The Progression of Aβ Proteins in the Retrosplenial Cortex Using an APP/PS1 Alzheimer’s Disease Transgenic Rodent Model

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The Progression of Aβ Proteins in the Retrosplenial Cortex
Using an APP/PS1 Alzheimer’s Disease Transgenic Rodent Model

by

Maha H. Rashid

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Requirements of Independent Study Thesis Research

Supervised by
Dr. Amy Jo Stavnezer
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Abstract

Alzheimer’s disease is the most common form of dementia in the world with a new case of dementia diagnosed every 3.2 seconds. For over 25 years the amyloid cascade hypothesis has existed as a mechanism of understanding and diagnosing Alzheimer’s Disease. The amyloid cascade hypothesis suggests that Alzheimer’s Disease is sequential in protein progression and proliferative from the medial temporal lobe to the rest of the brain. Hypometabolism of brain regions has also been correlated to plaque deposition and hypometabolism is observed in brain regions that make up the default mode network, specifically the retrosplenia cortex, which is not accounted for in the amyloid cascade hypothesis. The default mode network is the series of brain regions that are activated when the brain is at rest. To assess the validity of the amyloid cascade hypothesis, we tracked the rate of plaque progression in the retrosplenia cortex compared to other traditionally afflicted brain regions such as the hippocampus and entorhinal cortex with immunofluorescent staining of Aβ plaques in APP/PS1 transgenic mouse brains at 13, 16, and 28 months. Results showed that there is a significant difference in plaque deposition over time and between brain regions. The rate of progression at this age range in the retrosplenia cortex is consistent and higher than traditionally afflicted brain regions. Results of this study gives more insight into the role of Aβ of disease progression, as well as the validity of the amyloid cascade hypothesis.

Keywords: Alzheimer’s Disease, Aβ, Default Mode Network, Hypometabolism, Retrosplenia Cortes, Amyloid Cascade Hypothesis
Progression of Alzheimer’s Disease

**History and Prominence of Alzheimer’s Disease**

Dr. Alois Alzheimer was a prominent German doctor in the 19th century. His research interests spanned both neurodegeneration and psychosis. His interests were reflected in his work at a Frankfurt hospital for the mentally ill. In 1901, Auguste Deter, a 51-year-old woman, was admitted into the Frankfurt hospital. She became Dr. Alzheimer’s patient because she exhibited symptoms of severe cognitive impairment, aphasia, auditory hallucinations, delusions and psychosocial incompetence (Maurer, Volk, & Gerbaldo, 1997). In 1903, Dr. Alzheimer moved from Frankfurt to Munich to acquire a different position at a different psychiatric clinic. This move however did not distance his interest in Auguste. He maintained contact with her and her records until her death in 1906. After her death he performed an autopsy, which facilitated the discovery of unique characteristics that were dissimilar to the normal aging brain. Auguste’s brain, according to Dr. Alzheimer, had characteristic neurofibrillary tangles and senile plaques. Dr. Alzheimer later presented his research in 1907 about this neurodegenerative disease of the cerebral cortex. Since then much scientific research has focused on understanding these characteristic plaques and tangles. In fact, they have become a hallmark of this form of dementia known as “Alzheimer’s Disease” (AD) in homage to Dr. Alzheimer.

Auguste Deter had a very peculiar disorder in the eyes of Dr. Alzheimer. His research had previously focused on neurodegeneration, but he had never been exposed to someone with Auguste’s symptoms, especially at her young age. This early progression of AD was the first notable case study of its kind. We now know this early progression of AD has a genetic risk associated with it. Early onset AD only afflicts five percent of patients diagnosed with AD. Current literature suggests most individuals develop AD after the age of 60 (An et al., 2008),
and that there is increasing risk associated with increasing age, meaning that as life expectancy increases so does the prominence of the diagnoses associated with AD.

The age mediated type of AD is known as late-onset AD; symptoms begin after the age of 65 and increase with age. Late-onset AD, also known as sporadic AD, is the more common form of this dementia, affecting over 90% of those diagnosed with AD. True to its name, the cause of late-onset AD is still unknown, although it is thought to be a combination of environmental, genetic, and lifestyle factors. Conversely, the cause of early onset AD, also known as familial AD, which causes symptomology to occur before the age of 60, is genetic. Individuals with familial AD often have genes that put them at a higher risk for developing the disease. This is the likely cause of Auguste Deter’s AD. Different mutations on different chromosomes, such as chromosome 21, 14, and 1, can lead to AD. A mutation on chromosome 21 can cause a defect in amyloid precursor protein, while a mutation on chromosome 14 can cause abnormal presenilin 1 (PS1) protein, and a mutation on chromosome 1 can lead to abnormal presenilin 2 (PS2) protein (Ellenbroek & Youn, 2016). Each of these plays a part in the formation of the aforementioned plaques. The discovery of these genetic mutations has allowed researchers to study the progression of AD in nonhuman models.

One form of nonhuman models that researchers have focused on are rodents. They have created 160 different transgenic models of AD. Transgenic models, usually rodents, are genetically modified to simulate a disease state. Common models can look at disease states that range from Attention Deficit Hyperactivity Disorder and cancer types, to Parkinson’s disease. In the case of AD, many different transgenic models exist, each focusing on a different aspect of the disease. The progression of AD in each model is variable on both a cellular and behavioral level. Some examples of transgenic rodents’ models include APP/PS1, where researchers manipulate the amyloid precursor proteins and presenlin proteins associated with gamma secretase. It is
important to note that animal models cannot fully encompass the disease, nor the higher order processes that are afflicted by AD. However, they do provide a good preclinical background to a disease that has such a high clinical population. They also provide answers to basic cellular processes and treatment possibilities.

In the year 2000, there were 25 million people diagnosed with AD, and it is the fastest growing disease for individuals over the age of 60 (An et al., 2008). According to the World Health Organization (WHO) 63 million people will be diagnosed with AD by 2030 and 114 million will be diagnosed by 2050. There is a new case of dementia diagnosed every 3.2 seconds, which implies that every year there are 9.9 million new diagnoses (“Dementia statistics | Alzheimer’s Disease International,” 2017). Literature suggests that developed nations often have higher life expectancies and therefore are more stricken with AD diagnoses (An et al., 2008). In 2018 the cost to treat AD worldwide exceeded 1 trillion US dollars, which equated to about 1% of the world’s gross domestic product (GDP) (“Dementia statistics | Alzheimer’s Disease International,” 2017).

Not only does AD create a financial burden, but AD also creates a series of cognitive and life deficits for individuals and families affected by the disease. Memory loss, emotional instability, lack of coordination, and hallucinations are just some of the symptoms that exist for AD patients. These symptoms are not simultaneous but progress and develop as the pathology develops in the brain. Since it is a continuously worsening disease, family members often become caregivers for these individuals, which can have a large impact on the daily lives of those family members. Caregivers often feel a sense of guilt, grief and anger for their loved one. Interestingly, millennials are becoming the prominent caregivers in American society (“The Challenging Life of a Millennial Caregiver | Time,” 2018). These millennials often juggle full time employment with over 20 hours of unpaid caregiving. This causes unnecessary stress and
could lead to possible health implications later in life, bringing the possibility of a multigenerational epidemic to light.

