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NEUROCHEMICAL EFFECTS OF AMYLOID-BETA OLIGOMERS IN RATS

by

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NEUROCHEMICAL EFFECTS OF AMYLOID-BETA OLIGOMERS IN RATS

John J. Panos, Ph.D.

Western Michigan University, 2010

Alzheimer's disease is a progressive neurodegenerative disease characterized by memory loss and cognitive decline. Although the symptomology of Alzheimer's disease is well defined, its precise etiology remains elusive. Animal models are invaluable for understanding the pathogenesis of this devastating disease. Knowledge of the neurochemical actions of amyloid-β oligomers in specific brain structures is essential for validating animal models of Alzheimer's disease and for determining the most appropriate behavioral assays of memory. The specific aim of this project was to investigate the neurochemical effects of direct intracerebral infusion of amyloid-β oligomers in the rat. Experiment 1 investigated direct infusions of synthetic amyloid-β oligomers and experiment 2 examined cell-derived amyloid-β oligomer infusion in the rat prefrontal cortex. Neurochemical efflux was measured in the PFC using *in vivo* microdialysis and monoamine metabolite concentrations were determined using HPLC-EC. Results showed a reduction in 5-HIAA one hour post infusion of cell derived oligomers. This reduction is indicative of the involvement of the serotonergic system in Alzheimer's disease

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TABLE OF CONTENTS

Table of Contents—Continued

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Alzheimer's disease is a progressive neurodegenerative disease associated with aging, affecting millions of adults worldwide (Herbert, Scherr, Bienias, Bennett, & Evans, 2004). The World Health Organization (WHO) publication, the *International Classification of Diseases* (ICD-10, 2007), characterizes the symptomology of dementia in Alzheimer's disease as disturbances in cognitive function including memory, thinking, orientation, comprehension, calculation, capacity to learn, tasks involving judgment, and language (ICD-10: F00-F09). Type 1 and type 2 Alzheimer's disease are distinguished by age of onset. Type 1, or late onset, Alzheimer's disease, occurs in individuals after the age of 65 years; the progression is relatively slow and primarily characterized by deficits in memory (ICD-10: F00.1). Type 2, or early onset, Alzheimer's disease, is defined by multiple disturbances in cognitive function requiring higher cortical processes, with rapid deterioration in individuals before the age of 65 years (ICD-10: F00.0). The prognosis for individuals with all forms of Alzheimer's disease is devastating, requiring long-term assisted or institutionalized care. The negative prognosis produces a burden on both society and family members of persons afflicted with Alzheimer's disease.

Although the symptomology of Alzheimer's disease is well defined, the precise etiology of the disease remains elusive. Post-mortem studies of individuals diagnosed with Alzheimer's disease have aided in characterizing neuropathological and

neurochemical features of this degenerative disease. The pathology of the disease involves the formation of amyloid plaques (insoluble deposits of aggregated amyloid-β) that occur in the hippocampus, temporal, frontal, and parietal cortices of Alzheimer's disease patients (Walsh & Selkoe, 2004a).

Animal Models May Elucidate Pathogenesis of Alzheimer's Disease

Animal models are essential to understanding the etiology of Alzheimer's disease. Transgenic mouse models have been developed to determine the role of soluble Tau protein and soluble amyloid-β oligomers in the pathogenesis of Alzheimer's disease. These models are reviewed by Ashe and Zahs (2010). Alternative and complementary to transgenic mouse models are animal models aimed at determining the neural circuitry and neurochemistry of amyloid-β in the intact rodent brain (Stephan & Philips, 2005).

Several basic research endeavors may be formulated using *in vivo* animal models to investigate the pathogenesis of Alzheimer's disease. The first approach involves investigations of the neural pathways associated with Alzheimer's disease, while the second examines the components (low n-oligomers including monomers, dimers, trimers, and tetramers) of amyloid-β as they relate to the disruption of memory in animal models of Alzheimer's disease. Thirdly, the neurochemical effects of amyloid-β oligomers can be studied *in vivo*. With regard to the neural circuitry of Alzheimer's disease, new hypotheses for research can be formulated by reviewing the literature on neuroanatomical lesion studies and microinjection studies of amyloid-β (1-42) or the more soluble forms amyloid-β (1-40) amyloid-β (25-35). These studies are specific to structures associated with memory and are detailed below.

Izaki, Takita, and Akema (2008) demonstrated that ibotenic acid lesions of the hippocampal and prefrontal cortical pathway disrupt working memory in rats subjected to a delayed radial arm maze task. Furthermore, Izaki et al. delineated the role of the dorsal posterior hippocampus (dpHP) and the ventral hippocampus (vHP) in this type of memory. Significant deficits in memory were found following lesions in the prefrontal cortex (PFC) and dpHP. No significant effects on memory resulted from lesions of the vHP. The work by Izaki et al. has provided an initial reference point for the neuroanatomical structures of interest in the hippocampal-prefrontal cortical pathway that are associated with the disruption of memory in a delayed memory task. Therefore, we have selected the PFC as a target site in the current study.

