

Running head: ALZHEIMER'S AND AMYLOID BETA

1

Alzheimer's and Amyloid Beta: Amyloidogenicity and Tauopathy via Dyshomeostatic
Interactions of Amyloid Beta

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A Senior Thesis submitted in partial fulfillment
of the requirements for graduation
in the Honors Program
Liberty University
Fall 2020

Acceptance of Senior Honors Thesis

This Senior Honors Thesis is accepted in partial fulfillment of the requirements for graduation from the Honors Program of Liberty University.

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Abstract

This paper reviews functions of Amyloid- β ($A\beta$) in healthy individuals compared to the consequences of aberrant $A\beta$ in Alzheimer's disease (AD). As extraneuronal $A\beta$ accumulation and plaque formation are characteristics of AD, it is reasonable to infer a pivotal role for $A\beta$ in AD pathogenesis. Establishing progress of the disease as well as the mechanism of neurodegeneration from AD have proven difficult (Selkoe, 1994). This thesis provides evidence suggesting the pathogenesis of AD is due to dysfunctional neuronal processes involving $A\beta$'s synaptic malfunction, abnormal interaction with tau, and disruption of neuronal homeostasis. Significant evidence demonstrates that AD symptoms are partially due to aberrant $A\beta$, and further experimental research may focus on repairing or preventing the noxious effects of $A\beta$.

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Introduction to Alzheimer's Disease

Background: Amyloid Beta and Alzheimer's Disease

AD is the sixth leading cause of death in the United States, following accidents and strokes. Compared to other top ten leading causes of death in the United States, methods of preventing, curing, or slowing AD are exceptionally inefficient. In the United States alone, AD kills more than 93,000 people each year, which is more than breast cancer and prostate cancer combined. Additionally, there are over five million Alzheimer's patients in the United States or about one in ten people over the age of sixty-five (CDC, 2017). Furthermore, the incidence of Alzheimer's is increasing. From 1999 to 2014, deaths from Alzheimer's have increased an astounding fifty-five percent. Though the aging population is increasing, this alone cannot account for the fifty-five percent increase in deaths from AD. By 2050, an estimated sixteen million Americans will be diagnosed with Alzheimer's (CDC, 2017). Research in preventing and curing AD is some of the most vital research in the future of medicine. Currently, there are many hypotheses which attempt to explain the pathogenesis of AD. One of the most convincing hypotheses is the Amyloid- β hypothesis, which predicts that AD results from an accumulation of $A\beta$ protein in the interstitial matrix between the neurons of the brain (Qiu et al., 2015).

This thesis will review normal $A\beta$ function and contrast it with the abnormal function and accumulation of $A\beta$ that has become a characteristic component of Alzheimer's pathology. Specifically, $A\beta$ is known to result in synaptic interference, disruption of neuronal homeostasis, and abnormal interaction with tau. These interactions initiate the formation of the amyloid plaques and neurofibrillary tangles (NFT's) characteristic of AD. Considering these major

disruptions, this paper will review evidence that A β disruptions are closely associated with—and are likely an underlying cause of—the physical symptoms of AD.

This paper proposes that the Amyloid- β plaques and NFTs present in the brains of Alzheimer's patients result from the many toxic effects of A β and its dysfunction. While it is known that abnormal A β is present in amyloid plaques, the exact involvement of A β in the mechanism of cell death has yet to be determined (Murphy and LeVine, 2010). A β exists in multiple forms. Some forms have a propensity to polymerize and initiate plaque formation, but the involvement of A β plaques in cell death is still under investigation. Consequently, the mechanism of how specific isoforms of A β lead to cell death must be investigated. Specifically, research will be reviewed to determine the extent of evidence supporting or contradicting the involvement of A β in hallmark pathology of AD. Associations between A β , amyloid plaques, NFTs, and cell death will be examined in the context of the chronology of AD pathophysiology. Though this paper's intentions are primarily to demonstrate that A β -induced malfunctions are what eventually causes the physical symptoms of Alzheimer's, the mechanism by which it does so is also critical and will be given significant consideration.

The main objective of this thesis is to demonstrate the physical symptoms of AD are a result of A β -mediated synaptic malfunction, improper interaction with tau, and disrupted neuronal homeostasis. Additionally, this review will propose a method of how A β may lead to the neurodegeneration that is caused by Alzheimer's. This research holds important implications for Alzheimer's research, in that it seeks to clarify whether there is sufficient clinical and mechanistic data to conclude that A β malfunctions induce the physical symptoms of Alzheimer's. Specifically, if A β -mediated cellular and synaptic disruptions can be demonstrated

to be the primary link between the pathological and physical symptoms of Alzheimer's, then further research can focus on preventing and reversing these malfunctions. Historically, AD research has focused primarily on A β induced malfunction, however, this research seeks to present a more comprehensive representation of the disease and inspire novel research strategies.

Post-Translational Cleavage of the Amyloid Precursor Protein

Significant confusion has surrounded the research regarding A β and Amyloid precursor protein (APP). This confusion is primarily due to the two main avenues in which APP is processed. The differences between these two pathways are post-translational, they differ only in the cleavage point on APP, and by which enzymes they are cleaved by. The two pathways have been referred to as the non-amyloidogenic and the amyloidogenic pathway of APP processing. In the non-amyloidogenic pathway, APP is cleaved by α -secretase to produce two protein fragments: sAPP α and c83. sAPP α is proposed to have neuroprotective and neurotrophic properties. C83 is further processed by γ -secretase to produce p3 and AICD (APP Intracellular Domain). While no functions are currently known for p3, AICD may function as a transcription factor (Multhaup et al., 2015).

In the amyloidogenic pathway, APP is processed by γ -secretase and β -secretase to produce three peptide fragments: sAPP β , AICD, and A β . Functions of sAPP α and sAPP β can be considered identical. The last fragment in amyloidogenic processing of APP is that of A β , the namesake fragment of the amyloidogenic cleavage pathway and the main component in the amyloid plaques characteristic of AD (Pearson and Peers, 2006). The term amyloid is a broad term for an irregular aggregation of proteins. A β is thus named for its tendency to aggregate. When discussing A β , it is vitally important to specify the type of A β . A β exists in two basic

monomeric forms, A β 1-40 and A β 1-42; however, each of these monomers can also self-assemble into dimers, oligomers, and polymers (or fibrils) (Pearson and Peers, 2006). Unless otherwise specified, the studies reviewed in this thesis regard A β 1-42.