Despite the enormous number of people affected, treatment of AD is merely symptomatic. Many treatments that exist include cholinesterase inhibitors, N-methyl-D-aspartate (NMDA) receptor noncompetitive antagonists and vaccines. However, none stop the progression of the disease; they only minimize symptoms. These treatments are based on a theory known as the amyloid cascade hypothesis which attempts to identify the early biomarkers of AD. It has existed in scientific literature for 25 years but could be changing given recent research.

**Progression Theory of Alzheimer’s Disease**

The amyloid cascade hypothesis has played a very influential role in the AD research community (Karran, Mercken, & Strooper, 2011). There is a significant amount of evidence suggesting a key role of beta amyloid plaques in AD progression; however, the amyloid cascade hypothesis does not incorporate all aspects of the disease. The hypothesis proposes that AD progression is sequential and proliferative. It is sequential in the sense that the aggregation of proteins known as beta amyloid (Aβ) lead to the production of neurofibrillary tau tangles (NFT) through a series of intracellular events. AD is proliferative because past literature suggests that its neuropathology begins in the hippocampus and unidirectionally moves to other areas of the brain (Karran et al., 2011). To outline this progression, first I will develop the sequential aspect of the disease through the explanation of Aβ and its development. Then I will discuss the proliferative aspect of the disease by explaining brain areas affected by Aβ deposition and corresponding behaviors implicated. Finally, I will discuss the brain regions I believe are the first to be afflicted in AD due to the growing literature about the default mode network’s role in AD progression. I will explain the deficits in the amyloid cascade hypothesis by introducing the concept of metabolism and the important role it plays in the determination of AD.
Sequential. To understand the suggested mechanism of AD it is important to understand the component parts, beginning with Aβ. Aβ, a protein, is made from amyloid precursor protein (APP). APP is a single transmembrane protein that is highly prevalent in the brain. The exact function of APP is unclear; however, recent literature suggests that it plays a role in neuronal repair and axonal transport (Kametani & Hasegawa, 2018). Over expression in some transgenic mouse models has shown benefits to cell health and growth; however, lack of expression has not shown deleterious effects on the health of the organism (O’Brien & Wong, 2011), implying that APP does not play a critical function in the body. In addition to having an ambiguous role, it is metabolized in a complex manor by proteins known as secretases (O’Brien & Wong, 2011). These secretases have a central function in the creation of Aβ, specifically Aβ42.

APP cleavages are normally propagated by the α-secretase and γ-secretase, and some Aβ proteins exist in the normal brain. However, there are mutations that cause the secretases to favor proteolytic processing of APP (Hardy et al., 2002). Those mutations are the aforementioned presenilin mutations on chromosomes 1 and 14. Proteolytic processing refers to the function of proteases when they cleave one or more bonds on a specific protein to affect activity (Karran et al., 2011). This proteolytic processing is hypothesized to occur in the AD brain. There are two pathways that cause the breakdown of APP: amyloidogenic and nonamyloidogenic (Figure 1). The amyloidogenic pathway lead to the creations of Aβ, specifically Aβ42, while the other does not.

In the nonamyloidogenic pathway APP is first cut by α-secretase, which causes the production of two soluble protein fragments: carboxyl terminus α (CTFα) and soluble amyloid precursor α (sAPPα). γ-secretase then cuts the remaining transmembrane CTFα to produce soluble peptide p3 outside of the membrane and APP intracellular cytoplasmic domain (AICD) inside the membrane (Selkoe & Schenk, 2003). Each subunit of APP in the nonamyloidogenic
pathway has the ability to be metabolized by the body. The amyloidogenic pathway is slightly different.

APP is first internalized into an endosome. An endosome is an acidic compartment found in eukaryotic cells that helps facilitate the differentiation of material before degradation. After

![Diagram of APP cutting through amyloidogenic (B) and nonamyloidogenic pathway (A). B is the disease-based cleavage of APP while A is the “normal” cleavage of the disease. Adapted from O’Brian and Wong, 2011, p. 21.]

Figure 1. Amyloid precursor protein (APP) cutting through amyloidogenic (B) and nonamyloidogenic pathway (A). B is the disease-based cleavage of APP while A is the “normal” cleavage of the disease. Adapted from O’Brian and Wong, 2011, p. 21.

After internalization into the acidic membrane environment, β-secretase has the ability to cut APP into carboxyl terminus β (CTFβ) and soluble amyloid precursor β (sAPPβ). γ-secretase then cuts the CTFβ into AICD and Aβ. sAPPβ and Aβ then are released into the extracellular space (Selkoe & Schenk, 2003). The Aβ portion of APP that is produced by the β-secretase cleavage is not
soluble. This reordering of enzymatic cleavage creates the $A\beta_{42}$, which are the hallmark “sticky” proteins that exist in AD.

Along the amyloid path it is hypothesized that there is more $A\beta_{42}$ produced and the overproduction leads to an accumulation of $A\beta_{42}$. Some accumulation is not deleterious to the aging brain, in fact it is often found in most aged brains (Kametani & Hasegawa, 2018). The inability for the amyloid to be degraded along with its “sticky” nature leads to the development of oligomers and plaques. Oligomers are proteins that consist of a few monomer units and plaques are thought to be the buildup of those $A\beta_{42}$ oligomers. Those in turn lead to the lower function of neurons in the brain due to a decrease in neuronal signaling. These plaques exist extracellularly in relation to the cells.

These plaques, also known as senile plaques, have an influence on some deleterious aspects of brain function. Those include immune activation, synaptic transmission, and phosphorylation of tau proteins. First in regard to immune response, glia such as astrocytes and microglia become activated. Their role is to clear the amyloid from the synapse and extracellular space. This activation is known to cause inflammation in the brain. Inflammation can cause brain damage, specifically atrophy, which can accelerate the process of neurodegeneration. Patients with AD have been found to have higher levels of activated inflammasomes and proinflammatory cytokines (Frost & Li, 2017). In addition to inflammasomes and cytokines, cognitive decline has been associated with astrogliosis (Frost & Li, 2017). Astrogliosis is the abnormal increase in the number of astrocytes due to disruptions in the system. This is disadvantageous due to the inflammation caused by the overproduction of astrocytes.

Synaptic transmission is another aspect of the disease effected by $A\beta$. Individual $A\beta$ proteins along with plaques can influence transmission. Deficits in synaptic transmission can affect memory formation and day to day functioning. It is hypothesized that individual $A\beta_{42}$
proteins cause a sequential buildup of the non-soluble plaques, which cause decreased communication between synapses. The decrease in synaptic transmission begins to have an effect on tau, and its role in the cytoskeleton of the neuron. Tau is the protein that holds the microtubules in the axon together; therefore, a decrease in use will cause tau to phosphorylate off the microtubule. This aggregation of tau has historically led to tauopathies, which are characteristic of AD (Kaufman, Del Tredici, Thomas, Braak, & Diamond, 2018).

