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Near infrared light to promote synaptic resilience to Alzheimer's Disease neuropathology

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Near infrared light to promote synaptic resilience to Alzheimer's Disease neuropathology

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Dedication

This work is dedicated to my parents David Comerota and Mary Comerota, my brother James Comerota, sister-in-law Alexandra Comerota and all my family and friends. Thank you for your endless encouragement and support.

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Near infrared light to promote synaptic resilience to Alzheimer's Disease neuropathology

Abstract

Alzheimer's Disease (AD) is a multifactorial neurodegenerative dementia with no curative therapeutic options. One of the earliest impairments in AD triggering cognitive decline is the synaptic dysfunction induced by the selective targeting and interruption of the synaptic region by the small oligomeric form of amyloid beta (Aβ). Recently, the co-presence of Aβ oligomers (Aβo) and tau oligomers (tau-o) at the synapses has been suggested to exacerbate this dysfunction. Therefore, the development of therapeutics aimed at protecting the synapses from the toxic binding of both proteins at the synapses can preserve synaptic health and cognitive function. With this goal in mind, the present study investigated the transcranial application of near infrared light (NIR, 600-1000 nm) as a potential treatment for AD. The primary objective of this project was to determine the effect of NIR light treatments (670 nm; 90 sec/day for 4 weeks) on the dysfunctional synaptic impact of oligomers. To achieve this goal, we investigated the modulation of the synaptic Aβo and tau-o load, the susceptibility of synapses to Aβo and tau-o binding and the resulting impaired long-term potentiation (LTP). We also investigated multiple mechanisms induced by NIR light. We found a significant reduction of $A\beta_{1-42}$ at the synapses of NIR light treated APP transgenic (Tg2576) and 3xTg-AD mice. Further, 3xTg-AD and htau mice had reduced tau oligomers at the synapses and in the total protein extract after NIR light treatment. We further found a reduction in *ex vivo* synaptic Aβo binding and Aβo induced depressed LTP in wild type mice treated with NIR light while tau-o binding and resulting depressed LTP was not changed. In addition, we found increased efficiency of synaptic mitochondria, an upregulation of autophagy and increased inducible

heat shock protein 70 (HSP70) levels in the synapses of NIR light treated mice suggesting mechanisms contributing to NIR light induced synaptic protection and clearance of the toxic proteins. Collectively, these results support NIR light as a viable treatment option for AD by promoting the reduction of Aβ and tau pathology, as well as, synaptic resistance to Aβ oligomer binding thus alleviating the ensuing synaptic impairments.

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List of Abbreviations

α7nAChR	Alpha 7 nicotinic acetylcholine receptor
Aβ	Amyloid beta
AD	Alzheimer's disease
ADAS-Cog	Alzheimer's Disease Assessment Scale-Cognitive
APP	Amyloid precursor protein
CW	Continuous wave
FDA	Food and drug administration
HBK	HEPES-buffered Krebs-like
HSC	Heat shock cognate
HSP	Heat shock protein
LED	Light emitting diode
LTP	Long term potentiation
NDAN	Non-demented with Alzheimer's neuropathology
NFT	Neurofibrillary tangles
NIR	Near infrared
NMDA	N-methyl-D-aspartate
MCI	Mild cognitive impairment
MMSE	Mini mental state examination
mGluR5	Metabotropic glutamate receptor 5
PrP^c	Cellular prion protein
PSD95	Post synaptic density 95

Chapter 1: Background

ALZHEIMER'S DISEASE

Introduction

Alzheimer's disease (AD) is the most common age-related dementia that was first identified in 1907 by Alois Alzheimer. The first known patient, Auguste Deter presented with symptoms of memory impairment, delusions and disorientation. Further postmortem observation of her brain found extensive atrophy of the cortex and protein accumulations in the form of plaques and neurofibrillary tangles (NFT) providing the first description of what Dr. Alzheimer coined the 'disease of forgetfulness' (Alzheimer et al., 1995; Morris and Salmon, 2007). It took over 70 years after this first discovery for what is now known as Alzheimer's disease to be recognized as the major contributing dementia and one of the leading cause of death among the elderly (Katzman, 1976). Today, over 5 million people in the United States are living with AD costing nearly 259 billion dollars a year in health-related costs (Plassman et al., 2007; Hebert et al., 2013; Kelley et al., 2015). Because the age of the American population is rising and the lack of disease altering drugs, the number of AD cases is projected to reach 16 million by 2050 (Hebert et al., 2003).

Since the first observation of AD, the knowledge of the disease has grown immensely, however a resolving therapeutic has yet to be identified. The protein accumulations of plaques and tangles, observed in the post-mortem brain of Auguste Deter, were found to contain the proteins amyloid beta (Aβ) and hyperphosphorylated tau, respectively (Glenner and Wong, 1984; Brion et al., 1986; Grundke-Iqbal et al., 1986). The progressive accumulation of the proteins serves as hallmarks for the diagnosis of the disease (Braak and Braak, 1991; Thal et al., 2002). The underlying cause of these protein accumulations are still not well understood but have been linked to numerous factors. Genetic mutations or over expressions of proteins associated with Aβ production, such as, amyloid precursor protein (APP), presenilin 1 or presenilin 2, are factors in the development of a form of AD referred to as familial AD, which accounts for less than 5 percent of AD cases (Citron et al., 1992; Scheuner et al., 1996). Other factors such as obesity, Type II diabetes and related insulin resistance,

traumatic brain injury, among several others have been identified to contribute to a rising risk of the development of the more commonly occurring sporadic AD (Bomfim et al., 2012; Reitz and Mayeux, 2014; Gerson et al., 2016; Velazquez et al., 2017). The multifactorial nature of AD has challenged the development of effective therapeutics. Previous therapeutics have selectively targeted one of the many contributing aspects of the disease, however, in clinical trials failed to rescue or slow cognitive dysfunction in AD patients (Honig et al., 2018). Further understanding of these factors, their initiating mechanisms and the converging mechanisms inducing dysfunction will be imperative in the development of a therapeutic or combination of therapeutics that may modulate the multiple contributors to the disease progression.

Amyloid β protein processing

The Aβ protein is derived from the amyloidogenic cleavage pathway of the transmembrane protein, amyloid precursor protein (APP) (Kang et al., 1987). This pathway involves the successive cleavage of APP by beta (β) and gamma (γ) secretase producing an extracellular 40 or 42 amino acid Aβ peptide (Haass, 2004). APP also undergoes proteolytic processing by a second pathway known as the non-amyloidogenic pathway. Processing by this pathway involves APP cleavage by a third enzyme, alpha (α) secretase resulting in the release of two non-aggregating fragments, sAPPα and C83. The fragment C83 can be further cleaved by γ secretase producing the peptide, p3 (Sisodia, 1992; Kuhn et al., 2010). The amyloidogenic Aβ peptide is prone to misfolding and becomes highly susceptible to self-assembly into homogeneous protein aggregates, with $A\beta_{1-42}$ more prone to aggregation than $A\beta_{1-40}$ (Zheng et al., 2017). The continuous aggregation of Aβ monomers results in the production of various forms of aggregated Aβ including the oligomer, protofibril and fibrillar states. The accumulation of Aβ is thought to originate in the entorhinal cortex, an area involved in memory processes and highly connected to the hippocampal region, and spreads throughout the cortex as the disease progresses (Braak and Braak, 1991). However, some believe another area, the locus coeruleus, may be the earliest location of AD pathology development (Grudzien et al., 2007;

Kelly et al., 2017). Extracellular plaques observed in late stages of AD are composed mainly of fibrillar Aβ, which as a result has become the focus of the field for many years. However, the lack of correlation between Aβ plaque burden and cognitive impairments lead to investigation of the toxicity of other forms of Aβ (Erten-Lyons et al., 2009; Bjorklund et al., 2012). The oligomeric Aβ selectively targets the synaptic region and has been reported to be produced and associate with this critical region in the initial stages of AD (Gordon et al., 2002; Lacor, 2004), eliciting deficits that lead to synaptic dysfunction (Resende et al., 2008; Li et al., 2009; Wilcox et al., 2011). In addition, Aβ oligomers can be internalized by neurons through many different mechanisms including oligomer formed pores in the cell membrane (Lai and McLaurin, 2011; Serra-Batiste et al., 2016). Intracellular Aβ oligomers have been found to induce deficits contributing to overall synaptic dysfunction. The role of Aβ oligomers in the initiation of these mechanisms makes oligomeric Aβ and its mechanisms of toxicity a main focus in the understanding of AD initiation.

Tau protein accumulation

The microtubule associated protein tau (MAPT or simply referred to as tau), which comprises the neurofibrillary tangles is produced by alternative splicing of the exons 2, 3, and 10 of the tau mRNA. This alternative splicing results in six different isoforms of tau in the adult human brain, each with a differing propensity of contribution to disease progression (Espinoza et al., 2008). Under normal physiological conditions, tau is found in the axonal region of the neuron (Binder et al., 1985) and has many roles including acting as a stabilizing protein for microtubules. The tau protein undergoes several posttranslational modifications including phosphorylation, glycosylation, ubiquitination among others that alter its function (Wang et al., 1996; Song et al., 2015; Tracy and Gan, 2017). In AD, tau phosphorylation is elevated by 3 to 4-fold compared to normal physiological conditions resulting in increased dissociation from the microtubules (Wang et al., 2013). Previous studies have identified approximately 45 phosphorylation sites in AD hyperphosphorylated tau (Hanger et al., 2007). Of these sites, three (Ser262, Thr231, and Ser235) were found to decrease the

affinity of the tau for microtubules resulting in the increased release of hyperphosphorylated tau (Sengupta et al., 1998). After its dissociation from the microtubules, hyperphosphorylated tau is prone to misfolding and self-aggregation into oligomers, protofibrils and fibrils (von Bergen et al., 2005). Certain sites of phosphorylation, such as those at the C-terminal region were also found to contribute to the promotion of self-assembly of the protein inducing aggregation (Liu et al., 2007). In AD, hyperphosphorylated tau is also mislocalized to the dendritic and soma regions of neurons, resulting in elevated levels of tau oligomers at the synapses (Delacourte et al., 1990; Zempel et al., 2010). This region-specific accumulation has led to the investigation of toxic tau oligomers in the pathological spread and progression of AD. Tau oligomers act in a prion- like manner, inducing the misfolding and oligomerization of normal tau (Lasagna-Reeves et al., 2012a). It is believed that the synaptic terminals may serve as a gateway for the transmission of pathological tau from cell to cell (Lee et al., 2012; Tai et al., 2014). Combined with the tau oligomer's prion-like activity, elevated levels of the toxic tau at the synapses provides an opportunity to further the spread of the disease to unaffected cells. In addition, emerging research suggests tau oligomers induce synaptic dysfunction in a comparable manner as Aβ oligomers (Lasagna-Reeves et al., 2011; Kopeikina et al., 2013; Fá et al., 2016) and the two proteins interact in a synergistic manner resulting in exacerbated dysfunction in the development of AD (Pascoal et al., 2017).