A β Roles in Physiology at Homeostasis

Despite the overwhelming research dedicated to determining the cytotoxic effects of A β , many studies propose roles for A β or APP and its derivatives in normal physiology. Furthermore, several studies demonstrate that certain species of A β are even critical for neuronal survival. Multiple studies report that A β is found in the plasma and cerebrospinal fluid of non-AD individuals, indicating a possible homeostatic role for A β , or at least, as a byproduct of normal physiological processes. Plausible functions of the distinct species of A β range from immune defense via microbicidal effects, managing synaptic interactions and activity, acting as a neurotrophic factor or an antioxidant, involvement in neuronal growth or lipid metabolism, among other potential roles (Spitzer et al., 2016, Pearson and Peers, 2006, Cárdenas-Aguayo et al., 2014, Zinser et al. 2007). APP has a potential role in cell adhesion, neuronal growth, and formation of synapses (Cárdenas-Aguayo et al., 2014). Evidence of A β in normal brain physiology suggests that A β 's presence alone does not induce the destructive physiological malfunctions in AD.

Historical A β Pathophysiology

Plaque composition. Amyloid plaques, otherwise known as senile plaques, are one of the most well- documented characteristics of the AD affected brain. Amyloid plaques are composed primarily of the distinct species of A β (Pearson and Peers, 2006). Of the two forms, A β 1-42 has a higher affinity for aggregation, and is the main element of amyloid plaques.

Though A β 1-42 aggregates more readily, it is unclear if this equates to higher toxicity. It is also uncertain whether amyloid plaque formation is a result of processes which drive the pathophysiology of AD, or if the plaques themselves play a part in the pathophysiology of the disease (Qiu et al., 2015). In addition to A β , there are over four-hundred fifty proteins that have been found in amyloid plaques. These proteins are involved in cell adhesion, transport, cell structure, inflammation, phosphorylation and dephosphorylation, metabolism, and protein degradation (Liao et al., 2004). APP and tau filaments are also known to accumulate in these plaques (Cras et al., 1991). This thesis, however, will focus on the primary elements that compose amyloid plaques.

Species of amyloid beta and associated toxicity. A β 1-40 and A β 1-42 and their monomers, dimers, and polymers have been indicated in the pathophysiology of AD. Considerable AD research has focused on determining which form or forms of A β are the toxic species. This research is often contradictory, making it difficult to determine which species are likely to contribute to the physiological symptoms of AD. Historically, research has focused on the polymeric forms of A β due to their prevalence in amyloid plaques. This thesis will review the cytotoxicity of the various species due to recent discoveries and new insight pertaining to AD pathophysiology.

A β 1-42. Forms of A β 1-42 have been classically considered the more neurotoxic species of A β due to their increased tendency to aggregate (Qiu et al., 2015). Monomeric forms of A β 1-42 are able to produce reactive oxygen species and decrease cell viability, but significantly less than the oligomer and polymer forms of A β 1-42. A β 1-42 monomeric ROS production has been reported to be about 2.5 times lower than ROS production due to A β 1-42 oligomers and

polymers. Additionally, the monomeric form of A β 1-42 effects neuronal viability 10-40 times less than its other forms (Giovanna et al., 2009). Interestingly, the A β 1-42 monomer has not classically been considered toxic on its own; only upon oligomerization and polymerization has it been hypothesized that A β 1-42 exhibits detrimental effects (Qiu et al., 2015). However, in recent years new data has come to light that has made many researchers doubt the original interpretation of the amyloid hypothesis, or that A β plaques created by polymerization initiate neurodegeneration in AD.

Many studies that have examined the cytotoxicity of the different species of A β have found that A β 1-42 oligomers exert the greatest adverse effects on neurons (Sengupta et al., 2016). Apart from monomeric forms, A β 1-42 forms tetramers, pentamers, hexamers, and even dodecamers prior to forming larger oligomers and fibrils (Qiu et al., 2015). Interestingly, toxicity of oligomers has been reported to be inversely related to their size. As the size of an oligomer increases, the less harmful it is to nearby cells (Sengupta et al., 2016). In general, A β 1-42 oligomers have been the species indicated in A β ROS production and toxicity. Additionally, A β 1-42 oligomers are the only species of A β that exhibit the ability to form non-selective and voltage-independent ion channels. These oligomeric A β 1-42 species insert into membranes and disrupt ion homeostasis. Calcium dysregulation has been especially noted as a cytotoxic mechanism due to calcium's role in many cell-signaling pathways (Bode et al. 2016).

A β 1-42 fibrils and its aggregates have classically been considered to be involved in the pathophysiology of AD, perhaps because they are highly present in one of the most distinctive features in the AD brain, amyloid plaques. A β 1-42 fibrils form more readily than A β 1-40 fibrils, but are less cytotoxic than other forms of A β 1-42 and produce significantly less ROS, likely due

to their inability to enter cells (Sengupta et al., 2016). Regardless, A β 1-42 fibrils appear to be more of a byproduct of neuronal dysfunction, rather than a primary factor in the induction of cell death.

A β 1-40. Though A β 1-40 appears to be less cytotoxic than A β 1-42, many researchers believe it is involved in the pathophysiology of AD, while others believe A β 1-40 species are not toxic at all. In the last twenty years, few articles have shown A β 1-40 to have cytotoxic effects. Before this time period, it was hypothesized that A β 1-40 may form ion channels in neuronal membranes; however, more recent research indicates that A β 1-42 is the only form of A β able to do so. While A β 1-40 monomers are the most abundant isoform of A β in the human brain, it is generally accepted that this is not a toxic species of A β (Bode et al. 2016). In fact, several studies have shown that A β 1-40 may have neuroprotective properties via antioxidation or inhibition of lipid metabolism or aggregation of A β 1-42 (Pearson and Peers, 2006, Cárdenas-Aguayo et al., 2014, Qiu et al., 2015). A β 1-40 tends to fluctuate between monomers, dimers, trimers, and tetramers, but these do not easily polymerize. In vitro and in vivo models have successfully polymerized A β 1-40, but using much higher concentrations of A β 1-40 than are present in an AD brain. While A β 1-40 fibrils have been reported to be cytotoxic, they are still significantly less cytotoxic than A β 1-42 oligomers or fibrils (Okada et al., 2007). In transgenic models of A β 1-40 overexpression in mice, no amyloid plaques were found to be formed by A β 1-40 (Qiu et al., 2015). While A β 1-40 should not be completely disregarded in the study of AD, evidence suggests A β 1-42 may play a more significant role in AD pathophysiology than A β 1-40.