The neuronal cytoskeleton acts as structural support to the neuron but also transports nutrients and precursor proteins from the soma to the axon terminal. Tau acts as a connecting protein to microtubules that make up the cytoskeleton. Phosphorylation of tau proteins in AD is thought to occur because of Aβ42 build up. To understand Aβ42’s relationship to the progression of AD, it is important to understand the underlying description of a neurofibrillary tangle (NFT). NFTs are produced as a result of phosphorylation of the tau protein. This phosphorylation occurs as a result of increased calcium production in the brain. It is hypothesized that Aβ42 causes the creation of NFT because of its relationship with intracellular calcium (Hardy & Higgins, 1992). Intracellular calcium is necessary for the release of neurotransmitters into the post synaptic cell. NFT also lead to an increase in calcium which damages second messengers used in intracellular signaling inability for neurons to transport nutrients and neurotransmitters is detrimental to synaptic function and leads to many of the neurodegenerative properties associated with AD such as memory loss and cell death.

Three types of treatments exist to combat this, NMDA receptor antagonists acetylcholinesterase inhibitors and vaccines which work to decrease plaque buildup in the brain (Birks et al., 2006; Herline, Drummond, & Wisniewski, 2018). NMDA receptor noncompetitive antagonists block the binding of glutamate since it is assumed to be overactive in the AD brain (Danysz & Parsons, 2003). Cholinesterase inhibitors block the breakdown of acetylcholine,
which is hypothesized to improve neuronal signaling (Birks et al., 2006). Vaccines have also been created to slow down the progression of the disease, specifically by inhibiting the production of the senile plaques. Unfortunately, none of these treatments have worked in slowing or preventing the disease. This is due to their limited goal of only targeting amyloid beta plaques. Due to the treatments’ minimal efficacy, we can conclude that Aβ and tau are not the only factors that contribute to the progression of AD.

**Brain Regions Affected During AD**

Alzheimer’s pathology has often focused on the hippocampus because of the direct link to symptomology of memory loss associated with the disease. However, AD affects more than the hippocampus since other symptoms such as emotional instability, lack of coordination, and hallucinations exist in AD patients. In this section I will evaluate the effects of aforementioned Aβ pathology on different regions of the brain. The amyloid cascade hypothesis assumes that progression is proliferative from the inside of the brain out, more specifically from the medial temporal lobe to the cortex. To clarify we will first evaluate more subcortical brain regions such as the hippocampus, amygdala, corpus collosum, thalamus, and hypothalamus. Then we will focus on more cortical regions such as the temporal lobe, entorhinal cortex, frontal lobe, parietal lobe, occipital lobe, and cerebellum. This section will then specifically turn the focus to the posterior cingulate and retrosplenial cortex and their hypothesized prominent role in the progression and associated with the symptomology of the disease.

**Subcortical Regions**

The hippocampus has historically been linked to episodic memory formation and is thought to be the first brain region implicated in the disease (Figure 2). This form of memory can be exhibited in the remembrance of one’s 12th birthday party or one’s wedding. It builds throughout a lifetime to create the “self”. In AD, the Aβ plaques and tau lead to atrophy of the
hippocampus, causing memory loss and disorientation. In addition to memory loss, individuals with AD have difficulty forming new memories in regard to location and time (Rodrigue, Kennedy, & Park, 2009). Literature has shown that there is a negative correlation between hippocampal atrophy and episodic memory (Rodrique et al., 2009), suggesting that as the size of the hippocampus shrinks, the loss of memory correlated to events increases. Hippocampal atrophy has also been correlated with amygdala atrophy (Poulin, Dautoff, Morris, Barrett, & Dickerson, 2011).

The amygdala is rostrally adjacent to the hippocampus and is often regarded as the emotional center of the brain. It regulates emotions such as anger, pleasure, and aggression. Accumulation of Aβ and tau also leads to the atrophy of the amygdala. Literature has suggested

![Figure 2](image)

*Figure 2.* Representation of the progression of Aβ from the medial temporal lobe (MTL). The hippocampus is located in the MTL. Red arrows represent progression while blue circles represent hypometabolism. The cortical and subcortical regions represented by the blue are general and not specific brain regions. Adapted from Smith (2002).
that there is a relationship between increased atrophy and aberrant motor behaviors (Poulin et al.,
2011). Aberrant motor behavior is often described colloquially as an inability to sit still.
Although aberrant motor behaviors are not emotion-based symptoms, they are still thought to be
associated with the amygdala. This accumulation of plaques and tangles also often causes
emotional instability. More anxiety, stress, anger and paranoia are expressed when this brain
region is implicated (Poulin et al., 2011). Although the amygdala is the emotional center of the
brain, the thalamus is the sensory relay center, and important for all other brain function.

In addition to being known as the sensory relay system, the thalamus affects sleep
regulation and consciousness. Literature has often focused on the medial temporal lobe as the
hallmark for the initiation of AD pathology (Aggleton, Pralus, Nelson, & Hornberger, 2016);
however the thalamus and thalamic nuclei have the potential to be early initiators of the disease.
This is probably due to their high input and output of information to and from cortical regions.
Behavioral symptomology associated with Aβ progression has been thought to cause deficits in
hearing, touch, sleep, and sight. This could be indicative of thalamic damage.

The hypothalamus is involved with hormone regulation in the body. They control
metabolism, growth, mood, hunger, sleep, thirst and sex drive (Vercruysse, Vieau, Blum,
Petersén, & Dupuis, 2018). Atrophy to the hypothalamus has been shown to be present in a
subset of the AD population. A cellular explanation can be guided by the substructures that make
up the hypothalamus. The paraventricular nucleus, lateral hypothalamus, suprachiasmatic
nucleus (SCN), tuberomammillary nucleus and the supracholinergic nucleus have all been shown to be
affected by Aβ. Neurons containing orexin, in many of those regions are decreased in AD
(Vercruysse et al., 2018). Orexin is a hormone produced that regulates food intake; therefore, its
decrease in AD leads to loss of appetite. The SCN also has shown neuronal loss in AD patients,
suggesting and explanation for loss of sleep regulation.
The corpus collosum is the pivotal connection between the left and right cortical hemispheres of the brain (Ardekani, Bachman, Figarsky, & Sidtis, 2014). This structure is important for communicating information to other cortical areas and coordinating brain function. When the corpus collosum is implicated in AD there is often a loss of processing speed for motor function. Studies often focus on the size of the corpus collosum, but the shape is also important in the progression of the disease. Early in the disease there has been shown to be a significant difference between circular shape as compared to size, therefore creating a more prominent marker for early disease progression (Ardekani et al., 2014). Normally, the corpus collosum maintains a circular shape, however, in the AD brain the corpus callosum tends to lose its normal size and shape.

**Cortical Regions**

The temporal lobe is associated with comprehension of sound, including spoken word and memory for language. It is also the cortical input and output of the hippocampus. When implicated in AD it is often shown to be correlated to deficits in episodic and semantic memory. This means that although a person could be able to recognize an item, they would not remember the context of the item. For example, a person with AD could recognize a set of keys but could not remember the fact that they had seen them previously or where they would have been. The entorhinal cortex (EC), located in the medial temporal lobe, is important to memory function. It is extremely vulnerable to the plaques and tangles of AD pathology. It has also been found to be affected by atrophy early on in the disease (Du et al., 2003), making atrophy of the EC a possible biomarker for the early stages of AD. In fact, recent literature suggests that in addition to atrophy, “seeds” or prions of tau are first localized in the EC and the transentorhinal cortex. This initial aggregation of tau is thought to lead to the tauopathy expressed in AD brains.
The frontal lobe, associated with executive functions, inhibitory control and decision making, is also implicated in AD; it is. More specifically it is involved with voluntary movements, problem solving, impulse and emotional control, and execution of speech and writing skills (Hirono et al., 1998). When AD affects the frontal lobe, it is often associated with loss of motivation, inappropriate behaviors, and repetition of words or phrases. Pathology of AD in the frontal lobe is accompanied by symptomology that can include public urination, swearing, and eating and drinking nonfood items. The higher executive functions that are diminished by Aβ progression in the frontal lobe are paired with diminished behavioral functions in the parietal lobe.