Oligomeric Amyloid β and tau interaction

NEUROPATHOLOGY PROGRESSION

The co-presence of the two proteins Aβ and tau has recently emerged as a critical focus in the understanding of the development and progression of AD. One of the most common theories in AD progression is referred to as the amyloid cascade hypothesis, which describes the early accumulations of Aβ as the causative upstream regulator of the accumulation of hyperphosphorylated tau, an event that occurs much later in the disease and leads to cellular death (Hardy and Higgins, 1992). Consistent with this theory, it was found that the oligomeric form of Aβ promotes the pathological forms of tau

by increasing its phosphorylation and initiating the oligomerization and mislocalization of tau that contributes to the dysfunction associated with its pathological accumulations (De Felice et al., 2008; Lasagna-Reeves et al., 2010; Chabrier et al., 2012). Specifically, Aβ oligomers induce protein kinases known to contribute to the hyperphosphorylation of tau (Zheng et al., 2002). However, recent evidence shows that the accumulation of oligomeric tau occurs much earlier in the disease progression than previously believed (Lasagna-Reeves et al., 2012b). The early co-presence of both toxic Aβ and tau oligomers suggests a greater interaction with one another. This emerging theory is leading the field to not only understand the initial mechanisms contributing to the toxicity of each individual oligomeric protein but also their converging mechanisms and the interactive relationship. The initiation of major events involved in AD including synaptic dysfunction and mitochondrial impairment by Aβ and tau oligomers have been extensively studied with recent research focusing on the importance of the combined proteins in the exacerbated dysfunction seen in AD.

MITOCHONDRIAL DYSFUNCTION

The dysfunction of the mitochondria is an important contributing factor in the development of AD. Mitochondria are crucial organelles that play multiple roles in supplying and maintaining the bioenergetics of the neuron, as well as supporting the high energy demand of neuronal synaptic transmission processes (Weeber et al., 2002). When mitochondria function is impaired, the high energy demand of the synapses is no longer able to be supported, resulting in synaptic loss (Marta et al., 2013). Mitochondrial impairment is an early event in AD, however, there is much debate in determining the mechanistic order of mitochondrial dysfunction and accumulation of amyloid oligomers (Swerdlow et al., 2014). Early mitochondrial dysfunction has been found to exacerbate the progression of the disease by making the system, specifically the synapses more vulnerable to the toxicity of the oligomers (Pitt et al., 2009; Marta et al., 2013), as well as, inducing the hyperphosphorylation of tau (Melov et al., 2007). In addition, both dysfunctional tau and Aβ oligomers interfere with the mitochondria bioenergetic activity, further inducing dysfunction

resulting in the loss of health of the neuron and eventual cell death (Manczak and Reddy, 2012). While Aβ and tau induce similar deficits in the electron transport chain and the mitochondrion fission/fusion processes, in some case the mechanisms through which they act differ, suggesting an exacerbation of dysfunction when the two proteins are co-present (Eckert et al., 2011). The understanding of the impact of the mitochondria on AD progression is a vital aspect to identifying targets that have the potential to protect or regenerate the system from the toxicity related to disease.

Mitochondria's principle role of energy production involves a chain of four protein complexes that transfer electrons from high to low energy state, thus releasing necessary energy to establish a proton gradient allowing for the generation of ATP. Because of this essential function, mitochondria are found in elevated volumes at the high energy-dependent synapses (Nguyen et al., 1997). In AD, the protein complexes of the electron transport chain (ETC) become impaired resulting in the halting in the production of ATP and eventually leading to cellular death (Parker et al., 1994b). The activity levels of the complexes in the electron transport chain has been extensively studied due to its reduced activity levels in AD (Maurer et al., 2000). Studies show that the transfer of extracted mitochondrial DNA from AD platelet cells to control cells induces diminished activity levels of complex IV, also known as cytochrome c oxidase (Parker et al., 1994a; Sheehan et al., 1997; Swerdlow et al., 1997). The mitochondrial DNA repair systems are also impaired in AD patients, however, remain intact in Non-demented with Alzheimer's Neuropathology (NDAN) individuals (Taglialatela, unpublished). These individuals retain cognitive function despite ample presence of Aβ plaques and tau containing NFTs, however have reduced amyloid oligomers at the synapses (Zolochevska and Taglialatela, 2016). The intact mitochondrial DNA repair system in NDAN individuals suggests a role of Aβ and tau oligomers in the distribution and mutation of mitochondrial DNA that may contribute to the ETC dysfunction. Further, Aβ and tau are linked with interference of the functions separate complexes of the ETC. Aβ directly binds to and induces dysfunction of cytochrome c oxidase by membrane lipid peroxidation but may also impact the activity of complex I (Bobba et al., 2013). However, tau

selectively targets and induces the deregulation of complex I (Rhein et al., 2009). Together, tau and Aβ impedes the energy output of the ETC, reducing mitochondria function.

Mitochondria are highly dynamic organelles that constantly undergo fission, the separation of a mitochondria producing two separate organelles, and fusion, the joining of two mitochondria producing a single organelle (Scott and Youle, 2010). This process allows for rapid changes in the number and morphology of mitochondria in response to specific needs and changing energy demand of the cell (Sesaki and Jensen, 1999). The delicate balance between fission and fusion is imperative in the maintenance of healthy mitochondria. In AD, the morphology of mitochondria is altered with excessive amounts of fragmented mitochondria in the synaptic region suggesting an impaired ability of the mitochondria to undergo fusion (Wang et al., 2009). Both Aβ and tau have been linked to the impaired balance of the fission/fusion system. Specifically, the proteins, dynamin 1 like (DRP1), OPA1, Mfn1 are suppressed by the interaction with tau and Aβ reducing the machinery necessary for mitochondria to undergo fission and fusion (DuBoff et al., 2012; Reddy et al., 2017). Interestingly, modulation of the fission/fusion proteins prevents synaptic changes induced by Aβ oligomers (Wang et al., 2009) supporting the idea that mitochondrial health is imperative in the neuroprotection of synapses in AD pathology development. Therapeutics that target or increase the overall health of mitochondria can enhance this protection, slowing the progression of the disease.

SYNAPTIC DYSFUNCTION

The synaptic connections between neurons contribute to the complexity of the neuronal system and are the primary sites of communication between cells. The learning and memory processes is attributed to synaptic plasticity, the dynamic strengthening and weakening of the synapses (Takeuchi et al., 2013). One of the early events in the progression of AD is believed to be the impairment of synaptic function (Masliah et al., 2001; Scheff et al., 2007). The subsequent loss of synaptic spines correlates strongly with the progression of cognitive dysfunction (Robinson et al., 2014). The oligomeric forms of both Aβ and tau have been linked to the impediment of synapse

activity and have been widely studied in their respective roles in the induction of synaptic dysfunction with recent research focused on the converging mechanisms of the two proteins that could result in a synergistic interaction.

The selective association of Aβ oligomers with the synapses has been extensively studied as an initiating factor for early synaptic dysfunction in AD. Aβ oligomers specifically target the synaptic regions of neurons (Lacor, 2004; Koffie et al., 2009). The pre and post synaptic regions contain several of the identified binding partners of Aβ oligomers, including post synaptic density 95 (PSD95) (Pham et al., 2010), cellular prion protein (PrP^c) (Laurén et al., 2009), alpha 7 nicotinic acetylcholine receptor (α7nAChR) (Wang et al., 2000), metabotropic glutamate receptor 5 (mGluR5) (Um et al., 2013) and ephrin type-B receptor 2 and 4 (Cissé et al., 2011; Vargas et al., 2014), among others. The interaction of Aβ oligomers with these proteins induces disruption of the protein activity and can result in the activation of downstream pathways that disrupt the integrity of the synapse. One such event induced by the binding of Aβ oligomers with PrPc, is the activation of Fyn kinase which modulates levels of glutamate receptors on the membrane surface (Um et al., 2012). The toxic events of Aβ oligomers is not limited to interactions with membrane bound proteins. They have also been found to contribute to several other events that lead to excessive calcium influx, reactive oxygen species production and neuronal nitric oxide synthase hyperactivation (Tu et al., 2014). Aβ oligomers also contribute to excessive glutamate levels in the synaptic cleft by interacting with and impairing glutamate uptake, as well as, inducing glutamate release by astrocytes. The elevated levels of glutamate in the synaptic cleft results in glutamate spillover allowing for the transmitter to interact with extrasynaptic glutamate receptors (Li et al., 2011). The activation of these receptors is linked to impairments of long term potentiation (LTP), the electrophysiological equivalence to memory. Further, excessive activation results in the influx of calcium (Ca^{2+}) which initiates a cascade of events ultimately resulting in cellular death (Hardingham and Bading, 2010). The importance the Aβ oligomer synaptic interaction in the maintenance of cognitive integrity is highlight by a group of individuals referred to as Non-Demented with Alzheimer's Neuropathology (NDAN). These

individuals have the presence of AD pathology equivalent to that normally found in moderately to severely demented AD patients but maintain cognitive function comparable to age match healthy individuals (Zolochevska and Taglialatela, 2016). Upon further investigation of these individuals, it was found that while they have ample levels of Aβ oligomers throughout the brain, the synaptic fraction contained little to no detectable Aβ oligomers, an area of typically elevated levels in AD (Bjorklund et al., 2012). These individuals can be further utilized in the identification of innate mechanisms that contribute to resistance against the toxic association of Aβ oligomers with the synaptic region.