The A β 1-42 : A β 1-40 ratio. In recent years it has been proposed that the ratio of A β 1-42 to A β 1-40 is more important than individual levels of a single form of A β . In a typical non-

diseased human brain, the ratio of A β 1-42 to A β 1-40 is about 1:9 (Pauwels et al., 2011). However, in familial or early onset AD this ratio is elevated with mutations of the PS1 and PS2 genes (Sengupta et al., 2016). Nonetheless, only about 10% of AD cases are due to these inheritable mutations. This ratio has also been shown to influence oligomer growth, binding to neuronal membranes, synaptotoxicity, memory formation in animals, and neuronal viability (Johnson et al., 2013). While current studies are examining the effects of lifestyle factors such as diet and exercise on this ratio, results thus far have been inconclusive and further research is warranted. Furthermore, while research has demonstrated that A β 1-42 : A β 1-40 induces inflammation or oxidative stress, this relationship has only been demonstrated unidirectionally. Further research is required to determine the factors which influence the A β 1-42 : A β 1-40 ratio. Regardless, at physiological conditions, enough A β 1-40 is present to inhibit A β 1-42 polymerization, so with a decrease in the ratio of A β 1-42 to A β 1-40 the probability of forming toxic A β 1-42 oligomers and polymers decreases (Jan et al., 2008). Appropriately, as this ratio increases, neurotoxicity increases and cell viability decreases (Kuperstein et al., 2010).

Research Review

Synaptic Interactions of Amyloid Beta

Considerable research has been directed toward investigating the activity of A β at synapses. Several studies have demonstrated that certain forms of A β may play a normal role in modulating synaptic activity. Other studies suggest that A β could have negative interactions at synapses (Abramov et al., 2009, Pearson and Peers, 2006). Additionally, A β formation and secretion are influenced by synaptic activity (Kamenetz et al., 2003). Interestingly, AD has even been proposed to spread through the brain via synapses. A β is released at synapses and subsequently affects postsynaptic neurons via alteration of receptors and intracellular ion

concentrations, initiating intracellular processes which drive β -secretase cleavage of APP and, thus, production of $A\beta$ in the postsynaptic cell. Additionally, synaptic excitation increases APP processing towards $A\beta$ production. In AD, areas of increased neuronal activity correlate with amyloid deposition (Pignataro and Middei, 2017). Regardless, accumulation of $A\beta$ and synaptic degeneration is a crucial step in neurodegeneration and neuronal death, thus, synaptic interactions of $A\beta$ are critical in the pathophysiology of the disease.

$A\beta$ induced calcium dysregulation. There are numerous interactions of $A\beta$ at the synapse. A major synaptic disruption that occurs in AD is ion dysregulation. Particularly, calcium dysregulation has been proposed to play a role in the pathophysiology of AD. $A\beta$ has been proposed to dysregulate calcium levels in a variety of ways.

One of the mechanisms by which $A\beta$ may disrupt calcium concentrations is by directly inserting into the membrane and forming ion channels. $A\beta_{1-42}$ oligomers are the species suspected of this channel-forming ability. Ion channels formed by $A\beta_{1-42}$ oligomers disrupt ion homeostasis across the neural membrane with adverse downstream effects. Specifically, disruption of calcium homeostasis by $A\beta_{1-42}$ oligomer ion channels is thought to contribute to the physiological symptoms of AD. As $A\beta_{1-42}$ ion channels create pores in the neural membrane, both electrostatic pressure and the concentration gradient drive calcium ions into neurons, creating an excess of intracellular calcium (Bode et al. 2016). $A\beta_{1-42}$ also disrupts other channels in the neuronal membrane such as voltage-gated calcium channels and L-type calcium channels. $A\beta_{1-42}$ binding to voltage-gated calcium channels activates the channel, creating an abnormal influx of calcium into the cell. $A\beta_{1-42}$ also directly increases the amount of L-type calcium channels in neuronal membranes via association with the α_1C subunit of the L-

type calcium channel, increasing calcium influx into the cell. An increase in L-type calcium channels is either due to an A β 1-42 dependent increase of calcium channel trafficking to the membrane, or A β 1-42 inhibits removal of calcium channels from the membrane (D'Andrea, 2016). Additionally, A β 1-42 binds to and inhibits the α 7 nicotinic acetylcholine receptor, which mediates release of acetylcholine and calcium influx. This may cause a compensatory increase in other mediators of calcium, causing intracellular calcium levels to increase. Calcium homeostasis and acetylcholine are both sensitive factors involved in memory and cognition, so disruptions in these areas would impact memory and cognition (D'Andrea, 2016). In addition, A β 1-42 induces N-methyl-D-aspartate receptor (NMDAR) activation and subsequent calcium influx (West et al., 2001). Certainly, there are a number of established mechanisms by which A β 1-42 causes calcium influx. The question is then: how does increased intracellular calcium cause neurotoxicity and neurodegeneration? The broad answer to this question is by enabling or inhibiting calcium-dependent intracellular pathways (D'Andrea, 2016). In the normal physiology of a cell, calcium is highly involved in secondary messenger systems. In AD, however, many of these networks are defective. cAMP response element-binding protein (CREB) signaling for example, is disrupted in AD (D'Andrea, 2016). Increased intracellular calcium increases CREB phosphorylation at Ser133, Ser142, and Ser143 via activation of kinases such as Calcium/CaM-dependent kinase IV (CaMKIV) and MAPK. Increases in CREB phosphorylation leads to upregulation of target genes which may then produce gene products which further drive disease pathophysiology (Kornhauser et al., 2002). Many genes are regulated by CREB via phosphorylation by calcium/calmodulin (CaM)- dependent protein kinases (Wang et al., 2014). CREB-regulated genes are involved in intracellular events such as long-term potentiation,