The effects of central administration of amyloid-β in rodent models of memory have been investigated in behavioral studies. McDonald, Dahl, Overmier, Mantyh, and Cleary (1994) investigated spatial memory in rodents following bilateral microinjections of amyloid-β 1-40 (βA4) into the dorsal hippocampus (dHP). This study established that βA4 interferes with the process of memory consolidation. Sweeney, Luedtke, McDonald, and Overmier (1997) demonstrated that intrahippocampal injections of amyloid-β (1-40) increase post-delay errors in a radial arm maze procedure. Memory deficits were also observed in the Morris water maze after bilateral microinfusion of amyloid-β (25-35) into the CA1 region of the rat hippocampus (Chen, Wright, & Barnes, 1996). Intracerebroventricular (ICV) injections of amyloid-β (25-35) have also been shown to impair memory in rodents tested in a radial arm maze task (Holsher, Gengler, Gault, Harriott, & Mallot, 2007; Stepanichev, Zdobnova, Zarubenko, Lazareva, & Gulyaeva, 2006; Yamaguchi & Kawashima, 2001).

Neurochemical effects of centrally administered amyloid-β have also been documented. For example, *in vivo* neurochemical studies of amyloid-β (1-42) using reverse microdialysis have shown a significant increase in excitatory amino acid efflux at the microdialysis probe site (Harkany et al., 2000). Furthermore, microdialysis studies have demonstrated a decrease in dopamine concentrations after exposure to amyloid-β (1-42) in the prefrontal cortex (Trabace et al., 2007; Wu, Khan, & Nichols, 2007). An area of increasing interest that is in need of future investiagations is the functional link between the dopamine system and kynurenic acid in the central nervous system. Recent microdialysis studies in rodents have demonstrated that kynurenic acid lowers dopamine concentrations *in vivo* (Wu, Rassoulpour, & Schwarcz, 2007). Further relevance of kynurenic acid in Alzheimer's disease has been demonstrated by reports of postmortem kynurenic acid elevations in the brains of patients with Alzheimer's disease. Specifically, kynurenic acid was found to be elevated in the frontal cortex, hippocampus, putamen, caudate nucleus, and the cerebellum in Alzheimer's patients relative to age-matched controls (Baran, Jellinger, & Deecke, 1999). Because these brain regions also tend to accumulate high levels of amyloid-β in Alzheimer's patients, perhaps kynurenic acid is involved in the neurochemical actions of amyloid-β. The aforementioned amyloid-β studies of memory along with the neuroanatomical investigations of Izaki et al. (2008) and neurochemical investigations of J. Wu et al. (2007) and H. Q. Wu et al. (2007) have provided an estimation of the neuroanatomical structures and neurochemical pathways affected by central microinfusion of low-n oligomers of amyloid-β, that in turn may result in memory disruption.

Cell Derived Amyloid-β Oligomers

Recently, investigations of the small (low-n) soluble forms of amyloid-β have provided insight into progression of the associated neurotoxicity and cognitive dysfunction in Alzheimer's disease. These investigations have led to the emerging and controversial hypothesis that soluble amyloid-β oligomers are the primary cause of cognitive disruption and synaptic dysfunction in this progressive neurodegenerative disease (Walsh & Selkoe, 2004b). Of interest is the neurotoxicity associated with the naturally occurring amyloid-β oligomers secreted by cells expressing human amyloid-β precursor protein (APP). The 7PA2 Chinese hamster ovary (CHO) cell line is currently under investigation in our laboratory. The cell line referred to as 7PA2 is derived from CHO cells sodium dodecyl sulfate stably transfected with an APP_{751} cDNA expressing the V717F Alzheimer's disease mutation (Walsh et al., 2002). These cells contain amyloid-β monomers and **(**SDS)-stable amyloid-β oligomers including dimers and trimers, devoid of the larger insoluble amyloid-β aggregates (Walsh et al., 2002). To date, there are relatively few studies examining the effects of the 7PA2 cell line *in vivo*. Walsh et al. (2002) demonstrated that intracerebroventricular (ICV) injections of 7PA2 (1.5 ul) inhibited long-term potentiation in the hippocampus of anesthetized rats. In freely moving animals, ICV injections of 7PA2 have been shown to disrupt cognitive function in an alternating lever cyclic ratio (ALCR) operant task (Cleary et al., 2005; Poling et al., 2008). Furthermore, Poling et al. have demonstrated that ICV injections of 7PA2 disrupt working memory in a radial arm maze task.

To date, there are no published studies investigating the effects of 7PA2 infusions into specific neuroanatomical structures. The current study implemented *in vivo* microdialysis to investigate the behavioral and neurochemical effects of 7PA2 in the hippocampal-prefrontal cortical pathway of freely moving rats. Future studies should examine the neurochemical relationship between the catecholamines, amino acids, and the endogenous NMDA antagonist kynurenic acid. These studies may aid in elucidating the mechanisms of action of low-n oligomers of amyloid-β and provide valuable information to researchers developing targeted therapeutics for Alzheimer's disease acting on oligomers of amyloid-β.

Preparation of Synthetic Amyloid-β Oligomers

Stine, Dahlgren, Kraft, and LaDu (2003) originally described the preparation of synthetic oligomers from synthetic Aβ42 (California Peptide, Napa, CA). The authors used hexaflouroisopropanal (HFIP) to dissolve synthetic Aβ42. The solution was then evaporated to form an HFIP film containing synthetic oligomers. The film was then be dissolved in dimethylsulfoxide and diluted to the proper concentration with Ham's F-12 cell culture media. Western blot analysis confirms oligomers in the 13-15 kDa range.