Tau oligomers are suggested to induce similar synaptic deficits as previously observed by Aβ oligomers (Kopeikina et al., 2013), however, the mechanisms of impairment are not well understood. Alterations of dendritic morphology and reduction of synapses has been observed in mouse models of tau accumulation (Alldred et al., 2012) implying a direct effect of tau leading to synaptic impairment. Some studies suggest that the mislocalization of internal pathological tau interferes with the process of synaptic transmission by impairing the rate of vesicle release by interfering with the mobility of synaptic vesicles (Zhou et al., 2017). However, released tau oligomers may also contribute to synaptic impairments in the postsynaptic region. Indeed, the extracellular introduction of tau oligomers induces an increase of intracellular calcium levels in neuronal cultures (Usenovic et al., 2015) and resulted in impairment of LTP in cultured brain slices (Fá et al., 2016).

The combined presence of tau oligomers and Aβ oligomers at the synapses has led to a greater focus on gaining insight in the interaction between the two hallmark proteins of AD. Recently, a synergistic relationship between Aβ and tau oligomers was suggested to attribute to an exacerbation of synaptic dysfunction. An exacerbated impairment of LTP is observed after the application of a combination of suboptimal concentrations of Aβ and tau oligomers to brain slices. These results suggested an increased deficit induced by the co-presence of both proteins compared to the deficits induced by either of the amyloid oligomers proteins alone (Fá et al., 2016). In addition, the interference with mitochondrial dynamics by both proteins may be attributed to the reduction of

synaptic function, as discussed previously. The mechanisms contributing to this synergistic relationship are poorly understood. However, other interactions between Aβ and tau have been identified where the two proteins act concertedly. Specifically, it has been suggested that exogenous Aβ induced increased spine loss in explicit regions with elevated levels of missorted tau accumulations (Zempel et al., 2013). Further investigation is needed to understand the complex relationship between Aβ and tau oligomers in the induction of synaptic dysfunction. This intricate relationship suggests that future therapeutics should target the actions of both proteins to prevent loss of the synapses.

AD mouse models

Several transgenic animal models of AD have been developed to understand the pathological alterations that occur in the presence of the accumulation of both amyloid proteins and as a tool to investigate the reduction of such pathological events by potential therapeutics. Each mouse model has both positive and negative attributes and have been developed in attempt to create a better representation of AD as it occurs in humans. However, due to the genetic nature of these models, they best represent familial AD, the least prevalent form of AD, perhaps aiding in the difficult transition of therapeutics from mouse to humans. Nonetheless, these animal models have been invaluable in progressing the knowledge of AD and can be further utilized in new ways to continue expanding our understanding of the disease.

As a model of the Aβ pathogenesis, mutations in the APP processing pathway are utilized to promote Aβ accumulation. A few well characterized models introducing a mutated form of the human APP gene include the London mutation, Swedish mutation, and Florida mutation as outlined by figure 1.1. The Swedish mutation has an alteration in the 670 and 671 amino acids resulting in an increased affinity for β secretase cleavage, thus increasing the derivatives of the amyloidogenic pathway (Johnston et al., 1994; Haass et al., 1995). The London and Florida mutations are located near the γ secretase site, amino acid 717 and 716 respectively, and have been shown to promote cleavage at the

$A\beta_{42}$ site over $A\beta_{40}$ resulting in an increase in the highly aggregation prone $A\beta_{42}$ (Eckman et al., 1997; Muratore et al., 2014). Furthermore, the gene presenilin1, a unit of the γ secretase complex, has also been found to contain mutations that increase its activity resulting in elevated $A\beta_{42}$ levels (Citron et al., 1997). Along with the excess accumulations of Aβ in regions of the brain of interest in AD such as the hippocampus, these mouse models also display impairments in learning, working memory and declines in long term potentiation (Dineley et al., 2007a; Jung et al., 2011). AD mouse models have been developed to include one or a combination of these mutations resulting in various models that display progressive Aβ accumulation at various rates and ages (Oakley et al., 2006; Radde et al., 2006).

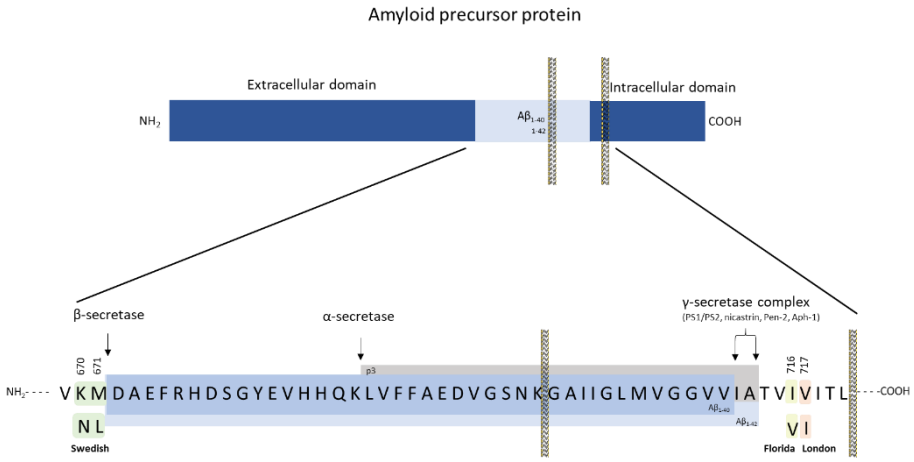


Figure 1.1 Amyloid precursor protein (APP) mutations.
A schematic diagram representing the sites of the three most commonly used APP mutation sites in the development of AD mouse models relative to cleavage sites of β and γ secretase.

Unlike mutations in the APP processing, tau mutations have not been found to be a factor in the development of familial AD (Roks et al., 1999). Other tauopathies such as frontotemporal dementia are associated with missense mutations in the tau gene (Spina et al., 2017). One such mutation, P301L, has been used to develop a mouse model that displays increases in the abnormal hyperphosphorylation of the tau protein (Alonso et al., 2004). The MAPT P301L mouse model has

been used as an AD model due to this increased tau accumulation. This mutation has also been combined with the Swedish APP and presenilin1 mutations to create a mouse model, 3xTgAD, that displays the co-presence of the Aβ and tau pathology (Oddo et al., 2003). This combination model provides the opportunity for greater insight into the development and relationship between the two proteins involved in AD and providing a complex model for the more accurate measurement of the effectiveness of therapeutics.

Current Pharmaceutical Therapies and Therapeutic limitations

Currently, two classes of pharmaceuticals are approved by the United States Food and Drug Administration (FDA) for patients diagnosed with AD. The first class are cholinesterase inhibitors which include the drugs Donepezil (Aricept), Rivastigmine (Exelon) and Galantamine (Razadyne) (Bond et al., 2012). These drugs inhibit the degradation of acetylcholine, thus increasing its concentration in the synaptic cleft, by halting the activity of the degrading enzyme cholinesterase (Colovic et al., 2013). Acetylcholine is an important neurotransmitter in the learning and memory processes, that has been found to be reduced by Aβ (Pedersen et al., 1996). The second class of drugs are N-methyl-D-aspartate (NMDA) receptor noncompetitive antagonist also known as, memantine (Namenda). This drug inhibits the activity of NMDA receptors, also referred to as glutamate receptors, specifically those located in the extrasynaptic region (Xia et al., 2010). As described previously, Aβ oligomers induce enhanced production of glutamate resulting in the hyperactivation of extrasynaptic glutamate receptors (Li et al., 2011). There have been many limitations identified in the efficacy of both classes of pharmaceuticals that have resulted in debates weighing the cost to benefits of prescribing these medications to AD patients. Both classes are aimed at reducing the manifested clinical symptoms of impaired cognitive function associated with AD that results in the reduced ability to perform daily tasks. However, since these drugs target downstream consequences of Aβ, as the disease progresses they are no longer able to compensate for the damage that is occurring in the system. Some individuals do experience cognitive improvements and increased quality of life

after the initiation of drug treatment, however, there is a large subset that display no cognitive improvements. In addition, these improvements are short lived, and the drugs quickly lose effectiveness as the disease progresses (Versijpt, 2014). In addition, many suffer adverse side effects ranging from headaches to gastrointestinal bleeding that limit the dose concentrations that can be tolerated (Kavirajan and Schneider, 2007; Casey et al., 2010; Kok et al., 2015). With the limitations of the current treatments, development of new disease modifying therapeutics is imperative.

Current research and clinical trials are being conducted on targeting multiple mechanisms to stop and reduce the pathological accumulations of the amyloid proteins. Several attempts have been made to develop Aβ target immunotherapies, both passive and active. Active immunotherapy involves the introduction of the Aβ peptide to stimulate the production of antibodies against the Aβ in attempt to clear the protein from the central nervous system. Passive, is the more commonly used for Aβ targeted therapy, involves the injection of exogenously produced antibodies (Röskam et al., 2010). Several antibodies have been developed that target different forms of Aβ. For example, Biogen's Aducanumab recognizes oligomer and fibril Aβ but not monomer forms, and Eli Lilly's Solanezumab recognizes Aβ monomers but not oligomer or fibrils (Doody et al., 2014; Sevigny et al., 2016). However, recently there has been a string of failed clinical trials involving Aβ targeting antibodies (Yiannopoulou and Papageorgiou, 2013) . Some studies resulted in minimal improvements that failed to reach statistical significance (Salloway et al., 2014). Other trials had to be terminated due to severe adverse side effects such as dangerous increases of neuroinflammation and other related abnormalities resulting in the swelling of the brain and small bleeds (Barakos et al., 2013; Doody et al., 2013). These results have shifted the focus to the importance of tau targeting or combination-based therapies. Several tau antibodies, specific for tau oligomers, are currently being developed and evaluated as the next step in the treatment of AD (Castillo-Carranza et al., 2015). However, some are concerned that adverse side effects will be induced by tau immunotherapies preventing the progression through clinical trials (Rozenstein-Tsalkovich et al., 2013). In addition, the importance of the interaction between Aβ and tau oligomers in the exacerbated dysfunction initiated by the co-

presence of the two proteins may suggest that the most effective future therapeutic for AD should target the actions of both amyloid proteins. Exploring the efficacy of alternative nonpharmaceutical treatments in the slowing of AD progression can provide valuable insights into safe options for the treatment of AD.