neuronal survival, circadian rhythms, exocytosis/endocytosis, and metabolism (Zhang et al., 2005). CREB-regulated genes are known to partially mediate trophic factors as well as synaptic plasticity (Hardingham et al., 2001). Calcium/CaM- dependent protein kinases have also been known to activate some adenylyl cyclases, thus increasing cAMP levels. An increase in cAMP increases CREB-dependent transcription (West et al., 2001). Thus, several mechanisms exist which may up-regulate genes containing CRE's. Interestingly, however, phosphorylated CREB is decreased in AD, leading to a reduction in expression of CREB-regulated genes (Reese and Tagliatela, 2011). This may lead to inhibition of CREB activation of pro-apoptotic processes, leaving the cell unable to perform controlled cell death (Reddy and Beal, 2008).

Calcineurin (CaN) is also heavily influenced by a dysregulation of calcium homeostasis in AD. Increases in intracellular calcium activate CaM, which then subsequently activates CaN (D'Andrea, 2016). An increase in CaN has been proposed to cause an increase in long-term depression and loss of memory (Berridge, 2013). CaN composes 1% of all proteins in neurons, so any disruption in its expression can have severe results. CaN itself has several effects at the synapse. CaN dephosphorylates voltage-dependent sodium channels, potassium channels, and L-type calcium channels, so its activation can affect ion concentration and membrane polarization. CaN is also heavily involved in synaptic release of neurotransmitters from the presynaptic neuron. It has been proposed to dephosphorylate proteins crucial to the release of glutamate as well as regulation of endocytosis. Thus, it decreases glutamate release and slows endocytosis. CaN is also known to dephosphorylate NMDARs, closing the channels (Reese and Tagliatela, 2011). The NMDA receptor has been indicated as crucial to forming memories. Furthermore, activation of CaN by CaM leads to phosphorylation of proteins resulting in synaptic loss of

proteins, inhibition of neurotransmission, neuroinflammation, tau phosphorylation and even cell death. In the AD brain, CaN has been shown to be overexpressed and CREB phosphorylation is reduced in the hippocampus. It is possible this is due to increased CaN, but other mechanisms are likely involved as well. CREB dephosphorylation is associated with loss of synaptic proteins (Reese and Tagliatela, 2011). CaN upregulation forms a negative feedback loop via dephosphorylation of nuclear activator of T-cells (NFAT) and a subsequent increase in RCAN1, a regulator of CaN activity. Since CaN is a regulator of CREB, this may lead to excessive transcription of CREB and subsequent cell death (Hoeffler et al., 2007). Furthermore, NFAT dephosphorylation is associated with neuroinflammation (Reese and Tagliatela, 2011). Additionally, regulator of calcineurin 1 (RCAN1) inhibition of CaN and activation of glycogen synthase kinase-3 β (GSK3 β) decreases dephosphorylation in tau and hyperphosphorylates tau, respectively, leading to further toxicity (Lloret et al., 2011). CaN also dephosphorylates the Bcl2-associated death promoter and pPP1, which are involved in inducing apoptosis and in neurotransmission, respectively (Reese and Tagliatela, 2011).

Another method by which calcium dysregulation may disrupt the synapse is by interfering with synaptic mitochondria. Damaged mitochondria produce free radicals which promote β -secretase cleavage of APP. This produces A β oligomers which further impair synaptic mitochondria function. A β oligomers may then be exported outside the cell where they polymerize and aggregate to form amyloid plaques. Meanwhile, damaged presynaptic mitochondria produce less ATP and are unable to sustain the metabolic demands of the presynaptic neuron. These events are hypothesized to interfere with presynaptic exocytosis and impair neurotransmission via glutamate and NMDARs (Avila, 2011).

Excessive intracellular calcium has also been demonstrated to induce apoptosis as well as creation of free radicals and reactive oxygen species. Mitochondria and the endoplasmic reticulum are both highly involved in these processes (LaFerla, 2002). Though excessive intracellular calcium may be caused by synaptic disruptions, the effects of intracellular calcium reach far beyond the synapse. These non-synaptic disruptions will be discussed later in this thesis.

Cholinergic disruptions of amyloid beta. Expression of muscarinic cholinergic receptors are also disrupted in AD. M1-M4 muscarinic receptors are expressed at lower levels in the hippocampi of AD patients compared to age-matched controls. Lower levels of M4 receptors have been particularly well characterized in AD. Further studies have demonstrated a decrease in M2 receptors compared to M1 and M3 receptors. M2 receptors have been proposed to inhibit expression of β -secretase, leading to a decrease in $A\beta$. Since these are under-expressed in the AD brain, $A\beta$ is more likely to accumulate. Stimulation of M1 and M3 receptors increases γ -secretase cleavage of APP, reducing levels of harmful $A\beta$. M1 may even reduce the toxicity of $A\beta$ through a GSK3-regulated pathway (El-Hassar et al., 2011). Regardless, there is certainly evidence for disruption of acetylcholine in AD synapses. Acetylcholine binds to postsynaptic muscarinic receptors, which are coupled to intracellular G-proteins. G-protein activation initiates several intracellular signaling pathways. While acetylcholine disruptions may play a part in the pathophysiology of AD, glutamatergic disruptions have been better characterized in the disease.

Glutamatergic disruptions of amyloid beta. $A\beta$ oligomers directly disrupt a variety of glutamatergic postsynaptic proteins such as NMDARs and metabotropic glutamate receptors (Ovsepian et al., 2018). These receptors are expressed at lower levels in AD patients.

Furthermore, research has demonstrated that exogenous A β treatment increases endocytosis of NMDARs in mouse and human cortical neurons. This may be a response to the increase in intracellular calcium caused by A β -dependent NMDAR activation (West et al., 2001).