Normally the parietal lobe is associated with interpretations of somatic sensation such as pain, pressure, and temperature. Different hemispheres of the parietal lobe have different functions and are implicated differently in AD progression. For instance, plaque progression in the left parietal lobe causes difficulty in math and writing, while plaque deposition in the right parietal lobe causes difficulty in interpreting faces, surroundings and objects. The parietal lobe also aids in spatial and motor memory; therefore, pathology in this region can often cause loss of fine motor movements such as writing and drawing.

The occipital lobe is associated with the visual system. The atrophy caused to the occipital lobe in AD is often thought to cause illusions, misidentification, misperception, and hallucinations. In addition to the psychiatric symptomatology, atrophied occipital lobe has been shown to cause lower cognitive scores, higher morbidity and increased cognitive decline (Holroyd, Shepherd, & Downs, 2000). Increased pathology in the occipital lobe has been thought to cause deficits in facial recognition, this is detrimental when paired with increased pathology in the temporal lobe, with deficits mentioned previously.
A brain region not often regarded for cognitive function is the cerebellum. This region is often associated with coordination, voluntary movement and balance. Recently it has been suggested that the cerebellum plays an important role in the alteration of emotion and cognition (Jacobs et al., 2018). \( \text{A}\beta \) is found in the molecular layer of the cerebellum but is not found in senile plaque form (Sepulveda-Falla et al., 2014). It is unknown why the discrepancy in \( \text{A}\beta \) exists, however the behavioral deficits are still present no matter the \( \text{A}\beta \) form. Buildup of this \( \text{A}\beta \) is thought to damage the universal cerebellar transformation, a theory suggesting that there is a relationship between motor movement and cognition in the cerebellum (Sepulveda-Falla et al., 2014). On a mechanistic level, the buildup of \( \text{A}\beta \) often causes a lack of balance and coordination, tremors, abnormal eye movements, and slurred speech. This is hypothesized to happen because of a loss of processing speed in the brain (Jacobs et al., 2018). This can be associated with aforementioned symptoms and adds yet another layer to the progression of the disease.

A previously mentioned subcortical region that is involved with cortical regions is hypothalamus. In addition to regulating hormones, it also regulates metabolism, which is affected in AD. Since the ORX neurons as well as the SCN are affected in AD, it has been suggested that they could also play a role in the hypometabolism found in the brain. That decrease in metabolism has been linked to \( \text{A}\beta \) deposition later in life, especially in the posterior cingulate cortex.

**Posterior Cingulate Cortex, Retrosplenial Cortex, and the Default Mode Network**

The posterior cingulate cortex (PCC) is a part of the limbic system that plays an important role in emotion regulation. Structures that most researchers agree make up the limbic system include the cingulate cortex, parahippocampal gyrus, the dentate gyrus, hippocampus, subicular complex, amygdala, septal area, and hypothalamus (Leech & Sharp, 2014). The PCC is
a part of the posteromedial cortex and the cingulate gyrus (Rajmohan & Mohandas, 2007), which are both heavily interconnected with other regions of the cortex. The cingulate cortex is primarily associated with autonomic functions as well as attentional functions like cognitive and emotional processing (Rajmohan & Mohandas, 2007).

Agreement about the function of the PCC is contentious, but one theory suggests that it is involved in internally directed cognition (Leech & Sharp, 2014). Cognitive tasks that demand attention are shown to decrease metabolism in the PCC. Metabolism, specifically glucose metabolism, is an important measure of energy consumption in the brain. Brain regions that have a higher metabolism during a task are thought to be essential to its function. Using PET and fMRI studies, researchers have evaluated the implication of metabolism during certain tasks. While researching “activated” states with higher glucose metabolism, they needed to create a control or “deactivated” state to compare region activation. Researchers noticed that the “deactivated” regions also had a high metabolism during non-task times (Buckner, Andrews-Hanna, & Schacter, 2008).

This “deactivated” region of the brain has become known as the default mode network (DMN). The DMN’s glucose metabolism is relatively high compared to other areas of the brain (Buckner et al., 2005). The DMN is a complex network of brain areas that become active when the brain is at rest. It gives insight to higher order processes that occur during that time such as remembering the past and imagining the future. It has been shown to be implicated in successful autobiographical memory concerning emotions related to individuals. Not only is it implicated in the retrieval of memory but the processing of memory as well. The DMN is thought to be a brain system or a series of interacting subsystems. Anatomy of the DMN is thought to include the inferior parietal lobe, posterior cingulate cortex, hippocampal sulcus, and retrosplenial cortex.
The retrosplenial cortex (RSC) is located caudally to the posterior cingulate cortex and corpus collosum in the human brain. Brodmann areas 29 and 30 are thought to be the RSC, and the PCC/precuneus is separated from the RSC by Brodmann area 23. Distinction of the RSC is important due to its distinct connectivity to the cortex, which could give rise to its function. The rodent RSC is connected to anterior thalamic nuclei, anterior cingulate cortex, hippocampal and parahippocampal regions (Powell et al., 2017). It is indirectly connected to the prelimbic cortex and literature studying its connection is limited (Powell et al., 2017). Studies evaluating lesions to the RCS in rodents found deficits not only in spatial memory but recency memory as well (Powell et al., 2017). In humans unilateral damage to the RSC has shown impairments in navigation, while bilateral damage has deficits of anterograde and retrograde amnesia (Vann, Aggleton, & Maguire, 2009). In rodents, more specific cytotoxic lesions to the RSC have not been involved in spatial learning and memory (Troy Harker & Whishaw, 2004). This could indicate different functions than previously anticipated.

The function of the RSC has remained unclear and varies between human and rodent literature, yet initial inquiries into the region suggest it plays a role in emotional regulation (Vann et al., 2009) because of its part in cingulate cortex activation. The autonomic changes that are evoked from said activation are thought to be a part of the Papez circuit. The Papez circuit is essential to emotional regulation because of the interconnectedness between the cingulate cortex and hypothalamus. In addition to emotional regulation, the Papez circuit is thought to play a role in learning and memory because of its damage in AD (Carlesimo, 2012). However, the focus on emotional function of the retrosplenial cortex limits exploration into other possible roles this brain area could have.

Alzheimer’s disease affects how the DMN functions (Buckner et al., 2008). In fact there has been a correlation between the hypometabolism of areas implicated in the DMN
PROGRESSION OF Aβ IN AD

(Figure 2) and Aβ deposition in AD (Buckner et al., 2005). This study evaluated nondemented individuals who were genetically at risk for AD and found that individuals at risk had lower metabolism in DMN brain regions. It is hypothesized that the interconnectedness of the PCC and DMN are lowered in the early stages of AD (Greicius, Srivastava, Reiss, & Menon, 2004). Therefore, metabolism could be an early biomarker of the disease before the clinical hallmarks of plaques and tangles occur. Additionally, individuals that had the genetic risk and eventually developed AD showed accumulation of Aβ in areas afflicted with the hypometabolism including the PCC. The impact of hypometabolism is not accounted for in the amyloid cascade hypothesis of AD formation.