In Vivo Microdialysis

The *in vivo* microdialysis technique provides a methodology to investigate neurochemical release localized in specific neuroanatomical structures. Microdialysis can be employed in a wide variety of species including birds, rodents, monkeys, and humans. When coupled to high performance liquid chromatography (HPLC) with an appropriate

detection method such as electrochemical (amperometric or coulometric), fluorescence, or mass spectrometry, several centrally active neurochemicals can be detected. The centrally active neurochemicals of interest include the monoamines and their metabolites, amino acids and acetylcholine. Additionally, peptides and cytokines can also be detected using these techniques.

To summarize the technique, a microdialysis probe is surgically implanted within the stereotaxic coordinates associated with a particular neuroanatomical structure of interest. A microdialysis probe generally consists of piece of cannula with an inlet line and fused silica exit line at one end and at the opposite end is a section of dialysis fiber with a molecular cut off weight greater than that of the analytes of interest. The typical fiber dimensions are 250 um or less in diameter and 1.0 to 4 mm in length. One end of the fiber is attached to the cannula and sealed at the opposite end with epoxy leaving an active surface area of appropriate length for transfer. Depicted in Figure 1, specialized commercially available probes are designed with a center channel that allows these probes to be used as combination probes and microinjectors (Bioanalytical Systems, Lafayette, IN). This type of probe allows for the delivery of compounds that will not readily cross the dialysis membrane with reverse microdialysis. The reverse microdialysis technique has technical considerations that may exclude its use for certain molecules. These restrictions include that the substance being infused must have a molecular weight below that of the molecular cut off weight of the dialysis fiber, and the substance must not have an attraction to the fiber that allows it to become bound to the fiber.
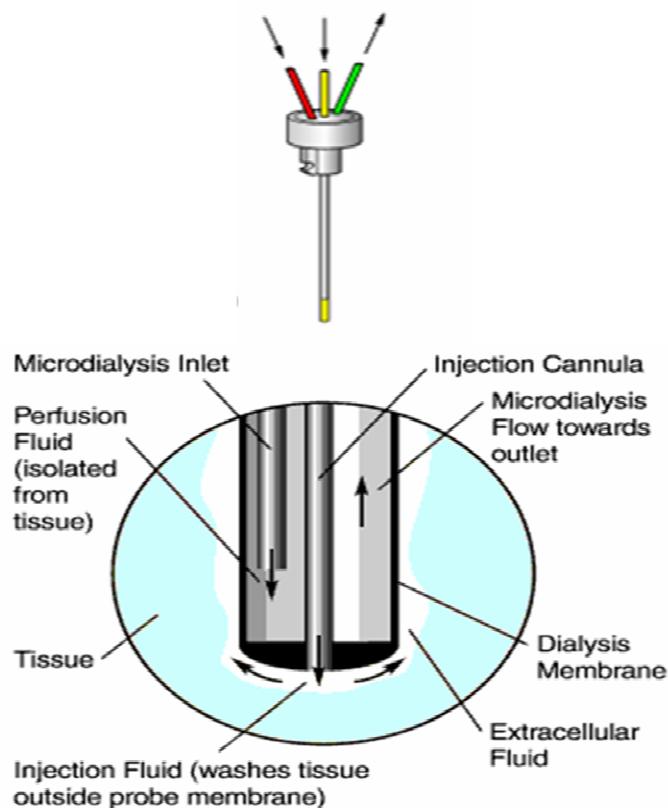
Figure 1. Diagram of a commercially available combination microdialysis probe/ microinjector. (Bioanalytical Systems, Inc., West Lafeyette, IN)

Following probe implantation, artificial cerebral spinal fluid (aCSF) is infused through the probe. Typical microdialysis flow rates range from 0.5 ul/min to 2.0 ul/min. Slower flow rates allow for greater transfer of neurochemicals across the active surface area of the probe. Flow rates and probe dimensions are chosen with respect to the neuroanatomical structure of interest, the detection method, and the temporal resolution required. Figure 2 illustrates the use of a combination microdialysis probe and injector while an animal's activity is monitored.