NEAR INFRARED LIGHT

Introduction

A novel noninvasive treatment option that has been proposed for patients with AD is the administration of transcranial near infrared (NIR) light, with wavelengths ranging from 600-1000 nm. The use of NIR light administration for medicinal purposes dates back to 1971 when Endre Mester observed the accelerated healing of wounds in mice that were exposed to a low energy Helium-Neon laser (Mester et al., 1971). Later the technique was applied to sores of bed ridden patients and a similar reduction of healing time was found (Mester et al., 1972). Since then, NIR light treatments have been investigated as a therapeutic for the reduction of pain and inflammation related to a variety of disorders including carpal tunnel syndrome (Chang et al., 2008), neck injuries (Chow et al., 2009), and arthritis (Lin et al., 2006). Most recently, the application of NIR light as a potential treatment has expanded to include brain related disorders and injuries such as, traumatic brain injury (Xuan et al., 2015), stroke (Oron et al., 2006) and Parkinson's disease (Johnstone et al., 2014). The 600-1000 nm wavelength window is an optimal window for the penetration of the light through biological material. The longer wavelength is minimally absorbed by chromophores found in water, hemoglobin and melanin allowing for the energy to diffuse deeper in tissue (Huang et al., 2009). Several studies have also measured the ability of this wavelength to move through dense tissue, such as bone and specifically skulls of several species of animals. They found approximately 40% of the light energy was transmitted through mouse skull depending on wavelength (Lapchak et al., 2015). In addition, the development of new modes of delivering light treatments such as, pulsed lasers and high-power light emitting diodes (LED) have contributed to improved penetration through dense tissue, increased

availability of devices and convenience of treatments. However, with these developments defining parameters of optimal effectiveness of NIR light is challenging. The wide array of injuries and diseases that benefit from NIR light administration has led to the extensive understanding of NIR light's mechanisms of action and potential other diseases, like AD, that can be improved by its administration.

Biological impact of NIR light associated with Alzheimer's disease

NIR LIGHT MECHANISMS

Several mechanisms that contribute to AD pathogenesis have been identified to be improved after NIR light treatment of various disease model systems. The primary mechanism of action of NIR light is believed to involve the activation of the enzyme cytochrome c oxidase in the electron transport chain located in the mitochondria (Tiphlova and Karu, 1991). Cytochrome c oxidase contains two copper and two heme iron centers that are optimally photostimulated between the wavelengths of 600-900 nm with absorption peaks at 606, between 613.5-623.5, 667.5-683.7, and 812.5-846 nm (Karu and Kolyakov, 2005). It has been proposed that this photostimulation increases activity level of the cytochrome c oxidase by releasing a nitric oxide block, thus promoting the establishment of the proton gradient by the electron transport chain resulting in an overall increased ATP production (Hashmi et al., 2010). In addition, a biphasic dose response is observed by NIR light suggesting that optimal beneficial effects occur at lower energy outputs and are diminished by high energy administration (Huang et al., 2009). Several studies have observed this increase in ATP levels (De Taboada et al., 2011), mitochondrial function (Lu et al., 2017) and nitric oxide levels (Mitchell and Mack, 2013) that support these collective event as a plausible primary mechanisms leading to the cellular protection afforded by NIR light. Multiple downstream responses can be further activated, such as transcription factors, that results in the upregulation of genes associated with stress protection. These precise responses that contribute to reduced sensitivity to toxins are not well understood. Studies utilizing AD models have found reduced proinflammatory markers (De Taboada et al., 2011),

increased chaperone expression levels (Grillo et al., 2013) and reduced impairment of mitochondria fission/fusion proteins (Lu et al., 2017). Although these studies also found a general reduction of Aβ pathology which may contribute to the reduction of these mechanisms. Further investigation to understand the downstream protective effects induced directly by NIR light treatment can result in the identification of other diseases that may be modulated by NIR light treatment.

AD PATHOLOGY

Many studies have been aimed at understanding the biological impact of NIR light on the development and toxicity of the Aβ and tau proteins. The NIR light treatment of both cellular and animal models have suggested an induction of a beneficial protective effect against the accumulation of these proteins. Aβ pathology particularly penetrating to the fibril form of Aβ, has been the main focus of these studies in the determining the effectiveness of the application of NIR light for AD. Four Aβ pathology transgenic mouse models containing different combinations of genetic mutations of APP and presenilin have been studied after NIR light treatment with various parameters of administration (differing in wavelengths, treatment schedule, source, energy density, mode and application sites) as described in Table 1. Three of these studies, initiated treatments at an age in which Aβ plaque formation is abundant, equivalent to later stages of the clinical manifestation of AD. Despite the different parameters applied in these studies, all found decreased Aβ levels after the given treatments (De Taboada et al., 2011; Purushothuman et al., 2014; Farfara et al., 2015) . On the other hand, Grillio et al. investigated an early but chronic administration of the treatment, initiating a 5-month long treatment schedule prior to known Aβ accumulation. Treatments were concluded one month after known plaque deposition is prominent. This study found a similar decrease of Aβ levels (Grillo et al., 2013).

Other animal models of AD have also been used to study direct effects of NIR light treatment on Aβ levels and induced dysfunction, as summarized in Table 2. In two separate studies rat models injected with exogenous Aβ were treated with differing NIR light protocols. The first study found

reduced Aβ levels after treatment once per day with a 627 nm LED for 14 and 21 days. However, no changes in Aβ levels were observed in the rats receiving 7 days of NIR light treatment suggesting a mechanism of clearance of Aβ proteins is dose dependent (da Luz Eltchechem et al., 2017). The second study administered NIR light treatments once per day by an 808 nm laser for 5 days. This study also did not find a statistically significant reduction in Aβ levels, but other mechanisms linked to Aβ toxicity, such as dendritic degeneration, oxidative damage, and proinflammatory cytokines, were decreased in NIR light treated rats. In addition, a decrease in endogenous hyperphosphorylated tau in the NIR light treated exogenous Aβ rats (Lu et al., 2017) was observed. Together, these studies support an overall improvement in Aβ burden (NIR light treatments) after pathology development by NIR light treatments.

Several experiments have also used a variety of cell lines exposed to Aβ before or after NIR light treatments to determine induction of neuroprotection against the toxicity of Aβ, as summarized in Table 3. Similar to experiments in animal models, all experiments differed in cell lines used, treatment schedule, source of the light, wavelengths, energy output and light mode. Studies investigating NIR light treatment administered after Aβ exposure found decreases in DNA fragmentation (Duan et al., 2003), decreased Aβ aggregates (Sommer et al., 2012) increased cell survival (Meng et al., 2013), among other parameters. This suggests NIR light induces neuroprotection against Aβ toxicity. In addition, three studies investigated the beneficial effects of NIR light pre-treatment (Yang et al., 2010; Duggett and Chazot, 2014; Farfara et al., 2015). In these studies, the cells were treated with NIR light prior to Aβ protein exposure. They found decreases in oxidative stress, proinflammatory markers (Yang et al., 2010), cell death (Duggett and Chazot, 2014) and increased phagocytosis of Aβ (Farfara et al., 2015). This suggests that early treatments, administered prior to toxic insult, induce mechanisms of protection.

Only one human mutated tau model has been used to determine the direct effect of NIR light on the accumulation of tau pathology. Near infrared light treated K3 mice, a mouse model with a human tau mutation found in frontotemporal dementia that accumulate hyperphosphorylated tau at 3

months of age (Ittner et al., 2008). After one month of NIR light treatments, these mice displayed a reduction of hyperphosphorylated tau and neurofibrillary tangles (Purushothuman et al., 2014). Further, reduced hyperphosphorylated tau levels were measured in two mouse models of Aβ pathology, however, differences in isoforms and alterations amino acids at the N-terminus in murine tau compared to human tau, raises the question of translatability of the possible effects to human AD (Andorfer et al., 2003). Many studies have also been conducted on TBI models, finding reductions in injury sites and inflammation markers (Huang et al., 2012; Xuan et al., 2014, 2015). While these study do not directly investigate tau levels, traumatic brain injury is linked to the production of tau oligomers that contribute to cellular death after injury (Hawkins et al., 2013).

COGNITION

Many of the previously described animal studies investigating the pathology development in AD mouse models after NIR light treatment also measured the coinciding functional behavioral changes in these animals. It was found that these animals have increased cognitive functions after NIR light treatment as tested by several behavioral tests including the novel object recognition test, Morris water maze and fear conditioning (De Taboada et al., 2011; Farfara et al., 2015; da Luz Eltchechem et al., 2017; Lu et al., 2017). These tests are common paradigms used to determine deficits in the hippocampal based learning and memory processes which become impaired during the progression of AD (Puzzo et al., 2014; Wolf et al., 2016). The enhancement of cognition in these AD mouse models suggests that NIR light treatment may improve functional deficits in AD patients.

Limited studies have been conducted in human patients diagnosed with AD or mild cognitive impairment (MCI) to determine if NIR light treatments improve cognition. To date, two studies have been published using differing devices for light delivery. One study used the Cognitolite Transcranial Photomodulation System which is a light helmet with 1060-1080 nm LEDs pulsed at 10 hertz (hz). One 6-minute treatment was administered daily for 28 days. The individuals were tested by the mini-mental state examination (MMSE) and the Alzheimer's Disease Assessment Scale-Cognitive

(ADAS-Cog) tests prior to and at the conclusion of the treatment regimen. A statistical difference was not measured in either tests compared to baseline test scores, however, a trend of improved scores was noted in tasks of executive function. This study had a limited number of participants (6 NIR treated, 3 placebo, and 2 withdrawn). The authors believed that statistical significance might be reached with increased group sizes (Berman et al., 2017). The second study used Vielight Inc.'s "intranasal" and "Neuro" devices that consist of 810 nm LEDs pulsed at 10 hz. The "intranasal" device is a single probe placed in the nose and the "Neuro" device is a headset consisting of four clusters of LEDs. Treatment included stimulation by the "Neuro" device twice per week, in addition to one daily 25-minute treatment by the "intranasal" device for 12 weeks. The study found a statistical improvement in MMSE and ADAS-Cog scores at the conclusion of the 12-week treatment compared to baseline scores. Other emotional improvements were also reported by the caretakers such as reduced aggression. It was also found that these scores rapidly declined within 4 weeks of withdrawing NIR light treatments (Saltmarche et al., 2017). Both studies had no reported side effects of the NIR light treatments. While this study provides promising evidence that NIR light enhances cognition in AD patients, further research must be conducted due to the small treatment group (5 individuals) and lack of a placebo group. There are currently two studies listed on clinicaltrials.gov for the investigation of NIR light treatments in human AD patients. Both are using Vielight Inc.'s "Neuro" devices, one to determine changes in AD biomarkers while the other is to replicate the previous results seen by Salmarche et al. in an expanded group size.