Function of the RSC in AD has been evaluated and suggested to also have initial hypometabolism in AD. A study looking at fluorodeoxyglucose PET found that individuals with Mild Cognitive Impairment, which often progresses into AD, had lower metabolism in the RSC (Vann et al., 2009). There are two theories that might explain the relationship between hypometabolism and AD. First, since the RSC is heavily connected with other brain regions affected by AD then those regions, such as the hippocampus, are affecting metabolism in the RSC. Second, it is suggested that the RSC itself is atrophied in AD, leading to the apparent glucose hypometabolism. This initial hypometabolism in the RSC suggests that it could be one of the first brain regions impacted in the disease, leading to the questioning of the amyloid cascade hypothesis on sequential development of the disease.

Disruption of RSC function leads to a loss in an individual’s ability to retrieve old information and acquire new information. This is because of its connection to the hippocampus through the DMN. In fact, the PCC and RSC are both crucial parts of the DMN. However, the PCC and RSC are not as well defined in nonhuman animal models, especially in rodent models. It is implied that the RSC can act as a quasi PCC in the brain. This is suggested because spatial
memory is affected when the RSC is lesioned in rodent studies. Additionally, the RSC has similar connectivity to the PCC in a rodent model, indicating their interchangeability. The role that the PCC and RSC play in the DMN is unclear but consistent. It is consistently activated and connected to similar regions in the brain. There are two brain areas consistently activated by the DMN are the prefrontal cortex and the PCC/RSC. Although DMN location changes with age due to development, the literature has indicated that the PCC/RSC do not change in the DMN, therefore implying its centrality in the network.

This study will focus on the progression of AD in the PCC/RSC in comparison to other brain regions that are historically associated with the disease progression. Through immunofluorescent staining of transgenic rodent brains, we will evaluate levels of Aβ deposition in those specific brain regions. As mentioned previously, the amyloid cascade hypothesis relies on the theory that AD progression is sequential and proliferative. It suggests that AD begins in the hippocampus with Aβ, which influences tau and proliferates to other regions of the brain. However, studies indicating hypometabolism in the DMN have been linked to AD meaning there is an interesting question about disease initiation and progression.

In addition to analyzing the PCC/RSC relative to the hippocampus, this study will also focus on the entorhinal cortex. Focusing on this brain region allows us to fully analyze the amyloid cascade hypothesis theory. Since recent literature suggests that the EC could possibly be one of the first brain regions afflicted in the brain, it would be interesting to measure progression in comparison to other brain regions that I am hypothesizing are also afflicted early. Additionally, sections of the subcortical region of the brain and other cortical regions will be measured to standardize progression in regions previously associated with early plaque development.
Literature reviewing the relationship with DMN and AD has only looked at static time points rather than progression of the hallmark proteins. By assessing progression of plaques at different time points later in the disease, we can analyze the rate of proliferation that is occurring in different brain regions. We will be able to determine whether progression follows the amyloid cascade hypothesis by measuring Aβ in the PCC/RSC and comparing it to the hippocampus, thalamus, and hypothalamus. If Aβ is progressing at a similar/increased rate in the PCC/RSC versus the hippocampus then we can hypothesize that the amyloid cascade hypothesis does not explain the disease progression fully.

This hypothesis also has broader implications for the role that our current unconscious mind plays on the consciousness of our future. The DMN has been thought to give rise to future thinking and reflection on past events. Those actions allow for the consolidation of memory and higher order processes like emotion regulation. If the DMN is one of the first areas affected by AD, then it can provide more insight into the behavioral symptomology experienced by those with AD. Lower metabolism in the PCC/RSC and Aβ deposition can give greater clarity into the memory dysfunction associated with the disease.

**The Current Study**

The transgenic rodent model used in this study was the APP/PS1 strain. Its nomenclature refers to the genetic mutation present in the model. The APP refers to the APP gene that causes the aforementioned amyloid precursor protein to be formed in excess. The PS1 refers to the Presenilin 1 gene, which overexpresses the γ-secretase protein causing amyloidogenic cleavage of APP. The characteristics of these mice include amyloid plaque deposition as early as 6 weeks with confirmed deposition within 5 months. Areas affected by the deposition include the hippocampus, thalamus, and striatum (Radde et al., 2006). Behavioral deficits, such as cognitive impairment, begin around 7 months in this model. Intricacies regarding amyloid beta deposition
are unclear between 9 and 18 months, therefore this study will focus on some time points that being in this range.

It is hypothesized that the PCC/RSC will have a similar level and rate of $A\beta$ deposition as compared to areas such as the hippocampus and entorhinal cortex. In addition to those brain regions, measurements from an arbitrary subcortical and cortical region will be used to create a baseline. Noting the equal and parallel progression is important for understanding the pathology of the disease. It is often thought that progression of the disease occurs in a sequential and proliferative domino-like process. First, noticing amyloid deposition in the hippocampus which transitions into plaque pathology that is thought to influence other areas of the brain. However, due to the hypometabolism of the PCC/RSC I hypothesize that $A\beta$ deposition could occur concurrently to the hippocampus.

To measure amyloid beta progression this study will use Thioflavin S staining techniques. Thioflavin S is a general beta amyloid stain, meaning its specificity for ab-40 or 42 is unclear. Past literature has used Thioflavin S due to its affordability and ability to stain beta amyloid plaques. To measure progression, three time points (13, 16, 24 months) will be assessed using immunofluorescent imaging and quantification techniques. Using these methods, we aim to paint a fuller picture of AD progression in less notorious areas of the brain.

The aim of the study is to change the conversation about the amyloid cascade hypothesis and the early stages of AD. Since the entorhinal cortex is also thought to be the first brain region affected then progression of $A\beta$ should be more than or equal to the PCC/RSC and the hippocampus. Although the time points in the present study do not evaluate early stages, they can give insight into the rate of progression. This can provide information about AD that had been previously overlooked. If metabolism is a biomarker for plaque deposition, then it can facilitate a conversation that is larger than what the amyloid cascade hypothesis encompasses. This study
could be one step in helping expand the scope of a long-standing theory to gain a broader understanding of the mechanisms that underlie such a complicated disease.

**Method**

**Subjects**

Procedures involved in this study were approved by the IACUC committee at Kent State University and The College of Wooster. Male APP/PS1 transgenic mice were used from the Cassedasus lab at Kent State University. Animals were maintained under temperature (72 F) and light controlled conditions (12hr light/dark cycle). Animals were aged to 13, 16, and 28 months of age.

**Perfusion and Slicing**

All perfusion and immunolabeling occurred at Kent State University. The animals were anesthetized using avertin. A transcardial perfusion was performed using a phosphate buffer saline (PBS). Brains were fixed with 4% paraformaldehyde (PFA) and went through a sucrose gradient. Brains were frozen in 2-methylbutane (C₅H₁₂), chilled in dry ice, frozen in Optimal Cutting Temperature (OCT) media, and placed into an -80F freezer until sliced. Brains were sectioned coronally (40 μm) on a cryostat microtome. Beginning at the hippocampus, every sixth tissue sample was collected. All samples were mounted on glass slides before immunostaining took place.