In Vivo Microdialysis

Microdialysis Pump

Microinjection Pump

Counter Balance Arm

Dual Channel Fluid Swivel

Locomotor Activity Chamber with Infrared Sensors

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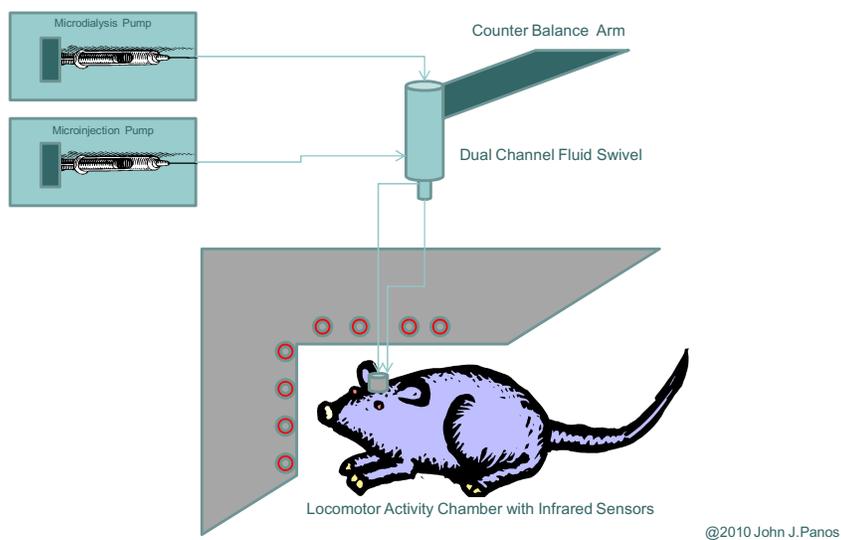
Figure 2. Diagram depicting microdialysis using a combination probe/injector.

High Performance Liquid Chromatography (HPLC)

The detection of monoamines using HPLC equipped with electrochemical detection is accomplished using an isocratic separation and a reverse phase column (Figure 3).

Monoamine Detection
by HPLC_EC
(Coulometric Detection)

Conditioning Cell

EC Cell

Pump

A

Injector

Autosampler

Column

Detector

Integrator
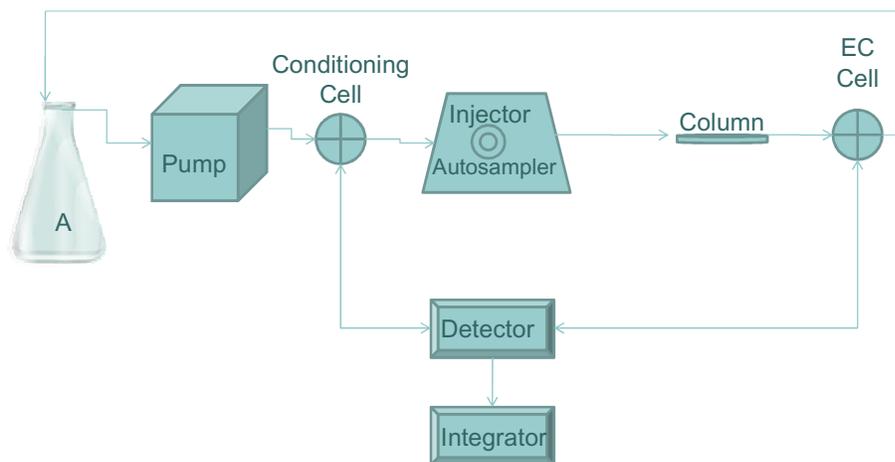
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Figure 3. Schematic diagram depicting an isocratic HPLC system used in the detection of monoamines.

CHAPTER II

METHODS

Synthetic Oligomer Study

Experimental Procedures

    Subjects. Male Sprague-Dawley rats approximately 70 days old were used in this study ($N = 16$). The experimental model employed 12 rats due to surgical complications and the mortality of four rats. Animals were singly housed in polycarbonate cages in an animal facility maintained on a 12:12 hour light/dark cycle and under conditions of constant temperature and humidity. Animals were allowed at least one week to acclimate to the colony environment prior to surgery. Animals were provided free access to food and water throughout the study, with the exception that food was not available immediately following surgery but was replaced once animals had fully recovered from anesthesia. The experimental protocol was approved by the Western Michigan University Institutional Animal Care and Use Committee (IACUC).

    Preparation of Synthetic Oligomer. Experimental subjects received one of two treatments: synthetic amyloid-β oligomer or Ham's F12 (control group). Synthetic oligimer Aβ42 HFIP film was resolubilized by the procedures indicated by Stine, Junbauauer, Yu, and Ladu (2011) (University of Illinois at Chicago). Briefly 0.0451 mg Aβ42 HFIP was dissolved in 2 microliters of sterile dimethylsulfoxide (DMSO), vortexed

11

(30 s) and sonicated for 10 minutes in a bath sonicator, yielding a 5 mM Aβ42 solution. The resuspended 5 mM Aβ42 solution was immediately diluted to a final concentration of 100 uM by adding 98 ul of cold Ham's F-12 culture media. The final 100 um solution was vortexed for 15 s and incubated for 24 hrs at 4° C.

Cell Derived Oligomer Study

Subjects

Male Sprague-Dawley rats approximately 70-90 days old were used in this study ($N = 16$) The model employed 11 rats due to surgical complications and the mortality of 5 rats. Experimental subjects received one of two treatments: amyloid-β oligomer 7PA2 DE23 (increased dimer concentration) or CHO⁻ cells (control group). The procedures for care and use of animals in this study were similar to those described above and this study was also approved by the Western Michigan University IACUC.