Regardless, extracellular A β oligomers have been demonstrated to directly activate NMDARs on post-synaptic neurons. A β oligomer activation of NMDARs induces further calcium influx into neurons and increases the probability of calcium induced neural toxicity as well as increases the probability of post synaptic neural firing (Texidó et al., 2011). Interestingly, prolonged NMDAR activation by A β results in high intracellular calcium, leading to an increase in vesicular merging with the neuronal membrane, and endocytotic transport of APP to late endosomes. APP is then cleaved by β -secretase to form the β -CTF. When this is further cleaved by γ -secretase it forms toxic A β peptides, adding to the neurotoxic load (D'Andrea, 2016).

Many studies have characterized the disruption of the glutamate system in AD. A β oligomers increase extracellular glutamate stores due to an induction of glutamate release and reduction of synaptic glutamate reuptake. Increased extracellular glutamate increases the likelihood that a neuron will fire. This build-up of synaptic glutamate may cause glutamate-induced excitotoxicity (Pignataro and Middei, 2017) However, these effects are likely after neurons lose sensitivity to the depressive effects of A β , as A β is well known to depress synaptic activity at physiological levels (Pearson and Peers, 2006). While the toxic effects of the NMDA glutamate receptor have already been discussed in detail, mechanisms of metabotropic glutamate disruption in the AD brain must also be discussed. Significantly, metabotropic glutamate receptor (mGluR) signaling is highly dependent on both intracellular and extracellular calcium levels in neurons. Synaptic calcium reduction leads to a significant reduction in postsynaptic

mGluR activity; thus an increase in intracellular calcium may lead to downstream inhibition of mGluR activity and disruption of glutamate mediated intracellular cascades (Revett et al., 2013). Group 1 mGluRs have been shown to increase intracellular calcium via IP₃ and ryanodine triggered calcium release from the ER, so a buildup of synaptic glutamate induced by A β likely adds to calcium dysregulation and cellular dysfunction (Schapiro et al., 2010). AMPA receptors, a type of ionotropic glutamate receptor, are also disrupted in AD brains. A β has been shown to increase endocytosis of these receptors, thus decreasing their effect at synapses. In fact, in rat models of AD, AMPAR decrease at synapses has been demonstrated to facilitate dendritic spine loss and long-term depression after A β treatment. Associated endocytosis of GluRs has also been shown to reduce synaptic AMPAR levels. Furthermore, A β interferes with calcium/CaM which is involved in AMPAR delivery to synapse. AMPARs are permeable to calcium, sodium, and potassium, so AMPAR disruption leads to neuronal depolarization. Thus, A β induces long-term depression in part by dysregulation of AMPARs (Revett et al., 2013).

Vesicular disruptions of amyloid beta. A β oligomers contribute negative effects on vesicle trafficking at the presynaptic side. Endocytosis is slowed, and synaptic vesicle recycling is impaired (Park et al., 2013). A β has been shown to impair Soluble NSF Attachment Protein Receptor or SNARE-dependent exocytosis via blockage of zippering (Ovsepian et al., 2018). A β has also been shown to decrease expression of dynamin 1 and synaptophysin, two proteins heavily involved in endocytosis, likely leading to a decrease in the formation of endocytic vesicles (Ovsepian et al., 2018). Decreased endocytosis and exocytosis can cause several deleterious effects in neurons from disruption of neurotransmitter and membrane protein levels to intracellular accumulation of toxins (Ovsepian et al., 2018).

Amyloid Beta Interactions with Tau/NFT's

The other major hallmark of Alzheimer's disease is that of intracellular NFTs. These NFTs are composed primarily of the hyperphosphorylated form of tau protein, a microtubule-associated protein (MAP). While normally positioned in axons, in AD pathology, tau is localized to dendrites (Murphy and LeVine, 2010, Su, et al., 1997). Phosphorylated tau has a significantly reduced ability to bind to microtubules and has a high affinity to polymerize with other tau molecules. Tau molecules first associate to form straight filaments and eventually these straight filaments join to form paired helical filaments (Revet et al., 2013). This review will center primarily on the relationship of A β and tau, with a focus specifically on mechanisms by which A β may contribute to tau pathology and NFT formation. Many studies have characterized a decrease in CSF A β 1-42 prior to increases in CSF tau and phosphorylated tau (Silverman et al., 1997). In accordance with this, PET brain imaging studies have revealed that clinically significant amyloid plaques form prior to cytotoxic NFTs, although NFTs are seen prior to overall amyloid plaque accumulation. This seems to suggest that A β oligomers induce initial formation of a small portion of NFTs, which in turn add to disease pathophysiology along with accumulation of extracellular A β . Coupled together, these elements induce extracellular amyloid deposits and subsequent formation of the majority of NFTs. Thus, this review proposes that extracellular amyloid disruptions precede significantly toxic intracellular tau pathology in AD (Blurton-Jones and LaFerla, 2006).

One of the main methods by which A β drives neurofibrillary pathology is by indirect phosphorylation of tau. Several proteins are disrupted by A β , with downstream phosphorylation of tau. For example, in rat models of AD, extracellular A β incites an increase in active and

phosphorylated ERK1 and ERK2 and their activity in hippocampi. These kinases are involved in several important neural functions such as modulation of synaptic plasticity, apoptosis, and neuroinflammation. Phosphorylated ERK1 and ERK2 induce hyperphosphorylation of tau. This occurs prior to amyloid plaque formation, suggesting soluble oligomers such as A β 1-40 and A β 1-42 are likely to play a role in disruption of ERK1 and ERK2 signaling and subsequent initiation of tau pathology (D'Andrea, 2016).

Another mechanism by which A β drives tau pathology is by promoting its aggregation. In a mutant mouse model, early accumulation of A β correlated with tau redistribution and tau hyperphosphorylation. A β and tau appear to first colocalize in dendrites and eventually localize around neuronal projections around amyloid plaques (D'Andrea, 2016). When this occurs, tau is phosphorylated by tau protein kinase II (TPK II). TPK II phosphorylation of tau is dependent upon A β concentration. Thus, not only does A β induce tau aggregation, but also hyperphosphorylation as well (D'Andrea, 2016).

