RAM is a very well-established experimental assay of memory which was used in the present study to examine the effects of Aβ oligomers on the cognitive performance of rodents in the hopes of advancing AD research.
METHODS

Subjects

Twenty-five male Sprague-Dawley rats purchased from Charles River (Portage, MI) were used as subjects. The subjects were approximately 1 year old at the beginning of the study, with an average weight of 448 grams, and had been handled extensively prior to the start of the study. Throughout the study the subjects were given unlimited access to water and were housed individually in plastic cages (24 cm long x 31.5 cm wide x 21 cm high) with metal grated tops. The subjects were housed in an animal colony room in Haenicke Hall at Western Michigan University. The colony room was maintained on a 12-h light/12-h dark cycle and kept at a constant temperature of 20-22°C and a constant humidity of 24%. Following surgery the rats were housed in a similar manner, except that the lids of the cages were changed to elevated tops to allow sufficient room for the animal's head cap and guide cannula.

Throughout the experiment the rats were weighed daily and were maintained at 85% of their free-feeding weight by limiting their access to Purina Rat Chow (Ralston-Purina, St. Louis). Enough food which would be sufficient to maintain the desired weight was delivered immediately after the final experimental sessions for that day had concluded, and this usually occurred about 20 hours before the first experimental session of the following day. This study was approved by the Institutional Animal Care and Use Committee at Western Michigan University and was conducted in accordance with the Guide for Care and Use of Laboratory Animals promulgated by the National Research Council (1996).
Apparatus

The experiment was conducted in an isolated room in Haenicke Hall with direct and private elevator access to the animal colony. The experimental apparatus was an eight-arm radial arm maze constructed of clear plastic. The walls of the maze were 18 cm high and each arm was 60 cm long and 12 cm wide. The arms radiated symmetrically outward from a circular start box which was 36 cm in diameter (see Figure 5, p. 23). Each arm was covered by a hinged plastic top which could be opened for easy cleaning. At the end of each arm was a small food cup which could be baited with various types of small edible food. Cocoa Pebbles cereal (Post Cereals, Battle Creek, MI) were used to bait the arms in this study.

The design of the maze was such that any or all individual arms could be blocked using plastic partitions which slid into the intersection between the start box and the individual arm entrance. The maze sat atop a perforated metal, and papers were placed beneath the floor to collect any feces or urine. The maze was encased by four opaque walls in order to prevent outside visual distractions. To provide visual cues for the rat, eight pieces of white poster board containing different designs in black ink (e.g., a straight vertical line, three squiggly horizontal lines) were affixed to the opaque walls surrounding the maze, and were located immediately behind the ends of each of the eight arms. The criteria for recording a choice or entry required that the rat run at least 53 cm down the arm, and this distance was marked by a clearly visible line scribed on the floor and sides of each arm.
Behavioral Procedures

All training sessions generally were conducted 6 days a week, at about the same time each day during the light portion of the light/dark cycle. Figure 6 shows an outline of entire experiment as it occurred. All sessions were observed and scored by a researcher and were video recorded as well. The researcher also timed each individual trial using a digital stop watch, and these data were also recorded. Data were recorded on specially created data sheets (Figure 7). The phase of the study (e.g., Acclimation/Shaping phase) and the day (e.g., day 1) was listed at the top of the data sheet, and the subject (e.g., Rat 1) was also listed. There was a space to record the total number of errors made and the time (i.e., duration). If blocks were used during a particular phase, the researcher would physically draw in the locations of the blocked-arms on the data sheet before the beginning of each session. This allowed the researcher to then go back and determine what type of errors had been made and to record this data at the end of each test session.

Habituation/Shaping

The habituation/shaping procedure occurred over the course of five days and was intended to familiarize the subjects to both the food reinforcer and the testing situation. On the first day of the habituation/shaping phase the food reinforcer was introduced to the subjects by simply placing a small handful of the cereal into the individual home cages of each rat. This was done to give the subjects experience with the novel food reinforcer. On day two of the habituation/shaping procedure the subjects were each individually placed inside the open maze and Cocoa Pebbles were scattered at the base of each arm (closest to the start box). Each rat was allowed to roam the baited maze
## Dissertation Running Schedule