**Immunostaining**

Staining of the slides occurred at Kent State University. To stain, mounted brains were sequentially placed into 1% Thioflavin S (100 mL), 70% ethanol (100 mL), 1% PBS 1 (100 mL), and 1% PBS 2 (100 mL) for 5 minutes each. Stained slices were taken out using aquamount and cover slips were adhered to the slices. Slices were allowed to dry overnight and refrigerated (4C) with minimal exposure to light.
Imaging

Using an immunofluorescent microscope at the College of Wooster, each brain region was imaged separately at 10X magnification. Totaling and threshold contrast data were collected from each brain region. Each brain region was captured using reference to a stereotaxic map (Figure 3A) and examples of images taken are shown in Figure 3B.

Quantification

ImageJ, provided by the National Institutes of Health, was used to image and quantify the plaque load in the PCC/RSC, ERC, hippocampus (A & B), and arbitrary cortical and subcortical regions (Appendix A). Areas of the relevant brain regions were “lassoed” using the ImageJ software (See Figure 3B, purple outlines). Within each region, a contrast was created, using the software, in order to differentiate the plaques from the background staining. Plaques were then identified based on brightness and quantified based upon a thresholding setting. In order to normalize to the size differences between the brain regions, the total number of counted plaques was divided by the lassoed area of each brain region.

Rate of Change. To determine rate of progression, over time the standard rate of change (ROC) formula was used, shown below.

\[
\frac{Plaque\ Area\ B - Plaque\ Area\ A}{Time\ B - Time\ A}
\]
Figure 3A. Part of a stereotaxic map with a Nissl stain included. This was one of the images used as a reference slide for the images taken in 3B. Each letter represents the colored area associated with a different brain region. (A) Retrosplenial cortex (B) hippocampus A (C) hippocampus B (D) entorhinal cortex (E) cortical region (F) subcortical region.
Figure 3B. Immunofluorescent Thioflavin-S microscope images. Rows indicate brain region while columns indicate time. These slices were used to measure Aβ concentrations across time. Purple outlines denotate brain area measured.
Results

The purpose of this experiment was two-fold, (1) to determine the amount of plaque in the retrosplenic cortex against other typically afflicted brain regions and (2) to measure the rate of progression in six different areas at early and late stages. The current study used statistical analysis set out to answer those questions in four ways: (1) a within subjects ANOVA determined general main effects and interactions of time and brain region; (2) a one-way ANOVA determined differences in plaque load at each brain region at each time point; (3) a repeated measure ANOVA determined the direction and consistency of plaque progression across time; (4) a rate of change derivation of the plaques qualitatively evaluated rate of change between brain regions over time. This facilitates the ability to better assess the amyloid cascade hypothesis in a holistic way.

A 3x6 within subject nested design ANOVA was used to determine amount of plaque in the specific brain region. There was a marginally significant main effect of time, $F(2,4) = 4.247, p = .103, \eta^2_p = .411$, across the three time points (Figure 4A), with increasing levels of plaque. There was a significant main effect of brain region, $F(5,10) = 11.601, p = 0.001, \eta^2_p = .998$, between the 6 brain regions, but no significant interaction, $p > .202, \eta^2_p = .554$. To determine where the significant group differences occurred, post hoc independent t-tests were performed. The subcortical region was significantly different from the cortical region, $p = .003$, entorhinal cortex, $p = .002$, hippocampus A, $p < 0.001$, and hippocampus B, $p < 0.001$, (Figure 4B). No other brain regions differed significantly, all $ps > .091$. 
Figure 4. Progression of plaque across time in different brain regions. (A) Represents plaque progression in a line graph and compares brain regions to themselves. Standard error bars not included to allow for clearer view of data points. (B) Represents average plaque deposition in each brain region at all time points. Bars with different letters denote significant difference, $p < .05$. 
To evaluate the differences between each brain region at each time point, separate one-way ANOVAs were conducted at 13, 16 and 28 months (Figure 6). This analysis was done to assess a cross-sectional window of plaque development to determine if plaque load was different across brain regions at each time point. This could provide insight into the later stages of the disease and plaque load at each time point. At 13 months (Figure 6A), there was a significant main effect difference between brain regions, $F(5,10) = 11.17$, $p = .013$, $\eta^2_p = .333$. Post-hoc LSD analysis determined a significant difference between the entorhinal cortex ($M = .0002553$, $SD = 4.22e-005$) and retrosplenial cortex ($M = .0001412$, $SD = 3.085e-005$), $p = .04$. There was
also a statistically significant difference between the subcortical region ($M = 6.989e-005$, $SD = 3.329e-005$) at 13 months and the entorhinal cortex ($M = 0.0002553$, $SD = 4.22e-005$), $p = .041$. Qualitatively there was a significant difference between the hippocampus and the retrosplenial cortex at 13 months of age.

At 16 months (Figure 6B) there was a significant main effect difference of brain region, $F(5,10) = 5.692$, $p = .051$, $\eta^2_p = .333$. Post hoc LSD tests demonstrated a significant difference between hippocampus A ($M = .000349, SD = 6.944e-005$) and the subcortical region, $p = .044$, as well as a significant different between hippocampus B ($M = .0003404, SD = .0001521$) and the subcortical region ($M = 9.648e-005$, $SD = 8.715e-005$), $p = .043$. The entorhinal cortex ($M = .0003488, SD = 5.225e-005$) also has a significant plaque accumulation in correlation to the subcortical region ($M = 9.648e-005$, $SD = 8.715e-005$), $p = .033$. At 28 months (Figure 6C), there is no significant difference across brain regions, $p = .333$, which is not surprising given the advanced age of the animals. However, there was qualitatively less plaque in the subcortical region, which is expected.

To better assess the proliferative aspect of the amyloid cascade hypothesis by tracking progression, repeated measures ANOVA’s were conducted for each brain region over time. There was a significant difference of plaque accumulation at the subcortical region across time (Figure 7F), $F(2,4) = 8.383$, $p = .037$, $\eta^2_p = .333$. Marginal significance exists in the retrosplenial cortex across time (Figure 7A), $F(2,4) = 3.817$, $p = .118$. Finally there was not a significant difference of plaque accumulation in the cortex across time, $F(2,4) = 3.17$, $p = .15$. Although there was not a statically significant difference in the retrosplenial cortex or cortex across time, there was qualitatively an increasing slope suggesting plaque progression. There was no change in the hippocampus A (Figure 7B) across time, $F(2,4) = 0.057$, $p = .946$ nor at the hippocampus
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B (Figure 7C) across time, \(F(2,4) = 1.408, p = .344\). The entorhinal cortex also exhibited no significant difference in plaque accumulation across time points, \(F(2,4) = 1.08, p = .422\).