While these results are promising, as mentioned earlier future studies need to expand the number of patients receiving NIR light treatment, as ongoing trials are attempting, but also provide proper placebo groups. Further, it is imperative to determine long term changes in cognition by NIR light treatment to gain an understanding of the efficacy of treatment in a chronic disease such as AD.

Table 1.1. NIR light treated Transgenic AD Models

Transgenic AD Models									
Model	Treatment initiation timeline	Treatment schedule	Source	Wave length	Energy density	Mode	Application site	Results	Study
APP mouse Human APP Swedish and London mutation	3 months old (After plaque accumulation)	2 mins per treatment 3x/week for 2 weeks	GaAIAs diode Laser	808 nm	CW; 1.2 J/cm^2 Pulsed; 1.2 J/cm^2 6 J/cm^2 12 J/cm^2	CW and Pulsed pulse duration = 2 ms rate=100 Hz	Trans-cranial	All parameters: ↓Aβ levels in CSF, plasma, CNS ↑memory ↓ inflammation markers (greatest change by Pulsed 2830 mW/cm^2) Pulsed 2830 mW/cm^2: ↑ATP levels ↑O_2 consumption	(De Taboada et al., 2011)
TASTPM mouse Human APP Swedish and PSEN1 M146V	2 months old (prior to Aβ accumulation)	6 mins per treatment 2 days biweekly for 5 months	LED	1072 nm	1.8 J/cm^2 5 mW/ cm^2	Pulsed 600 HZ Cycle 300 µs	Body	↑HSP27.HSP60, HSP70, HSP105 and P-HSP27 No change HSP40, HSP90 ↓phosphorylated tau ↓αB-crystallin, APP, $Aβ_{1-40}$, and $Aβ_{1-42}$ protein expression ↓PS-1 (independent of modifying transgene promotor)	(Grillo et al., 2013)
APP/PS1 mouse Human APP Swedish and PSEN1dE9	7 months old (After plaque accumulation)	90 s per treatment 5 days/week for 4 weeks	LED	670 nm	4 J/cm^2	CW	Trans-cranial	↓Aβ plaque burden, size and number	(Purushot human et al., 2014, 2015)
5xFAD mouse Tg6799; Human APP Swedish, Florida and London mutations PSEN1 M146L and L286V mutations	4 months old (After plaque accumulation)	Total of 6 treatments (10-day intervals over 2 months)	Ga-Al-As Laser	ns	1 J/cm^2 400 mW/ cm^2	CW	Bone marrow in medial tibia	↑memory (fear conditioning, NOR test) ↓Aβ burden (dentate gyrus of hippocampus	(Farfara et al., 2015)
K3 mouse tau with K369I mutation	5 months old (After hyperphosphorylated tau accumulation)	90 s per treatment 5 days/week for 4 weeks	LED	670 nm	4 J/cm^2	CW	Trans-cranial	↓phosphorylated tau and neurofibrillary tangles (neocortex and hippocampus) ↓oxidative stress (neocortex) ↑CCO expression (neocortex and hippocampus)	(Purushot human et al., 2014, 2015)

CW= continuous wave
LED= light emitting diode

Table 1.2. NIR light treated Nontransgenic AD Models

Nontransgenic AD Models									
Model	Treatment initiation timeline	Treatment schedule	Source	Wave length	Energy density	Mode	Application site	Results	Study
injected $A\beta_{25-35}$ rat	Intracerebral injection of $A\beta_{25-35}$ (fibrils) followed by light treatment	1 treatment of 200s for 7 days, 14 days or 21 days	LED	627 nm	7 J/cm^2 70mW	CW	Transcranial	14-day treatment: ↑spatial memory, OFT motor skills 21-day treatment: ↓Aβ levels, ↑OFT motor skills	(da Luz Eltchechem et al., 2017)
injected $A\beta_{1-42}$ rat	Intrahippocampal injection of $A\beta_{1-42}$ three hours later laser treatment began	1 treatment of 2 min/day for 5 days	Laser	808 nm	15 J/cm^2 25 mW/ cm^2	CW	Transcranial	↓dendritic degeneration (hippocampal CA1) ↓Mitochondrial fission/fusion imbalance, mitochondrial fractionation, depolarization, oxidation ↑ CCO activity, ATP ↓ G6PDH and NADPH oxidase activity, oxidative damage ↑ total antioxidant capacity ↓ gliosis, proinflammatory cytokines ↓ hyperphosphorylated tau ↓ cytosolic cytochrome c levels, caspase-9 and 3 activities ↑ spatial learning and memory	(Lu et al., 2017)

CW= continuous wave
LED= light emitting diode

Table 1.3. NIR light treated *in vitro* AD models

in vitro AD Models								
Model	Treatment initiation timeline	Treatment schedule	Source	Wave length	Energy output	Mode	Results	Study
Undifferentiated PC12 cells incubated with $A\beta_{25-35}$	$A\beta_{25-35}$ (20 μM) added to cells followed by light treatment	1 treatment of 30 mins or 60 mins	LED	640 nm	0.9-10 W/cm^2	CW	0.9W/cm2 for 60 minutes: ↓ cell apoptosis, DNA fragmentation	(Duan et al., 2003)
Primary astrocytes incubated with $A\beta_{1-42}$	Laser treatment followed by 4 hr rest, and 4 hr starvation period, then incubated with $A\beta_{1-42}$ (5 μM) for 2 hrs	1 treatment of 3 hrs	He-Ne Laser	632.8nm	16.2 J/cm^2 1.5 mW/cm^2	CW	↓oxidative stress ↓superoxide anions, NADPH oxidase ↓phosphorylation of cPLA2 ↓proinflammation markers (IL-1β and iNOS)	(Yang et al., 2010)
Human neuroblastoma (SH-EP) cells incubated with $A\beta_{1-42}$	Incubated with labelled $A\beta_{42}$ (200 nM) for 24 hrs prior to laser treatment	1 treatment of 1 min	Laser	670 nm	1 J/cm^2 17.36 mW/cm^2	Pulsed 1 Hz	↓Aβ aggregates ↑cell proliferation ↑ATP levels in light treated cells w/o Aβ	(Sommer et al., 2012)
SH-SY5Y, HEK 293T and differentiated PC12 cells incubated with $A\beta_{25-35}$	$A\beta_{25-35}$ (fibrils; 25 μM) added to media 30 mins prior to laser treatment	1 treatment of 2.5 mins	Laser	632.8 nm	2 J/cm^2 12.75 mW/cm^2	CW	SY5Y, HEK 293T and differentiated PC12 cells: ↓cell apoptosis ↑Akt/GSK3β/β-catenin pathway	(Liang et al., 2012)
Primary hippocampal neurons or SH-SY5Y incubated with $A\beta_{1-42}$ and $A\beta_{25-35}$	Aβ (25 μM) added to media 30 mins before laser treatment	1 treatment of 0.7, 1.25, 2.5 or 5 mins	He-Ne Laser	632.8 nm	0.5,1,2, and 4 J/cm^2 12.75 mW/cm^2	CW	2 J/cm^2: ↑cell survival, dendrite growth ↑activation of ERK/CREB pathway resulting in increased BDNF levels	(Meng et al., 2013)
Cath.a-differentiated cells incubated with $A\beta_{1-42}$	1st set of light treatments 24 hrs later incubated with $A\beta_{1-42}$ (fibrils) followed by 2nd set of light treatments, 24 hours later 3rd set of light treatment	5 treatments of 3 mins, 30 mins apart/day for 3 days	LED	1068 nm	5 mW/cm^2	Pulsed 600 Hz Cycle 300 μs	↓ cell death (3.5 μM, 4.5 μM, 5 μM, 10 μM and 25 μM of Aβ)	(Duggett and Chazot, 2014)
WT mice mesenchymal stem cells incubated with $A\beta_{1-42}$	MSC isolated, treated with laser 3 days later incubated with Aβ (12 μM) for 2 hrs	1 treatment of 20 s	Ga-Al-As laser	ns	1 J/cm^2 400 mW/cm^2	CW	↑phagocytosis of Aβ ↑CD11b	(Farfara et al., 2015)

CW= continuous wave
LED= light emitting diode

Gaps of knowledge

The expansion of NIR light treatment into human AD patients will be a challenging task due to the lack of acceptance and general knowledge of the technique in the medical field. While previous research suggests an overall reduction in Aβ and hyperphosphorylated tau accumulations in AD mouse models treated with NIR light, the underlying mechanisms are not well understood. In addition, previous experience with numerous pharmaceuticals that reduce pathology in AD mouse models but results in little cognitive change in human trials suggests that the observation of decreased protein accumulations may not be the optimal marker for effectiveness of the treatment in AD (Wilcox et al., 2011). The ultimate goal of determining the effectiveness of NIR light treatment for human AD patients will only truly be achieved by the pursuit of clinical trials. However, the clinical trial process is lengthy and often impaired by the expense and the overall challenge of recruiting a large cohort of individuals. The funding and recruitment process can be aided by the investigation of gaps of knowledge of NIR light administration and its modulation of key events leading to the cognitive decline associated with AD.

An important event that needs to be further understood is the ability of NIR light to modulate the association of Aβ and tau oligomers with the synapses and the resulting oligomer induced synaptic dysfunction. This can be achieved by utilizing the *ex vivo* administration of Aβ and tau oligomers to the synapses and brain slices of previously NIR light treated wild type mice. Further the wild type mice can be used to investigate proteomic and mitochondrial changes to discern the direct mechanisms that are induced by NIR light treatment. Previous studies have investigated alterations in the total mitochondrial dynamics in NIR light treated AD mice models, however, the parallel reduction of AD pathology could contribute to the changes observed.

Another aspect of AD that should be investigated to understand the effectiveness of NIR light treatments is the levels of synaptic Aβ and tau oligomers in a combined AD mouse model that integrate the human forms of both proteins. Previous research has suggested a decrease in the hyperphosphorylated tau in Aβ mouse models, however, these models do not express human tau

which include different isoforms compared to mouse tau, a factor that may change functionality of the tau accumulations (Andorfer et al., 2003). A combined transgenic model will better represent the effects of NIR light exposure on the amyloid proteins in a co-present system as seen in AD.