<table>
<thead>
<tr>
<th>Day</th>
<th>Activity</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>Acclimation to SR+</td>
<td>pellets in home cage</td>
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<tr>
<td>2</td>
<td>Acclimation/Shaping</td>
<td>pellet at base of arm</td>
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<td>3</td>
<td>Acclimation/Shaping</td>
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<td>4</td>
<td>Acclimation/Shaping</td>
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<td>Acclimation/Shaping</td>
<td>Pellet at end of arm</td>
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<tr>
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<td>Surgery Day</td>
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<td>***Recover Occuring</td>
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<td>72</td>
<td>Testing Day: 7PA2, CHO</td>
<td>blocked arms = 1,3,5,8</td>
</tr>
</tbody>
</table>

*** 5 Days of recovery***

**Testing Days = Infusions Delivered**

*By this point in the study the majority of the subjects had non-functional skull caps*

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**Figure 6.** Outline of the Dissertation Running Schedule.
Figure 7. Example of the Data Sheets Created for this Study.

The phase of the study and day are listed at the top of each sheet. The subject number is listed in the lower left hand corner of each maze depiction. This specific example is from the Acclimation/Shaping phase for rats 1, 2, 3, and 4.
freely for 5 minutes. The rat was then removed, and the maze was wiped down and baited for the next subject. The third day of habituation/shaping was similar to the second day, except that the food pellets were placed about 1/3 of the way down each arm. On the fourth day the food pellets were placed 2/3 of the way down the arm. Finally, on the fifth and final day of the habituation/shaping procedure the pellets were placed in the food cups at the end of each arm. Thus, while the rats were being habituated to the test situation, they were simultaneously being "shaped" to move further and further down each arm to obtain the food reinforcer.

Open-Arm Pre-Training

Open-arm pre-training sessions occurred daily over the course of 15 consecutive sessions, immediately following the habituation/shaping procedure. During the open-arm pre-training sessions all of the arms were open and baited. The session termination criteria required that the subjects would remain in the maze until either all 8 pellets had been consumed or 10 minutes elapsed, whichever occurred first. The subject was placed in the start box facing the same direction (arm 1) each time (see Figure 5). The timer was started as soon as the rat had been placed into the starting position. During this training procedure data were collected and recorded for the total response duration (e.g., 2:04 = 2 minutes and 4 seconds), the total number of errors (e.g., 4 errors), and the total number of correct responses occurring within the first eight responses (e.g., 6/8 correct). The average time required to complete the maze was 2:39 during open-arm pre-training session 1, and this time was reduced to 0:51 by the 15th session. The group mean for the total number of errors decreased from 2.08 errors during session 1, to 1.04 errors by the 15th session. The number of correct responses out of the first 8 responses rose from 6.8
correct to 7.4 correct between the first and last open-arm pre-training sessions. Guided by these performance improvements, training was able to progress to the more difficult, blocked-arm procedures.

**Blocked-Arm Training**

For blocked-arm training with a delay the plastic partitions were placed at the entrance of four of the eight arms of the radial arm maze. The sequence of arms that were blocked was chosen randomly from a grab bag which contained almost all the possible combinations printed on square cards. Combinations that included every other arm (e.g., 1,3,5,7 or 2,4,6,8) and those that included four arms in a row (e.g., 1,2,3,4 or 5,6,7,8) were excluded from the list of potential combinations which could be used. Each day the researcher would pull one combination from the bag, and this combination would be used for all of the rats that day. After a combination had been used, it was thrown out and no combination was ever used more than once.

During blocked training the subject was first placed in the starting position and allowed to visit the four unblocked arms which were baited with food. If the rat performed this sequence perfectly, each arm would only have been visited one time. Revisiting an already visited arm during this initial session was counted as an incorrect response. Once all four arms were visited the subject was removed from the maze for a designated period of time (delay). During the delay the researcher removed the partitions from the previously blocked and baited those arms. The arms which had just been visited were unbaited, and only those previously blocked arms now contained food. Following the delay, the subject was placed back into the maze and allowed to obtain the food reinforcers from the four previously-blocked arms.
Dependent Variables

There are two types of errors that could occur during post-delay sessions. The first type, retroactive errors, occurred when the subject visited an arm that was open during the pre-delay session and, therefore, was not baited during post-delay session. The second type, proactive errors, occurred when a subject visited an arm that was blocked during the pre-delay sessions more than once during post-delay session.