General Experimental Procedures

Stereotaxic Surgery

Prior to surgery, animals were administered atropine (1.0 mg/kg, IP) and subsequently anesthetized with sodium pentobarbital (50 mg/kg, IP). Once fully anesthetized, the scalp was shaved and the animal was placed in a stereotaxic apparatus. A small incision was made on the scalp and the periosteum was scraped from the top of the skull. A hole was drilled in the skull using a dental drill. Microdialysis MBR-2 probe guides (BioAnalytical Systems, West Lafayette, IN) were surgically implanted in the

prelimbic/infralimbic area at $+ 3.2$ AP, $+ 0.6$ ML, $- 2.4$ DV. Guides were anchored to the skull with skull screws and cranioplastic cement. Once the cannula was securely cemented in place, the animal was removed from the stereotaxic apparatus and allowed to recover from anesthesia before being returned to the animal colony. Following surgery, all animals were housed individually with elevated cage tops that provide sufficient space to accommodate the animal's guide cannula and head cap. The animals were monitored regularly during recovery from anesthesia. Animals were allowed to recover from surgical procedures for 3 to 5 days prior to the implementation of microdialysis and NOR procedures.

Microdialysis Procedures

An IBR-2 combination microdialysis probe and infusion cannula (BioAnalytical Systems, West Lafayette, IN) with a 2.0 mm length dialysis fiber membrane was inserted into the guide cannula and sterile filtered artificial CSF was perfused at a flow rate equal to 1.5 ul/min. Once inserted, microdialysis probes were allowed to stabilize for a 3-hour period before beginning sample collection. Dialysis samples were collected every 30 minutes and fractionated into equal aliquots for separate analysis of monoamines (40 ul) and amino acids (5 ul). Following the collection of at least six baseline samples, animals received a single microinjection of the appropriate treatment or control as specified above. The test materials were preloaded in the injectors and infusion lines so that they could be delivered without handling the animals. At the onset of the injection sample oligomers or vehicle were infused at a rate 0.5 ul/min for 4 minutes (total injection volume 2 ul). Six additional dialysis samples were collected over a 3-hour period

following infusions. Samples were stored in a –80° C freezer until HPLC-EC analysis was performed.

Locomotor Activity Data Collection

Locomotor activity sample collection was conducted in correspondence with each microdialysis sampling period in a VersaMax animal activity monitoring system (Accuscan Instruments, Inc., Columbus, OH). Data were analyzed for differences in total distance traveled. Statistical analysis was conducted using a repeated measures two-way ANOVA on measures of locomotor activity.

HPLC-EC Analysis (monoamines)

The microdialysis samples were analyzed using an ESA (Chelmsford, MA) CouloChem II HPLC system with autosampler and MD-TM mobile phase purchased from DIONEX Corporation (Sunnyvale, CA). HPLC-EC analysis was performed to assess monoamines and their metabolites in each microdialysis sample. Chromatographic analysis software ESA 501 (Chelmsford, MA) was used to analyze the concentrations of each analyte (DA, DOPAC, 5-HIAA, 5-HT, HVA, NE). A three-level standard calibration curve was determined to quantify levels of analytes in microdialysis samples. Data were analyzed as percent of control, which was calculated by dividing the analyte concentration (nanomoles) in the post-infusion samples by the average analyte concentration of at least three stable baseline samples. Repeated measures General Linear Model analysis was performed in each experiment.

Histology

Rats were euthanized with a lethal injection of sodium pentobarbital according to American Veterinary Medical Association panel on euthanasia guidelines (AVMA, 2007). Transcardial perfusion with a formalin solution was immediately performed following euthanasia. Brains were removed and stored in a formalin solution. Brain tissue was sectioned at a thickness of 60µm on a Vibratome. Examination of brain sections was performed to verify guide placement.

Statistical Analyses

Statistical analyses were conducted using MYSTAT 12 (SYSTAT Software, Inc., Chicago, IL) and GraphPad Prism 4.0 (GraphPad, Inc., La Jolla, CA). Neurochemical data and locomotor activity were analyzed by Repeated Measures Analysis of Variance (RM-ANOVA). Neurochemical concentrations were first transformed into baseline percentages. The baseline percentage for each analyte was calculated based on three or more stable baseline samples not varying by more than 10% in concentration. Post-infusion samples were transformed to a percentage of the average baseline. RM-ANOVA analyses of locomotor activity data were based on distance traveled in centimeters (cm). Total distance traveled is depicted in all locomotor activity graphs for data collected in conjunction with *in vivo* microdialysis. Based on graphical analysis of the neurochemical data, a single sampling period was selected to calculate 5-HIAA/HVA and DOPAC/HVA ratios. The time period selected was post-infusion period (P1). P1 refers to the sample

collected between 30 and 60 minutes after the infusion and represented the maximal

effect observed following infusion.

CHAPTER III

RESULTS

Neurochemical Results with Synthetic Oligomer

Analyses of monoamine metabolite data for 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-Hydroxyindoleacetic acid (5-HIAA) resulted in no significant effect of the synthetic oligomer ifusion for the 3-hour time period post-infusion (Figures 4, 5, and 6). Additional analysis of the Infusion (0 to 30 min) and P1 (30–60 min) post-infusion period yielded no significant differences. A two-sample t test (pooled variance) was conducted on the metabolite percent baseline ratios at P1 (60 min post-infusion) for 5-HIAA/HVA resulting in a significant group difference for the synthetic oligomer injection ($t(10) = -2.473$, $p < 0.05$). No significant difference was found for the DOPAC/HVA ratio following the synthetic oligomer infusion.