In addition, the identification of mechanisms induced by NIR light that contribute to the reduction of pathology that was observed previously will be imperative to understanding the benefits of NIR light. While mitochondria are believed to be the primary target of the cytochrome c oxidase activating NIR light, the link between increased mitochondria activity and reduced AD pathology is not well understood. Further, the health of the synaptic mitochondria has not been investigated. This insight will provide a clearer understanding on the health of the synapses and induced resilience to toxic insults after NIR light treatment.

The current project is aimed at determining if the damaging pathology that is associated with early AD development, specifically at the synapses, is modulated by treatments of NIR light. Additionally, we aim to gain a greater understanding of the implementation of NIR light in a combined Aβ and tau system and potential neuroprotective mechanisms induced by NIR light treatment. Together the current project aims to further the scientific understanding and acceptance of NIR light treatment as a viable therapeutic for AD.

Chapter 2: Methods

ANIMALS

Male and female transgenic 2576 (Tg2576) and htau mice and female 3xTgAD mice were utilized to evaluate *in vivo* Aβ and tau load at the synapses (n= 7, per experimental group). The NIR light treatment was initiated at 7 months of age for the Tg2576 mice, an age in which Aβ oligomers are abundantly found in the brain (Hsiao et al., 1996). The NIR light treatment was initiated at 12 months for the htau mice, an age in which tau pathology has developed throughout the brain (Duff et al., 2000). The 3xTgAD mice, which display Aβ and tau accumulations at 12 months of age, were treated with NIR light starting at 12 months of age to ensure development of both protein accumulations (Oddo et al., 2003). C57BL/6 wild-type male and female mice were employed for the *ex vivo* Aβ binding experiments (n=8, per experimental group), as well, as the electrophysiological properties after NIR light treatments (n=5, per experimental group, 2 slices per animal per experimental condition). Male and female Tg2576 and wild type mice were utilized for synaptic mitochondrial experiments (n=8, per group). All experimental protocols involving animals were approved by Institutional Animal Care and Use Committee of the University of Texas Medical Branch. All methods were performed in accordance with the guidelines and regulations of the committee. Animals were housed under USDA standards (12:12 hour light dark cycle, food and water *ad libitum*) at the UTMB vivarium. After the conclusion of the NIR light treatment, the mice were sacrificed by exposure to isoflurane and decapitated. The brains were quickly removed, dissected into major regions; frontal cortex, parieto-occipital cortex, hippocampus and cerebellum and stored at -80°C until ready for further analysis.

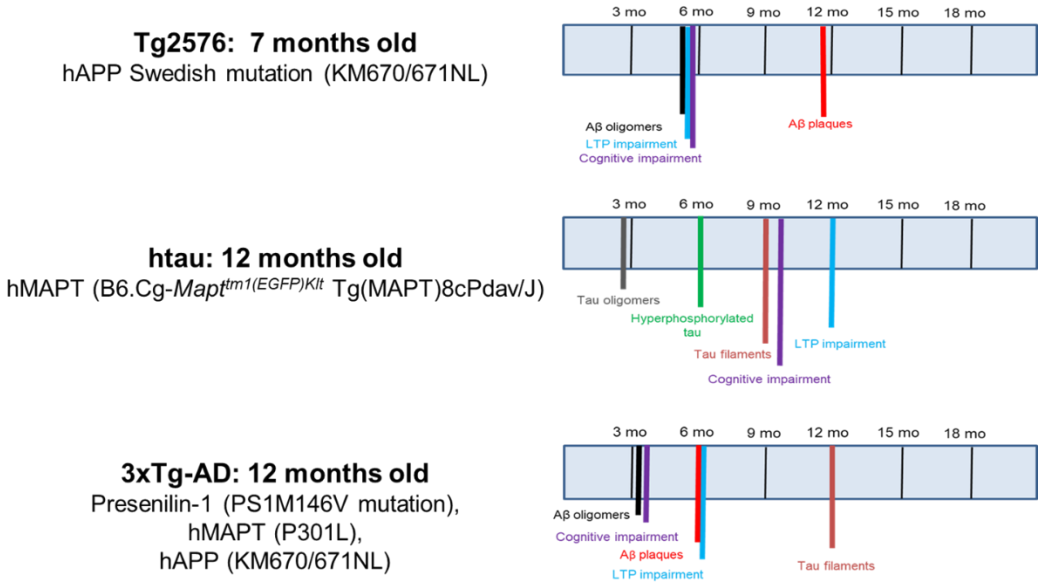


Figure 2.2. Timeline of pathology progression in AD mouse models.
A schematic diagram demonstrating the characteristic ages of protein accumulations and cognitive impairments of the three AD mouse models used in the NIR light treatment study. Figure adapted from the Alzheimer's Association.

NIR LIGHT TREATMENTS

One dose of the NIR light treatment consisted of a 90 second treatment from a 670 nm light-emitting diode (LED) device (WARP 10; Quantum Devices, Barneveld, WI, USA). Light energy emitted equated to 4 Joule/cm^2 per treatment. The treatment group received one dose per day, 5 days per week for 4 consecutive weeks. The mice were hand restrained and the light device was held approximately 1 cm from the top of the head. The body of the mouse was covered with aluminum foil to prevent light exposure to the periphery, as illustrated in Figure 2.1. The control sham light treated group was restrained in the same manner as the treatment group, however the light device remained off, as previously described (Purushothuman et al., 2014). The NIR light treatment was condensed to one week (4 treatments per day for 5 days) for wt mice treated for the electrophysiology experiment after it was determined that this condensed time period provided similar reduced binding effects as the 4-week treatment schedule (Supplementary Fig. 3.3.).

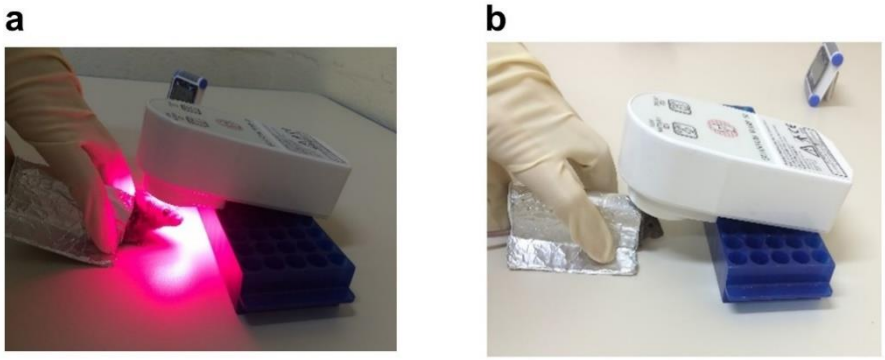


Figure 2.1. Near infrared light treatment procedure.
The mice were treated with NIR light from a 670 nm light-emitting diode (LED) device. (a) The device was held approximately 1 cm above the head of the mouse while aluminum foil was held over the body to minimize NIR light exposure to the periphery. The mice received 1- 90 second treatment per day for 5 days in a week over 4 consecutive weeks. (b) The control sham treatment group was held under the NIR light device in the same manner as the NIR light treated group with the device remaining off.

ISOLATION OF SYNAPTOSOMES

After the final NIR light treatment, the animals were sacrificed, and the brains collected as described above. To isolate synaptosomes, the tissue was homogenized in Syn-PER synaptic protein extraction reagent (ThermoFisher). A portion of the total homogenate was saved for biochemical analysis and the remaining portion was centrifuged at 1200 x g for 10 minutes at 4°C. The supernatant was collected and centrifuged further at 15000 x g for 20 minutes at 4°C, as per reagent instructions. The synaptosomes containing pellet were resuspended in HEPES-buffered Krebs-like (HBK) buffer or radioimmunoprecipitation assay (RIPA) buffer depending on future experiment. The quality of the synaptosomal isolation is routinely verified by Western blot and electron microscopy (Franklin and Taglialatela, 2016).

MEASUREMENT OF PROTEIN LEVELS

Western blot analysis

Western blot analysis was performed on the total protein extracts and synaptosome fractions to measure multiple protein levels. Separation of the proteins in the samples obtained was done by 12% gradient SDS- polyacrylamide gel (HSP70 proteins) or 4-20% gradient gel (Aβ, tau5, LC3A&B)

electrophoresis. The separated proteins were transferred to a nitrocellulose membrane (Bio-Rad) and incubated with the specific antibody such as 6E10 (total Aβ; BioLegend), Tau5 (total tau; ThermoFisher), HSP70/HSP72 and HSC70 (Enzo Life Sciences), LC3A&B (Cell Signaling) antibody overnight. The nitrocellulose membrane was then incubated with the appropriate fluorescent secondary antibody and imaged with an Odyssey infrared imager. The band densities were analyzed using Image J software, normalizing using the densities of the loading control obtained by reprobing the membranes for β-tubulin.

ELISA

Quantification of Aβ levels in the protein extracts was determined using an $A\beta_{1-42}$ specific solid phase sandwich enzyme-linked immunosorbent assay kit as described by kit directions (ELISA) (Invitrogen). The total tau levels were measured by ELISA analysis using the total tau antibody, tau5 (ThermoFisher). For the ELISA, samples were incubated at 4°C overnight on an ELISA plate with the coating buffer 0.1M sodium bicarbonate (pH 9.6). The plates were then washed with Tris-buffered saline with low Tween 20 (0.01%) (TBS-low T) followed by blocking with 10% nonfat milk. The plates received another washing step followed by an incubation with tau5 antibody (1:1000 in 5% nonfat milk in TBS-low T; ThermoFisher) for 1 hour at room temperature. Following a washing step, horseradish peroxidase-conjugated anti-rabbit IgG (1:10,000 in 5% nonfat milk in TBS-low T; Promega) was added to the plate and incubated for 1 hour at room temperature. The plates were again washed with TBS-low T and 3,3,5,5-tetramethylbenzidine (TMB-1 component substrate; Sigma-Aldrich) was added. After 15 minutes, 1 M HCl was added to stop the reaction and the plate was read at 450 nm.