Figure 7. Progression of plaque across time in each brain region. (A) Retrosplenial cortex at 13, 16, and 28 months (B) Hippocampus A at 13, 16, and 28 months (C) Hippocampus B at 13, 16, and 28 months (D) Entorhinal cortex at 13, 16, and 28 months (E) cortical region at 13, 16, and 28 months (F) subcortical region at 13, 16, and 28 months.
To determine rate of plaque progression between brain regions, to make a more direct comparison of change over time rather than volume of plaque a rate of change (ROC) formula was applied (See Method). A positive ROC suggests an increase in plaque accumulation between two time points, while a minimal or negative ROC suggests little or a reduction in plaque accumulation. Analysis of rate of change was conducted between 13 and 16 months, 16 and 28 months, and 13 and 28 months to evaluate ROC between each possible time point. A qualitative analysis (Figure 8) demonstrated that overall there was a higher rate of change in the retrosplenial cortex across all time points. When comparing ROC between 13 and 16 months there is a positive ROC in all brain regions except hippocampus A, and hippocampus B. When comparing 16 and 28 month there was slightly less dramatic ROC for all brain regions than between 13 and 16 months, however, Hippocampus A and Hippocampus B are still not demonstrating a positive ROC. There is a relatively equal ROC at the retrosplenial cortex, cortical region and subcortical region. This can be compared to Figure 7 where the retrosplenial cortex, cortical region and subcortical region are the only brain regions that increase.

![Figure 8](image_url)

*Figure 8. Rate of change for each brain region between different time points*
quantitatively between 16 and 28 months. The comparison between calculated ROC (Figure 8) and general plaque progression (Figure 7) increases the validity of the qualitative assumptions.

The greatest rate of change between 13 and 28 months was exhibited in the retrosplenial cortex. There was also a ROC in the cortical and subcortical region between 13 and 28 months but not as substantial as the retrosplenial cortex (Figure 8).

**Discussion**

The aim of this study was to compare the progression of Aβ plaques in the retrosplenial cortex to other brain regions traditionally afflicted by AD. The current study hypothesized that due to the suggested hypometabolism in the retrosplenial cortex in the early stages of the disease, specifically in the DMN, that there would be an increased rate of beta-amyloid plaque deposition. The current study hypothesized that the amyloid cascade hypothesis is partially false due to the hypometabolism in brain regions implicated in the DMN. Literature has suggested genetically at-risk individuals that developed AD showed accumulation of Aβ in areas with hypometabolism. The current study hypothesized that the PCC/RSC would have a similar level and rate of Aβ deposition as compared to areas such as the hippocampus and entorhinal cortex. Although the level of Aβ in the RSC did not surpass the hippocampus throughout the suggested timeframe, the rate of progression in the RSC, as compared to the ERC and hippocampus, was greater. This suggests that there was an effect of time on plaque progression as well as a difference in rate of progression between brain regions.

It is important to note a few limitations in the study before evaluating its results. First, although the timeframe analyzed in the current study gives insight into the possible rate of progression of the hippocampus in comparison to the RSC, it also creates a discrepancy between hypometabolism and rate of Aβ deposition. Meaning that the ages being measured are later in disease progression and that a correlation between hypometabolism and plaque deposition is
harder to correlate. This is because hypometabolism is thought to occur early in disease progression, rather than later. Second, Thioflavin-S is a general amyloid stain; therefore, the Thioflavin-S could have stained more than just the specific type of Aβ found in AD. Finally, while the mouse model used, APP/PS1, is a common model when studying AD, the lack of tau development could limit translational hypotheses. The limited translation is not specific to this particular mouse model, however, and is a consideration in many rodent models that are not also genetically manipulated to build tau tangles.

13 Months of Age

Results suggested that there were higher amounts of pre-existing plaque deposition in both sections of the hippocampus that were measured. The entorhinal cortex and cortical region seemed to have slightly less but similar amounts of pre-existing plaque deposition. There were lower levels of plaques at 13 months in the retrosplenial cortex and subcortical region as compared to the entorhinal cortex.

Previous literature is unclear about the exact amount and location of plaque deposition; however, it suggests that plaque deposition begins at 6 weeks in the cortex and 3 to 4 months in the hippocampus in the APP/PS1 transgenic mouse model (Radde et al., 2006). Literature fails to elucidate the exact rate of progression in each brain region previously mentioned. Higher plaque deposition in the hippocampus could imply early progression of beta amyloid plaques. Lower levels of plaques in the retrosplenial cortex suggest that rate of progression earlier in life was not as substantial as compared to the hippocampus. Therefore, later plaque deposition in the retrosplenial cortex suggests that the retrosplenial cortex is later to start in the amyloid cascade hypothesis.
16 Months of Age

Similar high amounts of plaque accumulation exist in the hippocampus, but by 16 months of age the entorhinal cortex had caught up and had comparable plaque deposition. The retrosplenial cortex increased the concentration of plaques at 16 months of age.

Previous literature is unclear about the specific beta amyloid plaque deposition at 16 months of age but suggests that there is loss in long term potentiation in the CA1 region of the hippocampus. This study shows that there is a consistent amount of beta amyloid plaques in the hippocampus at 16 months as compared to the 13-month-old brains. This may suggest a ceiling effect of beta amyloid later in disease progression (Gengler et al, 2010), at least as can be measured by Thioflavin-S.

Comparing plaque deposition at 16 months to behavioral data at the same age, it is interesting to note that amount of plaque deposition is not correlated to behavioral deficits (Radde et al., 2006). Therefore, Aβ is merely a biomarker for the disease rather than a perpetuator of the disease. This elucidates the amyloid cascade hypothesis once more, although there is more plaque deposition, it is not the sole proprietor of the disease.

28 Months of Age

There was a higher amount of Aβ plaque deposition in the retrosplenial cortex and cortical region at 28 months of age. Once again, the hippocampal and entorhinal region remained consistent with the amount of Aβ plaque deposition seen at previous time points. The subcortical region seemed to increase steadily but remained lower than all the other analyzed brain regions. There was no significant difference between regions at the time point, suggesting that regions with lower pre-existing plaque deposition have become equalized over the course of time.

The literature is very sparse on the molecular mechanisms that exist at this age. Most transgenic mouse models are considered very old due to the pathology that ensues. Due to that, it
can be assumed that the pathology is prominent in all stages of the brain, as exhibited in the data, and that the behavioral deficits are tremendous and life threatening at this point. The closest insight into pathology in the literature mentions neuronal loss at 17 months of age in the dentate gyrus (Rupp, Wegenast-Braun, Radde, Calhoun, & Jucker, 2011). The combination of $A\beta$ plaque deposition and neuronal loss can explain the behavioral deficits associated at this age, however the plaque deposition alone cannot.

**Rate of Change**

There were differences in rate of change in the retrosplenial cortex and cortical region while there was stagnation in the hippocampus and entorhinal cortex. Having a consistent rate of change in the retrosplenial cortex across time compared to the other brain regions analyzed gave insight into the role of the retrosplenial cortex in the greater scheme of disease progression. Additionally, given that there was no change across time in this study, but high levels of plaque accumulation in the hippocampus, there could have been a consistent rate of change earlier in the hippocampus. There are two ways to view the impact of plaque deposition rate: (1) plaque deposition could signify degeneration and therefore behavioral deficits (2) plaque deposition could signify disease progression, but nothing more.