As Poling and Byrne (2000) discuss, it is well established that drugs and other perturbations typically disrupt responding to a greater extent when stimulus control is relatively weak than when it is relatively strong. In other words, errors are more common when stimulus control is weak and less common when stimulus control is strong. Prior to the start of testing with 7PA2, different delays were examined to determine a value at which retroactive errors exceeded proactive errors. Both are assumed to be measures of "spatial working memory" (Chappell, McMahan, Chiba, & Gallagher, 1998). Essentially, the different delay durations were examined to determine whether or not the duration of the delay affected stimulus control.

Assessment of Various Delays

Three different delays were examined: 5 minutes, 30 minutes, and 2 hours. The data for the delay-accuracy curve were collected over the course of 6 days. The 5-minute delay was examined on days 1 and 4, the 30-minute delay was examined on days 2 and 5, and the 2-hour delay was examined on days 3 and 6. Pre-delay data were collect on the number of correct and incorrect entries in the first four arms. Post-delay data were collected on both retroactive errors and proactive errors. Data were also collected and recorded for the total number of errors across the entire session (i.e., pre-delay and post-
delay errors combined). The group means for the different variables examined proved to be similar across the 5-minute, 30-minute, and 2-hour delay durations, and at all of these values the number of retroactive delays exceeded proactive delays. The 2-hour delay was ultimately chosen for practical reasons (i.e., it allowed for easier sequential testing of subjects).

After the 6 days of blocked-arm training to determined delay curve, blocked-arm training continued for another 14 days using the chosen 2-hour delay interval. The 14-day group average for correct entries and errors in the first four entries during the pre-delay period are shown in Figures 8 and 9, respectively. The 14-day group mean for post-delay retroactive and proactive errors are shown in Figures 10 and 11, respectively. These data demonstrate the stability of responding and provide visual support for the researchers' decision to move on to the next phase in the research process and to schedule the surgeries.

Surgical Procedures

Prior to receiving any anesthesia, the rats were first given intraperitoneal (IP) injections of 1 mg/kg atropine to prevent bradycardia (i.e., slowed heart rate), which can sometimes result from anesthetization. Next, the rats were anesthetized via IP injections with 50 mg/kg sodium pentobarbital. Full anesthetization was indicated by the absence of the hind-paw pinch reflex. Once the rat was fully anesthetized a unilateral guide cannula (C313G, 22 gauge, Plastics One, Roanoke, VA) was surgically implanted into the dorsal lateral ventricle at stereotaxic coordinates of AP = - 0.4, ML = + 1.3, DV = - 4.2 (Paxinos & Watson, 1998) and anchored to the skull with skull screws and cranioplast cement.
Figure 8. Mean Correct Entries Made of the First Four Pre-Delay Entries.

Figure 9. Mean Errors of the First Four Pre-Delay Entries.
Figure 10. Mean Retroactive Errors.

Figure 11. Mean Proactive Errors.
Post Surgery Recovery and Retraining

Immediately following the surgeries the rats were returned to their individual home cages and allowed to rest for five days. The rats had fully recovered after five days of rest, and then post-surgery retraining began. Post-surgery retraining was done to ensure that the surgeries did not affect maze performance. After three days of retraining no differences were identified between pre- and post-surgery maze performance. The assessment of post-surgery maze performance was critical to the internal validity of the study because, without this assessment, one could not reliably distinguish between effects on performance due to the independent variable and those resulting from the surgery.

Injection Materials

The rats were given injections of either 7PA2 or vehicle control via their implanted guide cannulas. Concentrations of soluble Aβ oligomers that are physiologically relevant to that found in human brain (<$10^{-9}$ M) are contained in the culture media (CM) of Chinese Hamster Ovary cells (CHO) transfected with a human mutation of APP that causes early-onset AD (Podlisny et al., 1995). These cultured cells, called 7PA2 cells, excrete Aβ oligomers, primarily dimers and trimers, into the CM as the cells mature. The vehicle control, CHO-CM, contains extracellular components similar to those found in the 7PA2 CM, but without the Aβ oligomers. The 7PA2 and CHO- were obtained from Dr. Sylvain Lesné, an expert in the production of oligomers of amyloid-β from the University of Minnesota (e.g., Lesné et al., 2006).