Some mechanisms of tau phosphorylation depend upon A β 's ability to disrupt calcium homeostasis in neurons. For example, A β 's ability to bind to the α 7 receptor may also play a role in tau pathology as A β binding results in hyperphosphorylation of tau. Several studies have documented an interaction between the α 7 receptor and NMDARs. α 7 stimulation in glial cells upregulates GLAST, a glutamate transporter. This process is mediated by fibroblast growth factor and IP₃-calcium/CaMKII pathways. Thus, stimulation of α 7 nicotinic cholinergic receptors may lead to neuroprotective uptake and clearance of glutamate from the synapse. Excessive glutamate uptake in microglia may cause compensatory mechanisms to overproduce glutamate, hence the extracellular glutamate build-up in Alzheimer's disease (Akaike et al.,

2018). Excessive glutamate may then disrupt calcium homeostasis and lead to downstream phosphorylation of tau. mGluR2 has also been found in higher concentrations in the hippocampi of patients with Alzheimer's disease and are associated with an increase in phosphorylated tau. Likely, overexpression of mGluR2 in AD hippocampi increases intracellular calcium levels, initiating downstream activation of tau kinases and subsequent phosphorylation of tau (Revett et al., 2013).

Yet another protein that is disrupted by A β in Alzheimer's disease is Regulator of Calcineurin-1, also known as RCAN1. RCAN1 increases when cells are experiencing oxidative stress. Many forms of A β are known to induce oxidative stress and formation of reactive oxygen species (ROS), thus A β has significant potential to disrupt expression of RCAN1. Oxidative induction of RCAN1 overexpression has two potential consequences, inhibition of CaN leading to a decrease in dephosphorylation of tau, and upregulation of GSK3 β and subsequent hyperphosphorylation of tau. These actions coupled together represent a significant mechanism by which tau may become hyperphosphorylated (Lloret et al., 2011).

GSK3 β plays an additional role in tau pathology and NFT formation. As explained previously in this review, A β isoforms have been well characterized to increase intracellular calcium concentrations. This increase occurs via direct stimulation of NMDARs, calcium channels, or the ability of A β 1-42 oligomers to form ion channels directly in the neuronal membrane. An increase in calcium induces an increase in CaN/PP2B, which in turn induces an increase in GSK3 β activity and subsequent phosphorylation of tau and NFT pathology. Additionally, GSK3 β expression is elevated in multiple models of A β initiated tau pathology, reinforcing the concept of A β /GSK3 β induced tau pathology. In these models, inhibition of

GSK3 β decreases tau hyperphosphorylation (D'Andrea, 2016). Interestingly, A β 's ability to decrease NMDAR dependent LTP has also been linked to GSK3 β tau pathology (D'Andrea, 2016).

As many proteins are regulated by CaN, CaN clearly plays an important role in AD pathophysiology. Several studies have demonstrated that neurons near amyloid plaques as well as neurons with NFT's demonstrate a strong reaction when probed with CaN antibodies. This supports the hypothesis that CaN leads to downstream phosphorylation and hyperphosphorylation of tau. In accordance with this, the CaN activator calmodulin (CaM) is also over-expressed in the AD brain. This explains the imbalance of CaN kinase and phosphatase activity, as excess CaM over-activates CaN, tipping the balance in the favor of CaN activation. Interestingly, CaN has been proposed to interact with tau under normal circumstances, however, when calcium concentrations rise, CaM is activated and subsequently binds to CaN. This disrupts the interaction between CaN and tau and CaN is unable to dephosphorylate tau. Thus, excessive calcium leads to overactivation of CaN, and a subsequent decrease in tau dephosphorylation (Reese and Tagliatela, 2011).

Several additional kinases have also been found to be activated by A β 1-40 and A β 1-42. These kinases then phosphorylate tau directly or lead to downstream phosphorylation of tau. Other kinases which effect phosphorylation of tau include Fyn kinase, protein kinase c, calpain (p35 and p25) and cyclin dependent kinase (Revett et al., 2013, LaFerla, 2002). While these kinases may increase phosphorylation of tau, review of each kinase that does so is beyond the scope of this thesis.

Evidence suggests that tau phosphorylation occurs prior to neuronal loss. It is likely that

phosphorylation of tau and aggregation of A β are related and dynamic processes which are significantly intertwined. Phosphorylation of tau causes neuronal death via destroying microtubule function, thus, impairing axonal and vesicular transport and causing irregular neuronal morphology. Inhibition of this process appears to prevent a portion of A β cytotoxicity. Inhibition is achieved via interrupting tau kinase function. This has been shown to increase cell viability. Furthermore, tau knockout animal models have shown to produce A β cytotoxicity resistant mice (Revet et al., 2013).

Another mechanism by which A β may facilitate tau hyperphosphorylation is via activation and increased expression of stress induced proteins. One such protein is the mammalian target of Rapamycin, or mTor. mTor is an important protein involved in maintaining oxygen, metabolic rate, and nutrient levels (Tokunaga et al., 2004). mTor is regulated by nutrient and growth factor levels, as well as cellular stress. Though A β has been indicated in disruption of growth factors and nutrient levels, significant research has demonstrated its ability to distress cells. Particularly, A β has been shown to regulate ERK1 and ERK2 levels, which are also known to activate mTor. Furthermore, mTor is inhibited by GSK3 β , which is also disrupted by A β . Several studies have demonstrated mTor activity disrupts several kinases resulting in downstream phosphorylation of tau and even increases synthesis of tau itself (Tang et al., 2013). Together, these results indicate that cellular stress induced by A β may induce mTor overexpression and subsequently, tau pathology.

An abundance of research illustrates the interplay of A β , calcium dysregulation, and tau disruption. In AD, microtubules are severely disrupted, and phosphorylation of tau has been shown to have many negative downstream effects. Primarily, phosphorylated tau is believed to

interact with unphosphorylated tau decreasing tubulin formation of microtubules. Phosphorylated tau itself also does not bind microtubules, decreasing the amount of viable tau present in the cell. On the other hand, dephosphorylation of tau has been shown to rescue functionality of tau in vitro. Regardless, phospho-tau regulated disruption of microtubule assembly has been proposed to disrupt axonal transport and possibly induce retrograde neuronal degeneration (Alonso et al., 1994). Furthermore, tau paired helical filaments have been shown to inhibit neuronal proteasomes, causing a further increase in toxic protein accumulation. Tau paired helical filaments and subsequent NFT formation do in fact correlate with the severity of disease (Keck et al., 2003). Finally, phosphorylation of tau has been shown to increase the detrimental mitochondrial effects $A\beta$ has in neurons (Quintanilla et al., 2014). As a result of this mass of research on the role of tau in AD, dephosphorylation of tau is currently a major therapeutic aim in AD.