Figure 4. Percent baseline values for DOPAC in the PFC, comparison of Ham's F12 culture media (control injection) to Aβ42 synthetic oligomer. (*Note:* error bars indicate S.E.M.)

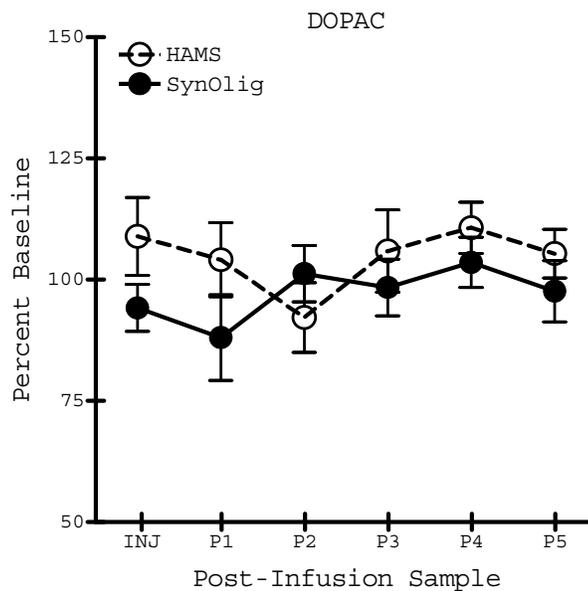*Figure 5.* Percent baseline values for HVA in the PFC, comparison of Ham's F12 culture media (control injection) to Aβ42 synthetic oligomer. (*Note:* error bars indicate S.E.M.)

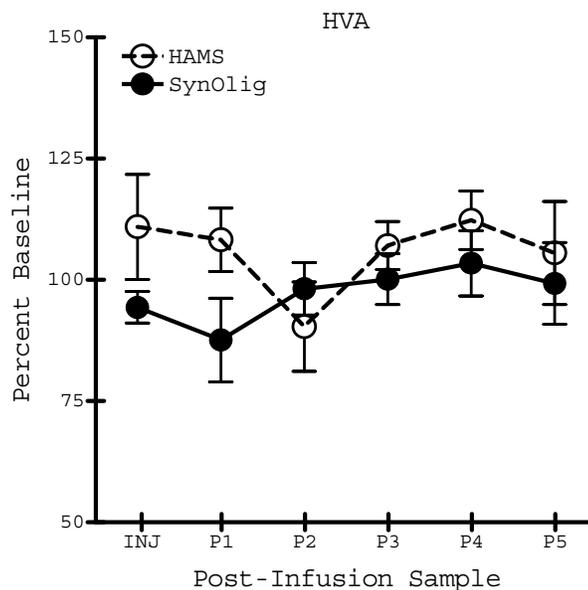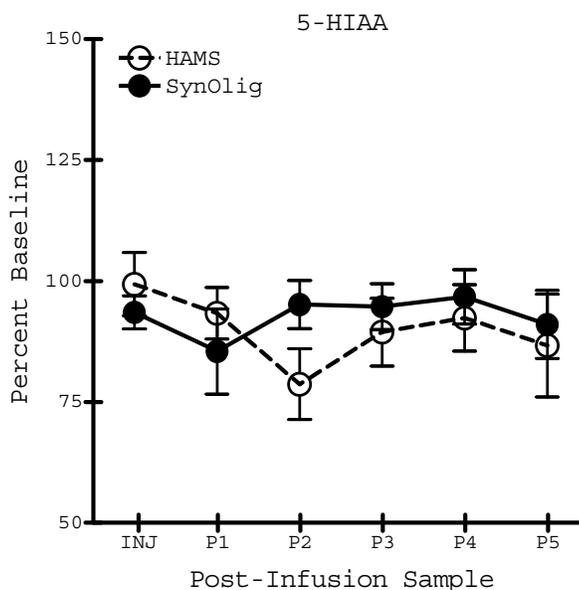*Figure 6.* Percent baseline values for 5-HIAA in the PFC, comparison of Ham's F12 culture media (control injection) to Aβ42 synthetic oligomer. (*Note:* error bars indicate S.E.M.)

Neurochemical Results 7PA2 Cell Derived Oligomer

For the 7PA2 cell derived oligomer study, RM-ANOVA on the injection sample through post-injection sample 5 (p5) yielded a significant difference for the analysis on DOPAC for the treatment by sample interaction $F(5, 35) = 2.544$, $p < 0.05$. No other significant results were found for DOPAC, HVA or 5-HIAA following the cell derived oligomer injection (Figures 7, 8, and 9). Although, there appeared to be a trend for metabolites to have a reduced efflux for at least 90 minutes post injection. Further analyses were run on subsets of the data assuming the effect was small and brief. RM-ANOVA was used to analyze the data for the injection (0 to 30 minutes post-infusion) and the post-infusion sample P1 (30 to 60 minutes post-infusion). A significant treatment

effect for 7PA2 vs. CHO⁻ (control) was found for 5-HIAA ($F(1, 7) = 11.225$, $p < 0.05$).

The reduction of 5-HIAA is shown for sample points labeled INJ and P1 in Figure 9.