Immunofluorescence

Immunofluorescence was performed on post fixed (4% paraformaldehyde in 0.01 M PBS, pH 7.4) cryosectioned brain slices of the 3xTgAD and htau mice that received NIR light or sham treatment. First the slices were washed in phosphate-buffered saline (PBS) followed by permeabilized

with 5% normal goat serum, 0.3% Triton X-100 and 0.05% Tween-20 in PBS for 1 hour at room temperature. After a wash with PBS, the slides were incubated overnight at 4°C with primary antibodies. The primary antibodies used were the tau oligomer specific antibody, anti-T22 (1:500; Dr. Rakez Kayed lab) and the total tau antibody, anti-tau 5 (1:1000, ThermoFisher). The slices were then washed with PBS and incubated with Alexa-conjugated secondary antibodies (1:400; Life Technologies) for 1 hour at room temperature. Finally, the slices were washed in PBS and coverslips were mounted using Vectashield mounting medium containing DAPI (Vector Laboratories).

MEASUREMENT OF GENE EXPRESSION LEVELS

Total RNA was isolated from the hippocampus of the sham (n=7) and NIR light treated (n=9) 3xTgAD mice utilizing the RNA isolation kit (Qiagen). Quantitative polymerase chain reaction (qPCR) was conducted to determine mRNA levels of Atg5, as previously described (Ali et al., 2016). The fold expression was calculated relative to the beta-actin gene.

EX VIVO AMYLOID B OLIGOMER BINDING

Aβ oligomer preparation

Human Aβ oligomers were prepared from lyophilized synthetic Aβ aliquots (0.3 mg) dissolved in 0.2 ml of 1,1,1,3,3,3- Hexafluro-2-propanol (HFP). The HFP- Aβ mixture was then added to 0.7 ml of H_2O. A cap with four holes was placed on the tube and the sample was stirred by a magnetic stir bar under a fume hood for 48 hours. The sample was used immediately after the 48 hours of stirring (Kayed, 2003). For the flow cytometry analysis of Aβ oligomer binding to the synaptosomes, Aβ oligomers with a Flour 488 tag were utilized. These Aβ oligomers were prepared by adding $Aβ_{1-42}$ peptide with a Flour 488 tag (AnaSpec, Inc) to the HFP-Aβ mixture described above. This mixture was then added to 0.7 ml of H_2O and spun, as described. Western blot and dot blot analysis using A-11 antibodies (Aβ oligomer specific) are used to determine the quality of oligomerization (previously described by Reese (2008) (Reese et al., 2008).

AB OLIGOMER BINDING CHALLENGE

The *ex vivo* binding of Aβ oligomers to the synaptosomes of mice treated or not with NIR light was evaluated using flow cytometry. Synaptosomal fractions of male and female wild type mice receiving NIR light treatment were prepared and resuspended in HBK buffer. Synaptosomal fractions from multiple animals in each group were combined in separate tubes. Pooled samples were then aliquoted into 8 separate tubes per group containing 100 mg of total protein per tube determined by bicinchoninic acid (BCA) assay. To perform analysis using flow cytometry, the synaptosomes were prepared and aliquoted as described in the ELISA analysis, however, the synaptosomes were treated for 60 minutes with Aβ oligomers tagged with HyLite Fluor 488 (AnaSpec) in various concentrations ranging from 1 μM to 10 μM. The synaptosomes were then centrifuged and washed multiple times with HBK buffer and resuspended in PBS. Synaptosomes were analyzed by size gating using size standard beads (Spherotech, Inc.) Gylys et al. (2004) (Gylys et al., 2004). Data was acquired by a Guava EasyCyte flow cytometer (EMD Millipore) and analyzed using Incyte software (EMD Millipore).

EX VIVO TAU OLIGOMER BINDING

Tau oligomer preparation

Prepared recombinant tau oligomers were obtained from Dr. Rakez Kayed's laboratory. The tau oligomers were produced as previously described (Lasagna-Reeves et al., 2010). Briefly, recombinant tau monomer protein was added to 1xPBS to obtain a concentration of 0.3 mg/ml. $A\beta_{42}$ oligomers seeds were added to the tau mixture and incubated on an orbital shaker for 1 hour at room temperature. The produced tau oligomers were used as seeds in a new batch of tau monomers to produce a new batch of tau oligomers. This protocol was repeated three times to ensure the elimination of the original Aβ seeds resulting in the production of tau oligomers. Each batch of oligomers is tested using dot blot with T22, a tau oligomer specific antibody, Western blot analysis and atomic force microscopy (AFM) to verify the quality of the tau oligomer preparation.

Tau oligomer challenge

Synaptosomes isolated from mice receiving NIR light or sham treatment were resuspended in HBK buffer. Using the bicinchoninic acid (BCA) assay, 50 mg of total protein in our synaptosomal preparations was determined and aliquoted from each individual animal. 50 nM of tau oligomers was then added to each synaptosome preparation and allowed to incubate for 1 hour at room temperature. The samples were then centrifuged and washed with HBK buffer three times to thoroughly remove any unbound tau oligomers. The total protein levels were again measured by BCA and equal amounts of protein was analyzed by tau5 ELISA analysis, as described above.

ELECTROPHYSIOLOGY

The wt mice were anesthetized with isoflurane and were perfused intracardially with a NMDG solution containing; 93 mM NMDG, 2.5 mM KCl, 1.2 mM NaH_2PO_4, 30 mM $NaHCO_3$, 20 mM HEPES, 25 mM glucose, 5 M sodium ascorbate, 2 mM thiourea, 3 mM sodium pyruvate, 10 mM $MagSO_4 \cdot 7H_20$, 0.5 mM $CaCl_2 \cdot 2H_2O$, and 12 mM N-acetyl L-Cysteine. Brains were harvested and sliced in the NMDG solution followed by a 10-minute recovery period in 35°C NMDG solution. The slices were then maintained in a modified HEPES holding aCSF (92 mM NaCl, 2.5 mM KCl, 1.2 NaH_2PO_4, 30 mM $NaHCO_3$, 20 mM HEPES, 25 mM Glucose, 5 mM sodium ascorbate, 2 mM thiourea, 3 mM sodium pyruvate, 2 mM $MgSO_4$ $7H_20$, 2 $CaCl_2$ $2H_20$, 12 N-Acetyl L-Cysteine). For recording the slices where held in an artificial cerebrospinal fluid (aCSF) (124 mM NaCl, 2.5 mM KCl, 1.2 mM NaH_2PO_4, 24 mM $NaHCO_3$, 5 mM HEPES, 12.5 mM glucose, 2mM $MgSO_4 \cdot 7H_20$ and 2 mM $CaCl_2 \cdot 2H_20$) (Ting, 2014). All solutions were supplemented with 95% O_2/5% CO_2. Prior to recording, the slices were incubated with 200 nM of Aβ oligomers for 1 hour at room temperature. Field excitatory post-synaptic potentials (fEPSPs) recordings were performed by stimulating the Schaffer collateral pathway. The stimulating electrode of 22 kΩ resistance was placed in the cornu ammonis 3 (CA3) region and the recording electrode was located at the junction of the alveus and cornu ammonis 1 (CA1). High frequency stimulation consisting of 3- 100 Hz trains for 1 second duration with 20 second intertrain intervals was be used to induce LTP. The traces were analyzed with Clampfit 10.6 software (Molecular Devices).

SYNAPTIC MITOCHONDRIAL MEMBRANE POTENTIAL AND ABUNDANCE

The synaptosomes were isolated from frozen brain tissue as described earlier. The synaptosomes were then immediately treated with Mitotracker green FM (Invitrogen) and MitoSense Red (1,1',3,3,3',3'- Hexamethylindodicarbocyanine iodide) (EMD Millipore) for 15 minutes at 37°C. MitoTracker is a fluorescent dye that diffuses across the mitochondrial membrane and reacts with thiol groups of specific mitochondrial proteins (Presley et al., 2003). The fluorescent dye MitoSense correlates with mitochondria membrane potential (Mattiasson et al., 2003). The synaptosomes were washed twice with HBK buffer and fluorescent emittance was acquired by a Guava EasyCyte flow cytometer (EMD Millipore) and analyzed using Incyte software (EMD Millipore).

NOVEL OBJECT RECOGNITION

After the conclusion of NIR light treatment regimen, the htau mice underwent cognitive testing using the novel object recognition (NOR) paradigm. The novel object recognition protocol included three phases; habituation phase, a training phase and an object recognition phase, as described previously by our lab (Taglialatela, 2009). The habitation phase included of two sessions, on two different day each 10 minutes in length, in which the animals were allowed to freely explore the open field arena. During the training phase, the animals were placed in the same arena with the addition of two identical objects. The animals were allowed to freely explore for 10 minutes. 24 hours after the training phase, the test phase was initiated. During the testing phase, the animal was placed again in the arena for 10 min with one familiar object previously explored in the training phase and one novel object differing in color and shape, but sharing a common size and volume. Trials were recorded and time spent exploring each object was measured using ANY-Maze software. Exploration was defined by head orientation within 2 cm of the object or physical contact with the object. The object discrimination ratio (ODR) was calculated by the following formula:

ODR= (Time exploring specified object)/ (time exploring novel object+ time exploring familiar object)

STATISTICAL ANALYSIS

The statistical analysis of the data was analyzed using SPSS software. Student's t test was used to determine statistical significance in the protein level experiments. The calculated object discrimination ratios of the NOR behavior test were analyzed by the one-sample t test to determine difference from chance (0.50). The one-way ANOVA with Dunn's post hoc test was used to determine statistical significance between the calculated fEPSP percentage of each condition in the electrophysiology experiment.