When evaluating the impact of plaque deposition through the first lens, increased rate of plaque progression in the retrosplenial cortex, and general cortical region, can add to the explanation of other behavioral deficits associated with AD. These brain regions are primarily associated with autonomic functions as well as attentional functions like cognitive and emotional processing. In a human population, inhibition of these processes through degradation are often associated with later stages of the disease (Leech & Sharp, 2014). Evaluating plaque deposition through the second lens suggests that part of amyloid cascade hypothesis is accurate due to the later progression of plaque in the retrosplenial cortex.
Amyloid Cascade Hypothesis

The results of this study leave the reliability of the amyloid cascade hypothesis unclear. The amyloid cascade hypothesis relies on the theory that AD progression is sequential and proliferative. It is sequential in the sense that the aggregation Aβ leads to the production of NFT through a series of intracellular events. AD is proliferative because past literature suggests that its neuropathology begins in the hippocampus and unidirectionally moves to other areas of the brain (Karran et al., 2011). In the current study, due to the pre-existing Aβ deposition in the hippocampus, it could be suggested that AD is proliferative. The increased rate of progression in the retrosplenial cortex occurs later in the disease, suggesting that rate of Aβ is higher in the hippocampus earlier in the disease.

Hypometabolism associated with Aβ deposition cannot be directly linked in the current study due to the age of the transgenic mice. Literature is inconsistent about hypometabolism based off of brain region. Literature suggests that there is a direct link between hypometabolism in the DMN and plaque deposition in AD (Buckner et al., 2005). However, in some cases the plaque deposition is not a direct effect of the hypometabolism. A study evaluating hypometabolism in hippocampal subdivisions of early AD patients found no consistent difference between AD and healthy patients hypometabolism using and FDG-PET (Choi et al., 2018). However, there was still normal plaque accumulation observed in the AD patients. Therefore, suggesting that hypometabolism can become a biomarker and perpetuator AD in some brain regions while not being the complete biomarker for other brain regions.

In the current study, the use of APP/PS1 transgenic mice prevented us from assessing the sequential aspect of the amyloid cascade hypothesis. In the APP/PS1 mouse model, there is a substantial amount of Aβ development and accumulation. However, throughout the life span of the transgenic mouse, no NFT phenotypes are developed. This reaffirms previous literature
disassembling the clear link between Aβ development and tau development (Kametani & Hasegawa, 2018). If the amyloid cascade hypothesis were completely valid, then this mouse model would have developed tau as a result of Aβ accumulation, but the mouse model must be further genetically manipulated for this to happen, unlike the human.

The behavioral phenotype of APP/PS1 also gives insight into the amyloid cascade hypothesis. Aβ deposition begins as early as 6 weeks, with confirmed deposition within 5 months. However, behavioral deficits, such as cognitive impairment, do not begin until around 7 months in this model (Radde et al., 2006). Additionally, the neuronal loss and deficits in APP/PS1 do not begin until 15 to 17 months of age (Rupp et al., 2011). The large discrepancy between deposition and behavioral deficits gives more insight in the role that Aβ plays in disease progression.

**Future work**

AD is a complicated disease with no clear and causal beginning. Each study that is published aids in increasing the literature and information available for the AD community. Future work should focus on the earlier progression of the disease since this study elucidated later stages. Analysis of plaque deposition at a greater number of time points would be beneficial and help create a clearer picture of the rate of progression of Aβ. In addition to measuring Aβ, it is important to look at other factors that could also be implicated in the disease.

Measuring glucose metabolism in the earlier stages of the disease could also guide the hypothesis of the current study. Evaluating hypometabolism, not only in the retrosplenial cortex, but in the hippocampus as well as entorhinal cortex can provide greater insight into the role of metabolism in AD. In addition to these measurements, parallel behavioral assays measuring learning, memory, and social deficits over the course of the disease would aid in a more comprehensive analysis. Finally, using a different mouse model could be beneficial. Observing
similar molecular and behavioral phenotypes across different transgenic mouse models increases the ability for the basic science research to become translatable.

**Conclusion**

The current study set out to question the amyloid cascade theory and hypothesized that the PCC/RSC would have a similar level and rate of Aβ deposition as compared to areas such as the hippocampus and entorhinal cortex since there was hypometabolism in the DMN. Although the retrosplenial cortex did not have the same rate of progression as typically afflicted brain regions, it gave insight into the rate of plaque accumulation in AD during later stages of the disease, which was sparse in the literature. Interestingly, there was a high pre-existing amount of plaque in the hippocampus and entorhinal cortex, suggesting that they could have had a similar rate of progression, but that it could have occurred earlier in AD.

Initially, the current study suggested that the hypometabolism associated with a brain region would be a biomarker and suggest later plaque accumulation. Since literature suggested higher levels of hypometabolism in the DMN, it initially deconstructs the proliferative theory underlying the amyloid cascade hypothesis (Buckner et al., 2005). This is the case because the theory suggests that the disease progresses from the hippocampus and moves outwards. The current study contradicts previous literature in a sense because we found preexisting accumulation in the hippocampus and later plaque accumulation in the retrosplenial cortex. Literature has also found minimal hypometabolism in the hippocampus but substantial plaque accumulation. Therefore, this suggests that hypometabolism can be used as a biomarker for the disease but not a biomarker for plaque accumulation. Additionally, plaque accumulation can be a biomarker for the disease but not a factor that is causative in disease symptomology.

The insight gained in this study creates a platform to conduct more research in the future regarding hypometabolism, rate of progression, and plaque deposition in different transgenic
mouse models. The literature has expanded greatly since Auguste Deter’s case study in 1901, but so have the possible answers to the conundrum of Alzheimer’s Disease. As we progress in the scientific literature to become more interdisciplinary, I am confident that we will begin to narrow the scope of the comprehensive question that is Alzheimer’s Disease.


Danysz, W., & Parsons, C. G. (2003). The NMDA receptor antagonist memantine as a symptomatological and neuroprotective treatment for Alzheimer’s Disease: Preclinical
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https://doi.org/10.1002/gps.938


Appendix A

Written Steps for Quantification of Plaques Using ImageJ

- Open FIJI version of ImageJ
  - This allows you to edit while ImageJ itself does not
- Open desired image
- Click image → 8 bit
  - This allows you to process the image effectively as well as use the threshold feature
- Click Image → Adjust → Threshold
- Adjust the first box
  - I made it 50 since it seemed to work the most uniformly
- Make sure that it is on Red in the drop-down menu
- Click apply
  - The plaques should turn white
- Click Process → Noise → Despeckle
  - This removes extra noise in the picture and gets rid of the line drawn
- Go to tool bar and click irregular polygon tool
  - Make a shape for desired area you want to quantify
  - Make sure to make polygon to not include the line
- Once polygon connected and complete click Analyze → set measurements → make sure to click area
- Click → Analyze → Measure
  - This should be the area of the polygon in pixels
- Copy and paste area into excel sheet
- With polygon still on image Click Analyze → Analyze particles
  - First box (Size and pixel^2) set to: 5-Infinity
    - This allows for it to pick up anything that is 5 pixels and up and hopefully doesn’t pick up noise
  - Keep second box the same
  - Show: drop down to outlines
    - This lets you see what it is quantifying to make sure that it is getting the desired plaques
  - Check the following boxes: Display Results, Clear Results, Exclude on Edges, Include Holes
    - Click OK
- This should give you an output and show you the plaques quantified. Take the number of plaques and insert into excel
- Divide number of plaques by area to get a standard area.
Appendix B

Visual Steps for Quantification of Plaques Using ImageJ

A

B

C

D

E

F

G

H

I

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