Figure 7. Percent baseline values for DOPAC in the PFC, comparison of CHO⁻ media (control injection) to 7PA2/DE23 cell derived oligomers. (*Note:* error bars indicate S.E.M.)
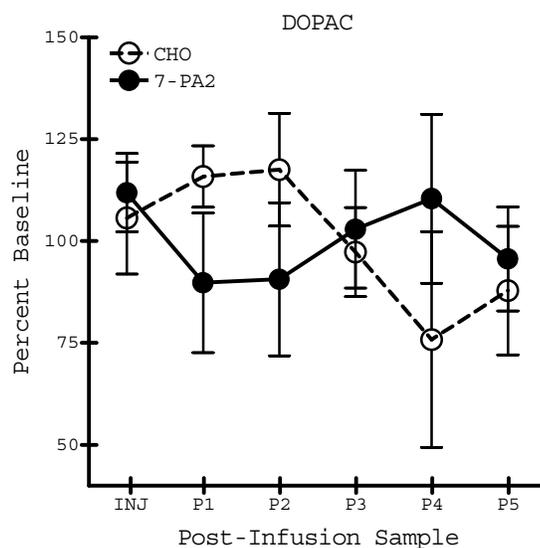
.

Figure 8. Percent baseline values for HVA in the PFC, comparison of CHO⁻ media (control injection) to 7PA2/DE23 cell derived oligomers. (*Note:* error bars indicate S.E.M.)
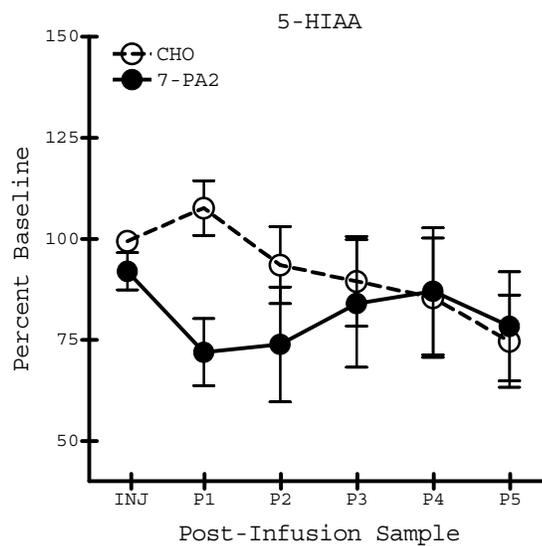
Figure 9. Percent baseline values for 5-HIAA in the PFC, comparison of CHO⁻ media (control injection) to 7PA2/DE23 cell derived oligomers. (*Note:* error bars indicate S.E.M.)
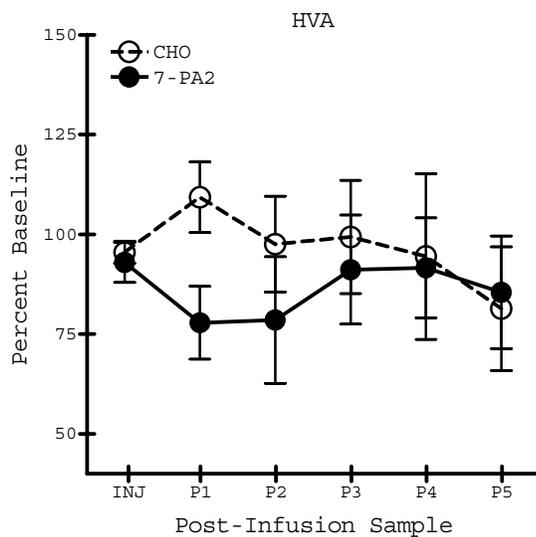
Locomotor Activity Results

Analyses of locomotor activity data by RM-ANOVA indicated that both cell derived and synthetic oligomer microinjections into the PFC had no significant effect on locomotor system activation (Figures 10 and 11).

Figure 10. Distance traveled (cm) representation of locomotor activity for the Aβ42 synthetic oligomer and Ham's F12 vehicle. (*Note:* error bars indicate S.E.M.)

Figure 11. Distance traveled (cm) representation of locomotor activity for the 7PA2/DE23 cell derived oligomers and CHO⁻ vehicle. (*Note:* error bars indicate S.E.M.)