Injection Procedures

As was previously mentioned, no sessions were arranged for eight days immediately following cannula implantation surgery. Following the five days of
recovery and three days of retraining, one session was conducted each day, as described above. Immediately after the pre-delay part of the session ended, all of the rats received an intracerebroventricular (ICV) injection of either 7PA2 CM or CHO- CM (n = 10 per group, with rats randomly assigned to the 7PA2 and CHO- groups). Two hours after the injection, they began the post-delay portion of the daily (test) session, as described previously. The two-hour delay between injection and post-delay test session has previously been shown to produce errors under the ALCR procedure (Cleary et al., 2005). Micro-infusion pumps (CMA/100, CMA, Stockholm, Sweden) were used to slowly infuse 20 μl of 7PA2 CM or CHO- CM slowly through 28 gauge injectors (C313I, Plastics One) into the lateral ventricle at a maximum rate of 5 μl/minute.

The initial test session under 7PA2 and CHO- CM was followed by 3 weeks of training and testing sessions (see Figure 6). During this time both the 7PA2 group and the CHO- group received 6 days without any injections, followed by 1 day with an ICV injection prior to the second part of the daily session. Animals that received 7PA2 CM initially always received 7PA2 CM in subsequent injections and animals that initially received CHO- CM always received CHO- CM subsequently. Two rats in the 7PA2 group did not receive the third injection because their cannula skull caps had loosened and, as a result, were no longer functional. By the last test day over half of the subjects had non-functioning skull caps, therefore, the data from this day were not included in the final outcome of the study. Following behavioral testing, the rats were euthanized and cannula placements were confirmed by histological examinations.
RESULTS

The performance of the treatment (i.e., 7PA2 CM) group can be compared to that of the control group (i.e. CHO- CM) by examining the mean (± SEM) number of proactive errors, retroactive errors, and total errors made by rats during the three test sessions in which 7PA2 CM or CHO- CM was administered 2 hours earlier and comparing these data to the data collected during the three control sessions immediately preceding those test sessions (Figure 12). The performance of the two groups of rats during the three control sessions immediately preceding test sessions was comparable. Specifically, the mean control value of total errors was 2.10 for the 7PA2 group and 2.33 for the CHO- group. Each group made relatively few proactive errors (M = 0.30 and 0.33 for the 7PA2 and CHO- groups, respectively) and substantially more retroactive errors (M = 1.80 and 2.00 for the 7PA2 and CHO groups, respectively).

In contrast to the comparable performance levels achieved by both groups during the control sessions, performance during test sessions differed in the two groups. While the performance of the CHO- CM group during experimental sessions was comparable to control-session performance, the performance of the 7PA2 CM group was not. For the CHO- CM group (i.e., the control group) the mean values for the number of proactive (0.27), retroactive (2.0), and total (2.37) errors occurring two hours after ICV injections of CHO CM did not differ appreciably from these control values. Statistical analysis by means of Wilcoxon signed rank tests indicated that control and injection means for proactive, retroactive, and total errors did not differ at the 0.05 alpha level (W = -7, -6, -9; p = 0.56, 0.56, 0.64, respectively). However, for the rats that received 7PA2 CM (i.e. the experimental group) the mean numbers of proactive (0.57), retroactive (2.55), and total
Figure 12. Mean Proactive, Retroactive & Total Errors: Treatment vs. Control Group.
(3.125) errors made during sessions preceded by injections were substantially above control levels. Wilcoxon signed rank tests indicated that the differences in means were statistically significant at the .05 level for proactive, retroactive, and total errors ($W = -28, -47, -55; p = 0.0156, 0.0137, 0.002$, respectively).

Table 1 provides data for each of the three injections of CM from 7PA2 and CHO- cells. Here, the mean number of errors emitted during sessions immediately following injections is expressed as a percentage of the mean number of errors emitted during the preceding control session, not preceded by an injection. These data indicate that the disruptive effects of the Aβ oligomer-containing 7PA2 CM did not increase with repeated exposure, but rather were largest following the first injection.

Table 1

*Errors During Sessions Preceded by Injections Expressed as Percent Control*

<table>
<thead>
<tr>
<th>Error Type</th>
<th>Proactive</th>
<th>Retroactive</th>
<th>Total</th>
</tr>
</thead>
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<tr>
<td>Administration</td>
<td>1 2 3</td>
<td>1 2 3</td>
<td>1 2 3</td>
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<tr>
<td>7PA2 Group</td>
<td>500 150 *</td>
<td>223 125 135</td>
<td>243 129 173</td>
</tr>
<tr>
<td>CHO Group</td>
<td>67 100 75</td>
<td>139 82 127</td>
<td>129 84 116</td>
</tr>
</tbody>
</table>

*no errors were emitted during the control session*
DISCUSSION

The results of this study indicate that oligomers of amyloid-β significantly affect the performance of rats tested in a RAM by increasing proactive, retroactive, and total errors. Administrations of CM containing Aβ oligomers produced significant memory impairments, whereas no such impairments in memory were produced by those administrations of CM not containing the Aβ oligomers. These results suggest that Aβ oligomers were responsible for the observed effects on memory, and that the observed memory impairments were not the result of extraneous factors such as the IVC injections or non-specific components of the CM.