Additional Homeostatic Disruptions of Amyloid Beta

Increase in intracellular calcium: non-synaptic effects. Intracellular calcium accumulation leads to neurotoxicity through a variety of non-synaptic pathways. These include ER disruptions, mitochondrial disruptions, and ROS production. In fact, when intracellular calcium is artificially increased in cultured neurons, $A\beta$ accumulates, tau becomes hyperphosphorylated, and cells eventually die. Likely, intracellular calcium levels play a key part in the neurodegeneration seen in AD. Furthermore, many of the genes affected in familial AD are related to calcium signaling (LaFerla, 2002).

Additional mechanisms of $A\beta$ toxicity involve neural ER and mitochondria. While these organelles have very distinct functions within the cell, they each play a role in the regulation of

intracellular calcium. In AD, the ER is heavily impaired by A β . In several in vivo studies, A β stimulates IP₃ and Ryanodine receptors in the ER, causing a significant efflux of calcium from the ER. This depletion of ER calcium stores in part induces the unfolded protein response, which will initiate apoptosis if chronically activated. ER stress may even further increase APP cleavage by β -secretase inducing further A β production. Nonetheless, while mitochondria are often near the ER, mitochondria take up extracellular calcium (Li et al., 2015). Increases in mitochondrial calcium disrupt mitochondrial membrane potential and function. Severe disruption of the mitochondrial membrane potential induces opening of the mitochondrial transition pore, which releases ROS and proapoptotic substances, such as apoptosis inducing factor and cytochrome c into the cell (Redza-Dutordoir and Averill-Bates, 2016). Disruption of mitochondrial membrane potential disrupts the electron transport chain, and thus, ATP production decreases. A β has also been found to directly disrupt mitochondrial protein transport. Specifically, A β treatment is associated with a decrease in complex IV in the mitochondrial membrane, decreasing mitochondrial ability to meet the metabolic demands of the neuron (Chen and Zhong, 2014). This disruption is also associated with an increase in ROS, particularly, production of the superoxide ion (Ferreiro et al., 2008). ROS damages mtDNA further compounding the effect of mitochondrial membrane potential disruption (Redza-Dutordoir and Averill-Bates, 2016).

A β may also disrupt the balance of mitochondrial fusion and fission. In mouse models, mice producing high levels of A β were found to have lower mitochondrial ATP production, an increased rate of fission and associated proteins, and a decrease in fusion and associated proteins. This creates a deficit in axonal and synaptic mitochondria leaving these areas vulnerable to metabolic deficiency. Transgenic mice overexpressing A β not only demonstrated a fission and

fusion imbalance, but also increases in ROS production and associated changes in oxidative markers such as superoxide dismutase and 4-hydroxynonenal. Interestingly, a decrease in mitochondrial proteins appears prior to mitochondrial membrane failure and oxidative stress. Oxidative stress further exacerbates mitochondrial dysfunction and feeds back to continue β -secretase cleavage and A β production. Mitochondrial dysfunction and A β toxicity are thus closely associated. In A β transgenic mice, mitochondrial dysfunction and A β deposition increase as the mice age. Furthermore, ROS facilitates tau hyperphosphorylation, increasing tau pathology (Pagani and Eckert, 2011). In addition to mitochondrial ROS production, metal accumulation and inflammation also contribute to neuronal oxidative stress. A β binds directly to copper and iron, creating an accumulation of metal and producing hydrogen peroxide, further promoting oxidative stress (Chen and Zhong, 2014). Furthermore, activated microglia and astrocytes continually produce ROS as cellular byproducts (D'Andrea, 2016). Extracellular oxidative stress can also induce cell death via induction of transmembrane cell death receptors such as TNF receptor 1, TRAIL receptor 1 and 2, and the Fas receptor (Redza-Dutordoir and Averill-Bates, 2016). Regardless of the source of ROS, oxidative stress disrupts mitochondrial and cell function propelling the cell further toward death.

Amyloid disruptions of the ubiquitin-proteasome system. Apart from the detrimental effects of intracellular calcium disruption, another mechanism through which A β may disrupt neuronal homeostasis is via disruption of the Ubiquitin proteasome system. HIP-2, otherwise known as UBE2K, is an ubiquitin conjugating enzyme which is suspected to contribute to A β disruption of the ubiquitin proteasome system. In both in vivo and in vitro studies, A β 1-42 treatment induced a significant overexpression of HIP-2/UBE2K in neurons. HIP-2/UBE2K's

activity is required for A β 1-42 induced inhibition of the ubiquitin proteasome system and subsequent neurotoxicity. A β 1-42 has been proposed to inhibit proteasomal degradation of aberrant protein leading to a buildup of dysfunctional protein in affected cells. Protein aggregation induces the apoptotic marker apoptosis signal regulating kinase 1 and the c-Jun-N-terminal kinase, increasing the likelihood of neural apoptosis. Additionally, UBB+1, a dysfunctional form of ubiquitin, has been found to associate with UBE2K and collect nearby. UBB+1 has been proposed to inhibit the proteasome by binding dysfunctional proteins prior to ubiquitin, and disabling the proteasome from removing the polyubiquitin chain and degrading the protein, leading to accumulation of dysfunctional protein and subsequent cytotoxicity (Song et al., 2003). Furthermore, HIP-2/UBE2K inhibition of the proteasome has been shown to activate Caspase-12 in mice both by increasing levels of Caspase-12 mRNA and more indirectly via inhibition of proteolysis of Caspase-12 and activation of Caspase-12 via endoplasmic stress induced by proteasomal inhibition. Though the human homolog of Caspase-12 does not currently have any known functions, other caspases may have similar defects when activated by A β in humans (Song et al., 2008).