CHAPTER IV

DISCUSSION

This study was the first to compare the neurochemical effects of synthetic amyloid-β oligomers and the dimer enhanced cell derived amyl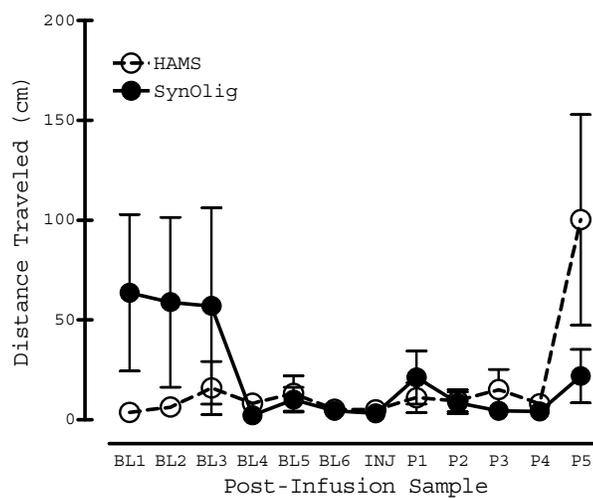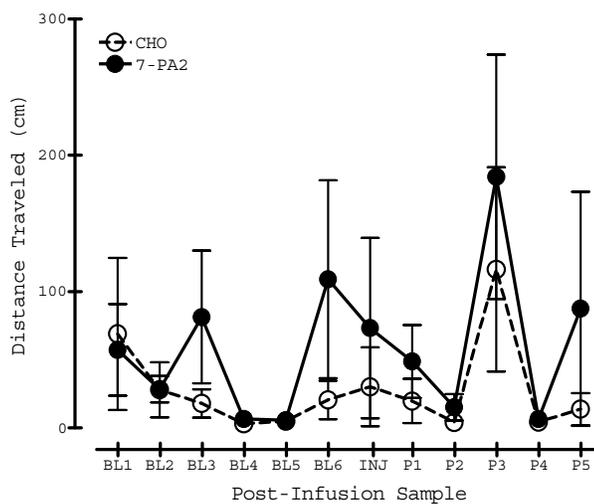oid-β oligomer 7PA2/DE23 in the rat pre-frontal cortex. The study employed a novel methodology of delivering the amyloid-β to the target brain region through the microdialysis probe. This method allowed for the sampling of neurotransmitter efflux directly at the site of oligomer action in the prefrontal cortex. Other studies have combined microdialysis in the hippocampus and used separate intracerebroventricular (ICV) injection techniques to deliver synthetic amyloid-β oligomers (Watanabe et al., 2008) and 7PA2 cell derived oligomers (O'Shea et al., 2008). O'Shea et al. demonstrated that an acute 20 ul ICV injection of the cell derived oligomer 7PA2 (CM) increased glutamate in the hippocampus when measured in the dialysite for 260 minutes post injection. No effect was demonstrated for the amino acids aspartate and GABA. Watanabe et al. (2008) infused amyloid-β oligomers (200 pmol/20 ul) daily for 7 days via bilateral ICV cannulae in combination with a model of cerebral ischemia. The authors demonstrated that high potassium evoked release of acetylcholine was reduced. While the methods employed by these investigators allowed for sampling of neurotransmitters at the probe site, global distribution of the oligomer via the ventricular system does not allow for determination of specific neuroanatomical sites of action.

The methodology of the current study also differs from the aforementioned microdialysis studies with respect to the volume of amyloid-β infused. The current methodology employed low microliter infusions (2 ul) to avoid dilution of normal neurotransmitter levels and tissue damage at the injection site. An additional advantage of the probe/injector combination used in the current study is that it allows for compounds to be completely delivered in a designated volume unlike reverse microdialysis. With reverse microdialysis, the transfer rate across the membrane needs to be accounted for to estimate the amount of a particular compound to be infused in the aCSF, thus requiring larger quantities of the compound. The combination probe/injector allows for compounds that exceed the MWCO of the dialysis fiber (30 kDa) to be infused, an important consideration for future investigations with compounds like Aβ*56(~56 kDa).

The results of the current study indicate both synthetic oligomers and cell derived oligomers may affect monoamine metabolites in the PFC. The effects appear to be more pronounced with the 7PA2 cell derived oligomer compared to the Aβ42 HFIP synthetic oligomer. Moreover, these effects appear to be relatively short-lived (<90 minutes) with the greatest effect of 7PA2 being on the monoamine metabolite 5-HIAA within 60 minutes following injection. The effects of the synthetic oligomer injection could be demonstrated only in the 5-HIAA/HVA ratio and may be attributed to lower ratios for two of the control animals. It is unclear whether the effects are related to monoamine metabolism or the availability of the monoamine serotonin (5-HT). However, these results indicate the involvement of the serotonergic system in the effects of an amyloid-β oligomer.

Previous studies indicate alterations in brain monoamines may be associated with the neuropathology of Alzheimer's disease. For example, studies examining monoamine metabolites in the cerebrospinal fluid of Alzheimer's patients have demonstrated decreases in 5HIAA and HVA (Sjögren, Minthon, Passant, Blennow, & Wallin, 1998). In a review of pharmacological treatments for Alzheimer's patients by Raskind and Peskind (2001), atypical antipsychotics are suggested as the initial choice for treating psychosis and agitation in Alzheimer's disease. Moreover, their review of the empirical research indicates low dose treatments with risperidone or olanazapine provide the optimal therapeutic to adverse effects ratio. These atypical antipsychotics exhibit affinity for both serotonergic and dopaminergic receptors.

The results of the current study are relevant to further explorations regarding the role of monoaminergic changes in Alzheimer's disease and may provide insight into a novel mechanism of action related to Aβ oligomers and serotonin. Future studies elucidating monoaminergic mechanisms in the actions Aβ oligomers may lead to the development of novel pharmacotherapeutic approaches to the treatment of Alzheimer's disease.

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Appendix A

Institutional Animal Care and Use Committee
Letter of Approval

WESTERN MICHIGAN UNIVERSITY

Centennial
1903-2003 Celebration

Date: February 11, 2009

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

From: Robert Eversole, Chair

Re: IACUC Protocol No. 09-01-02

Your protocol entitled "Neurochemical Effects of Beta-Amyloid Oligomers" 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: January 14, 2010