Aβ oligomers are small clusters of accumulated β-amyloid plaques, and the presence of these plaques in the brain is considered to be a hallmark of AD. These β-amyloid plaques are a prime suspect in AD because they are sticky fragments that tend to accumulate in stages. The first stage is formation small clusters called oligomers, then chains of clusters called fibrils, then mats of fibrils called sheets, and the final stage is clumps of sheets called plaques (Alzheimer’s Association, 2006). These stages are thought to disrupt brain cells by clogging cell-to-cell communication, which activates the immune system triggering inflammation, and ultimately resulting in cell death (Mrak & Griffin, 2001). The evidence to support this amyloid hypothesis comes from several sources. First, all of the rare genes which have been identified that virtually guarantee a person will develop AD increase either the production or accumulation of β-amyloid (Alzheimer’s Association, 2006). Second, mice which have been genetically engineered to carry the genes associated with inherited AD develop amyloid plaques and develop symptoms which mimic those of AD sufferers (Janus et al., 2001). In addition, Aβ fibrils
are known to kill neurons in a cell culture, suggesting that these fibrils might initiate AD neurodegeneration (Klein, Grant & Finch, 2001). However, it has recently been determined that the Aβ fibrils are not the only form of Aβ relevant to AD, and that small, pre-fibrillar assemblies of Aβ (i.e., Aβ oligomers) are also deleterious (Walsh & Selkoe, 2007). The present study sought to examine further the potentially causative role of Aβ oligomers in AD neuropathology.

In order to examine the role that these oligomers may have in the development of AD, it is important to use a tried and true animal model of memory. The RAM was used in the present study because it is a well-established animal model of spatial working memory in rodents (Chappell et al., 1998; Chrobak et al., 1995; Levin, 1988; Olton, 1985; Wenk, 2007) that has proven to be sensitive to the effects of a variety of toxicants and drugs of abuse (Levin, 1988). Furthermore, the RAM is useful in examining aspects of both short- and long-term memory relevant to AD.

Cleary et al. (2005) reported previously that naturally produced Aβ oligomers from 7PA2 cells, but not Aβ monomers or non-Aβ CM, produced cognitive impairment in rats tested under an ALCR procedure. Aβ oligomer-induced errors under ALCR have been associated with reference memory (Cleary et al., 2005), and such errors in the Morris Water Maze (Lesné et al., 2006) also are thought to be related to reference memory.

Under the RAM procedure in the present study, oligomers of Aβ significantly increased proactive and retroactive errors in rats. Both measures are assumed to assess spatial working memory (Chapell et al., 1998). Errors did not increase when CM containing no Aβ oligomers was administered. Therefore, it appears that the Aβ
oligomers *per se*, not ICV injections or non-specific components of the CM, were responsible for the effect. In sum, the present data provide further evidence of the deleterious cognitive effects of oligomers of Aβ and extend those effects to a well-established animal model of spatial working memory, the RAM (Chappell et al., 1998; Jaffard et al., 2000; Olton, 1985; Wenk, 2007). This extension is significant because a deficit in working memory has been considered to be one of the earliest detectable symptoms of AD and is a clinical hallmark of the disease (Selkoe, 1991; Small et al., 2007; Walsh et al., 2005).

In the study by Cleary et al. (2005), in which rats responded under an ALCR schedule, Aβ oligomers increased perseveration errors (i.e., errors to 201% of the control level and switching errors to 146% of control). Switching errors occurred when the subject did not alternate to the other lever after reward or switched from the correct lever to the incorrect lever before completing the response requirement and getting rewarded. Perseveration errors occurred when the subject ‘persevered’ on the incorrect lever after responding once on the incorrect lever. In the current study in which rats were tested in a RAM, Aβ oligomers increased Proactive and Retroactive errors to 190% and 142% of control, respectively. Thus, the degree of cognitive disruption observed under the two behavioral procedures was comparable in magnitude.