A β and neuroinflammation in AD. After review of several mechanisms by which A β disrupts neuronal homeostasis, a crucial final consideration is the efficiency of the neural defense mechanism against the extracellular perturbations which A β plaques present. In an attempt to reduce these effects, neuroinflammation, or gliosis is initiated. In gliosis, microglia and astrocytes function to eliminate extracellular depositions of debris. In this process, microglia and astrocytes accumulate around debris to form glial scars. In AD, gliosis occurs in areas adjacent to amyloid plaques. Glial cells then release proinflammatory cytokines (such as tumor necrosis

factor- α , interferon- γ , and interleukin-1) and oxidants (superoxide and nitric oxide) which further induce neural death. As neural inflammation and related cell death are secondary outcomes of A β plaques, inhibition of inflammation alone does not stop the neurodegenerative effects of A β . In mice models of AD, however, inhibition of neuroinflammation with non-steroidal anti-inflammation drugs (NSAIDs) has been shown to inhibit plaque formation, decrease AD-like symptoms and A β concentrations. Furthermore, patients with a history of NSAID use demonstrate a significant decrease in microglia. This evidence suggests that inhibition of inflammation may decrease secondary neural death and thus slow neurodegeneration in AD. Though A β does provoke neuroinflammation, it is a secondary event in the pathogenesis of AD, and it only exacerbates neural cell death. Furthermore, when neurons undergo apoptosis or lyse due to intracellular A β accumulation further neuroinflammation is triggered, creating a cycle of positive feedback in which inflammation initiates neural death which provokes further inflammation and so on. Additionally, some studies report that proinflammatory cytokines can increase expression of β -secretase, increasing A β formation and hyperphosphorylation of tau, pushing cells further along in the pathophysiology of AD (D'Andrea, 2016).

Conclusions

Alzheimer's disease is a physiologically devastating disease. While there are many hypotheses of the pathophysiology of the disease, the hypothesis that has come closest to explaining the research is the Amyloid- β hypothesis. This hypothesis states that extracellular A β accumulation in the form of extracellular plaques initiate the pathophysiology of the disease. While the evidence no longer appears to support this exact hypothesis, the contents of this thesis have demonstrated that a similar mechanism likely initiates the pathophysiology of Alzheimer's

disease. While amyloid plaques have been shown to disrupt neuronal function, they do not appear until late in the stages of Alzheimer's disease. The research conducted in this study suggests that the cytotoxic A β 1-42 initiates a cascade of synaptic and intracellular events leading to downstream neuronal death and neurodegeneration.

Synaptic Interactions of Amyloid Beta

The synaptic interactions of A β 1-42 represent some of the most devastating effects of amyloid in AD. A β 1-42 oligomers have been shown to disrupt calcium homeostasis via a variety of pathways. Prolonged increases in intracellular calcium wreaks havoc at the synapse, leading to downstream genomic and neurotransmitter effects, phosphorylation of tau, creation of reactive oxygen species, and further proliferation of A β 1-42 in neurons. Cholinergic, glutamatergic, and vesicular effects further amplify the effects of A β 1-42. Combined, these downstream effects all contribute to a mechanism of A β 1-42 induced neuronal cell death.

Amyloid Beta Interactions with Tau/NFT's

A β 1-42 interacts indirectly with tau via a variety of pathways. A β 1-42 effects phosphatases and kinases and intracellular calcium with consequent hyperphosphorylation of tau. While phosphorylation of tau disables its incorporation into microtubules, phosphorylated tau also associates with non-phosphorylated tau, further decreasing the pool of tau available for incorporation into functional microtubules. These effects interfere with axonal transport and induce toxic accumulation of proteins and even increases the detrimental effects of A β . This thesis suggests that AD is not a disease simply resulting from amyloid disruptions, but also, A β 's antagonism of and synergy with the detrimental effects of phosphorylated tau.

Other Homeostatic Disruptions of Amyloid Beta

Apart from the various effects of A β 1-42 at the synapse and its interactions with tau, A β 1-42 has many additional effects which contribute to neuronal death. While synaptic effects play a large role in neuronal death, A β 1-42 initiates significant intracellular effects as well. Intracellular calcium dysregulation depolarizes mitochondria and induces ER and mitochondrial dysfunction, ROS production, and inflammation. Additionally, A β 1-42 disrupts the ubiquitin-proteasome system, causing aberrant protein accumulation and additional cytotoxicity. Oxidative stress and ubiquitin-proteasome disruptions further impair the normal functioning of neurons, adding to the catastrophic interactions of A β 1-42 at the synapse and with tau.

While the classical amyloid hypothesis does not explain the current research on the physiology of AD, the hypothesis should not be completely discarded. According to the classical amyloid hypothesis, amyloid plaques composed of aggregations of A β induce the pathophysiology of AD. As research on AD has progressed, amyloid plaques have come to be seen as downstream effects of disruptions of normal cell biology. This thesis has demonstrated that cellular disruptions of A β precede the formation of both amyloid plaques and NFTs. This thesis also provides evidence that A β disruptions induces extracellular and intracellular consequences which induce the pathophysiology of AD. There are several approaches which may prove to be successful in mediating the adverse effects of A β in the AD brain. Prevention of AD should be the primary focus of AD research. This begins with APP processing. Stimulation of α -secretase cleavage of APP and inhibition of β -secretase cleavage prior to or early in disease pathology would likely prevent the physiological abnormalities of AD. A β clearance or degradation in early stages of AD would also cease progression of the disease. As a majority of

AD research focuses on reversing the neurodegeneration characteristic of the disease, experimental research must be redirected to address preventing the disease entirely or stopping the disease in its early stages. While reversal of neurodegeneration, induction of neurogenesis, reformation of neural pathways, and complete cognitive restoration are all ideal and noble endeavors, it may prove beneficial for AD research to explore alternative approaches to defeating the neurological atrocity that is Alzheimer's disease.

Acknowledgements

I would like to thank Dr. Isaacs and Dr. Schweitzer for their contributions in the editing of this thesis. I would also like to thank Dr. Townsend for her patience, energy, and support throughout the process of this thesis.

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