Although they differ substantially in complexity, both the ALCR and RAM procedures involve spatial memory, in that successful performance requires subjects to move from place to place and to base their movements on the consequences of prior actions. It is tempting to suggest that oligomers of Aβ generally impair rats' spatial memory, but an unpublished pilot study from our laboratory failed to find a disruptive
effect of oligomers of Aβ (ICV) in rats exposed to a delayed-nonmatching-to-position procedure, which also involves spatial memory (Vardigan et al., 2006). It is not clear why Vardigan et al. failed to demonstrate memory impairment, but it was the case that the rats had received extensive training and demonstrated high levels of accuracy at all delays. Moreover, a correction procedure, intended to reduce position bias, was in effect. Either of these variables may have reduced the sensitivity of the procedure. Nonetheless, Vardigan et al. did find increased errors with injections of the prototypic amnestic drug scopolamine.

Although the current study expanded the class of behaviors affected by Aβ oligomers, the range of conditions under which memory is disrupted and the specific nature of the disruption is still largely unexplored. It is, however, clear that the disruptive effects of oligomeric Aβ are not limited to measures of reference memory and common animal behavior tests may be sensitive to these effects under some conditions.

The present data are consistent with those reported by Cleary et al. (2005) in that the effects of Aβ oligomers were transient, that is, there was no evidence of significant cognitive impairment on the day following the injection under either the RAM or the ALCR lever pressing task. Moreover, there was no evidence that oligomeric Aβ was neurotoxic in the present study, because there was no increase in errors as a result of repeated exposure. In fact, the oligomers appeared to act similarly to pharmacological agents that are cleared over time. This aspect of the oligomers' action is important because it offers hope that cognitive deficits due to Aβ oligomers in people with AD may be reversible. Reversible effects were also reported in an animal model by Holscher et al. (2007), who found that three daily ICV injections of soluble Aβ(25-35) produced short-
and long-term memory impairment in rats tested in a radial-arm maze on post-injection days 12-20, but not on post-injection days 3-11 or 20-28. The difference in the time-course of effects with soluble synthetic Aβ(25-35) and with oligomers of naturally derived Aβ(1-42) from 7PA2 cells is noteworthy and merits investigation with respect to mechanism.

In sum, the present findings provide further support for the conclusion that 7PA2 cells disrupt memory and that, because there was no evidence of significant cognitive impairment on the day following the injection, the memory disrupting effects of the Aβ oligomers are transient. Furthermore, there was no evidence of enduring neurotoxicity, because errors did not increase as a result of repeated exposure. To the contrary, oligomers appeared to act similarly to pharmacological agents which clear over time. However, the range of conditions under which memory is disrupted by 7PA2 remains to be determined, and further research in this area is certainly warranted.

Taken together, these results are both encouraging and confounding. On the one hand, these results seem to suggest that cognitive deficits resulting from Aβ oligomers may be reversible. On the other hand, it is troubling that the Aβ oligomers did not produce significant cognitive impairments on the days immediately following injections, nor did repeated exposure produce enduring toxicity. These findings may suggest that it is not just the over-production of Aβ oligomers which leads to neurotoxicity. Perhaps the AD individual has some other feature which makes clearing Aβ oligomers more difficult. Regardless, future research in this area is definitely warranted.

As average life expectancy continues to increase, so too does the need to find a cure for diseases of old age, such as AD. Before cures can be developed, however,
researchers must first determine what causes the neurodegenerative symptoms of AD. The present study adds additional evidence to the growing body of research suggesting that Aβ oligomers disrupt memory and may play an important role in the development of AD. Although these oligomers do appear to play a role in the development of AD, it is important to understand that they probably are not the only causative factor of the disease. Regardless, it seems that the RAM is a good animal model to use in future studies examining the disruptive effects of Aβ oligomers in rats.
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Appendix

Western Michigan University Animal Care and Use Committee
Approval Form
Date: October 12, 2005

To: Alan Poling, Principal Investigator
    Lisa Baker, Co Principal Investigator

From: Robert Eversole, Chair

Re: IACUC Protocol No. 05-09-01

Your protocol entitled “Behavioral Effects of Beta-Amyloid Precursors in the Rat” has received approval from the Institutional Animal Care and Use Committee. The conditions and duration of this approval are specified in the Policies of Western Michigan University. You may now begin to implement the research as described in the application.

The Board wishes you success in the pursuit of your research goals.

Approval Termination: October 12, 2005