Chapter 3: Near infrared light decreases synaptic vulnerability to amyloid beta oligomers[1]

INTRODUCTION

Alzheimer's disease (AD) is the most common and severe age-associated neurodegenerative disorder for which there is currently no effective therapeutic intervention. One of the key events contributing to the development of the cognitive decline that marks the clinical profile of AD is the selective targeting and disruption of the synapses by small, soluble amyloid beta (Aβ) oligomers, the most toxic form of the Aβ protein. Aβ oligomers binding to synapses have been shown to induce many morphological and physiological changes that collectively lead to the loss of cognitive integrity including retraction of synaptic spines (Shankar et al., 2007; M. Vargas et al., 2014), decreased pCREB and increased calcineurin protein levels (Dineley et al., 2010) and reduced long term potentiation (LTP) initiation (Walsh et al., 2002; Li et al., 2011; Fá et al., 2016). Furthermore, we recently reported a group of individuals referred to as **N**on- **D**emented with **A**lzheimer's **N**europathology (NDAN) that provides evidence supporting the correlation between the presence of Aβ oligomers at the synapses and the retention of cognitive function. NDAN individuals maintain their cognitive integrity despite the presence of Aβ plaques and neurofibrillary tangles at an extent comparable to demented AD patients. By comparing these individuals to AD patients, one can infer properties that may protect these individuals from the cognitive dysfunction normally associated with AD pathology. We showed that while NDAN individuals displayed similar levels of soluble Aβ oligomers throughout their CNS, contrary to demented AD patients, they had synapses that were devoid of Aβ oligomers (Bjorklund et al., 2012). This suggests the possibility that synapses of NDAN subjects are resistant to Aβ oligomers and illustrates that absence of Aβ at synapse is a key event

[1] Previously published in Scientific Reports: Comerota MM, Krishnan B, Taglialatela G (2017) Near infrared light decreases synaptic vulnerability to amyloid beta oligomers. Scientific Reports 7 Available at: http://www.nature.com/articles/s41598-017-15357-x.

associated with preservation of cognitive integrity. Taken together this evidence suggests that eliciting a protective mechanism resulting in synaptic resilience to binding of Aβ oligomers similar to NDAN individuals would be the most effective protection against Aβ oligomer driven toxic synaptic dysfunction. With this goal in mind, in this chapter we investigated the effect of a treatment with near infrared light (NIR, 600 to 1000 nm wavelength) in increasing synaptic resilience to Aβ oligomers. NIR light treatment administered in a noninvasive transcranial application has been suggested to photostimulate the mitochondrial chromophore, cytochrome c oxidase, resulting in increased ATP formation (Karu, 2010b; Begum et al., 2015; Yu et al., 2015). Mitochondrial dysfunction is a pathological event occurring in early stages of AD. Much evidence suggests dysfunctional mitochondria events such as increased reactive oxygen species (ROS) production (Sheehan et al., 1997; Wang et al., 2016), cytochrome c deficiencies (Kish et al., 1992; Manczak and Reddy, 2012), and mutated mitochondrial DNA (Linnane et al., 1989; Gredilla et al., 2012) contributes to the exacerbation of AD pathology (Swerdlow et al., 2010, 2014). Notably, studies have described the intimate relationship between reduced Aβ oligomer binding at synapses and increased mitochondrial function (Pitt et al., 2009). Thus, it is not unreasonable to hypothesize that NIR light treatment could effectively promote synaptic resilience to Aβ oligomer binding. To test this hypothesis, we investigated the ability of NIR light to reduce synaptic susceptibility to Aβ oligomer binding and, as a result, increase synapse function. We utilized wild type (wt) mice to determine the impact of NIR light treatment on the binding of Aβ oligomers to isolated synaptosomes and long-term potentiation (LTP) in the hippocampus of these NIR light-treated wt mice in the presence and absence of Aβ oligomers. To determine light-driven changes in the synaptic presence of endogenous Aβ oligomers we also investigated the $A\beta_{1\text{-}42}$ load at isolated synapses of NIR light-treated Tg2576 mice that overexpress human amyloid precursor protein (APP) and progressively accumulate Aβ in their CNS (Hsiao et al., 1996; Kawarabayashi et al., 2001; Dineley et al., 2007b; Duffy et al., 2015). We further investigated synaptic mitochondria in wt and Tg2576 mice after NIR light treatment. We found that NIR light treatment reduced *ex vivo* Aβ oligomer synaptic binding in wild type mice that

was paralleled by a retention of long term potentiation induction. We further found that after NIR light treatment, the levels of $A\beta_{1-42}$ at the synapses was significantly reduced in 6-month-old Tg2576 mice. These changes were in conjunction with a retention and increase in the synaptic mitochondria health after NIR light treatment in both wt and Tg2576 mouse models.

RESULTS

Decreased susceptibility of synapses to toxic Aβ oligomers binding in wild type mice treated with NIR light.

To investigate the effects of NIR light on synaptic sensitivity to Aβ, we studied the *ex vivo* Aβ oligomer binding on isolated synaptosomes from wild type mice receiving NIR light treatment. The NIR light treatment consisted of a 90 second exposure **(Figure 2.1)** administered once a day, 5 days a week for 4 weeks as detailed in Methods. Using flow cytometry analysis, we performed a binding curve with various concentrations of Aβ oligomers labeled with Flour 488 (from 1 μM to 15 μM). As shown in Figure 3.1, we gated for synaptosomes based on size, using appropriate standards (Spherotech, Inc.) **(Figure 3.1)**. The parameters were set to include particle sizes that are typical of synaptosomes which range from 1-5 μm, as previously described by Gylys et al. (2004) (Gylys et al., 2004). This size parameter insures that the particles included in the analysis are synaptosomal elements. This method allows us the ability to exclude any nonspecific binding of Aβ oligomers onto non-synaptosomal particles that may be present in the synaptosomal prep. We found that the pooled (3 pooled samples per group, consisting of 3 individual mice per sample for a total of 9 mice per group) hippocampal and cortical synaptosomal fractions from mice treated with NIR light had decreased Aβ binding at the concentrations of Aβ oligomers used **(Figure 3.2a, b)**. We further confirmed this reduced binding affinity using the an $A\beta_{1-42}$ specific ELISA analysis **(Supplementary Fig. 3.1)**. These results strongly suggest that NIR light treatment reduces synaptic susceptibility to Aβ oligomer binding. By further analyzing the data using Scatchard plot analysis **(Figure 3.2c, d)**, we found that the maximum binding capacity (B_{max}) was reduced in the group receiving NIR light treatment as compared to the sham-treated group in both the hippocampus (NIR-treated B_{max} =

63.14+/- 17.46 μM and sham treated group B_{max} = 87.58 +/- 8.40 μM, $p<0.05$) and the cortex (NIR-treated group B_{max} = 79.99 +/- 3.18 μM and sham treated group B_{max} = 98.07 +/- 9.84 μM, $p<0.05$). On the other hand, no changes in the affinity (Kd) of Aβ binding were observed between groups for either the cortex or hippocampus (Kd= 5.7 +/- 1.95 μM and 9.00 +/- 5.08 μM, respectively, for the sham-treated group and Kd= 5.12 +/- 1.97 and 8.14 +/- 4.22 μM, respectively, for the NIR-treated group). The smaller B_{max} along with unchanged Kd indicates that there was a reduction in the number of binding sites at the synapses in the group that received NIR light treatment.

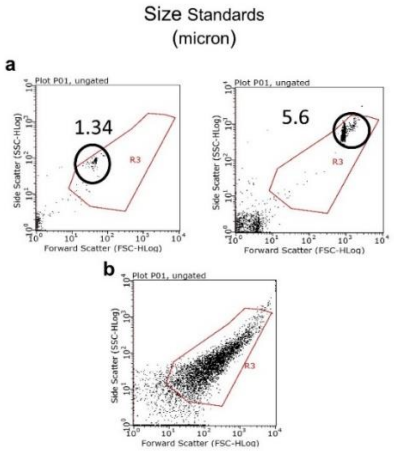


Figure 3.1. Flow cytometry gating parameters.
(**a**) Size beads (circled) ranging from 1-5.6 μm, the average size range of synaptosomes, were used to determine the gate (red box). (**b**) The size gate was applied to the particle distribution of mouse synaptosome preparation to exclude non-synaptosomal particles.

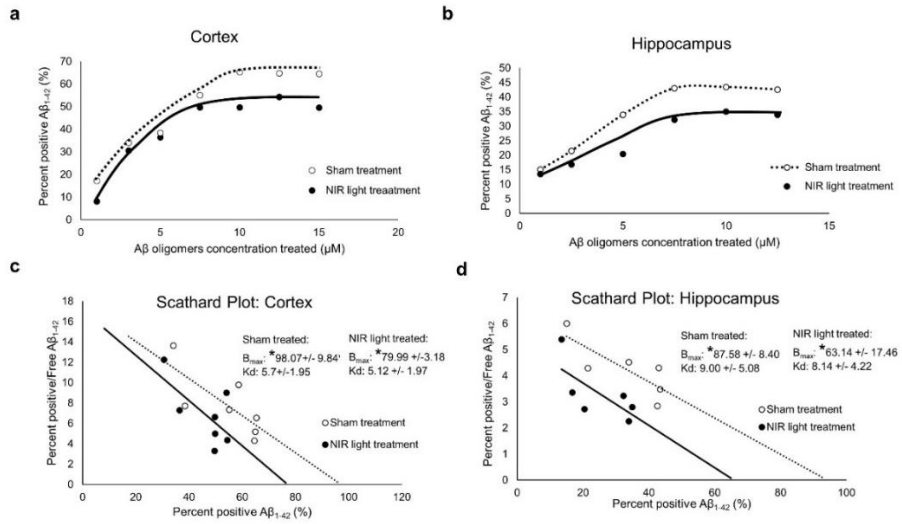


Figure 3.2. Flow cytometry analysis of NIR light treatment Aβ oligomer binding curve. Groups of pooled synaptosomes from (**a**) cortex and (**b**) hippocampus of WT mice receiving NIR light treatment (black square) and sham treatment (white circle) were challenged with increasing concentrations of Aβ oligomers tagged with HyLite Fluor 488. The percent of synaptosomes with bound Aβ oligomers was determined by flow cytometry analysis. (**c**) Scatchard plot analysis of Aβ oligomer binding show decreased B_{max} values but not Kd values after NIR light treatment in (**c**) cortical synaptosomes and (**d**) hippocampal synaptosomes from WT mice (n=9 per treatment group; 3 independent pooled samples of 3 mice per group). Statistical significance was determined by Student's two tailed t-test analysis on three separate binding curve analysis of three to four pooled samples per group. *$p<0.05$.

We also performed the *ex vivo* Aβ oligomer binding at a single concentration (5μM) on synaptosomes prepared from each individual wt mouse (n=8; per group) to further demonstrate changes in binding properties due to NIR light treatment. Consistent with the results described above, we found that in the parieto-occipital cortex, frontal cortex, and hippocampus there was a significant reduction of $A\beta_{1\text{-}42}$ positive synaptosomes of about 7%, 16%, and 6%, respectively, in the samples prepared from NIR light treated mice as compared to the control, sham-treated animals **(Figure 3.3a-d)**.

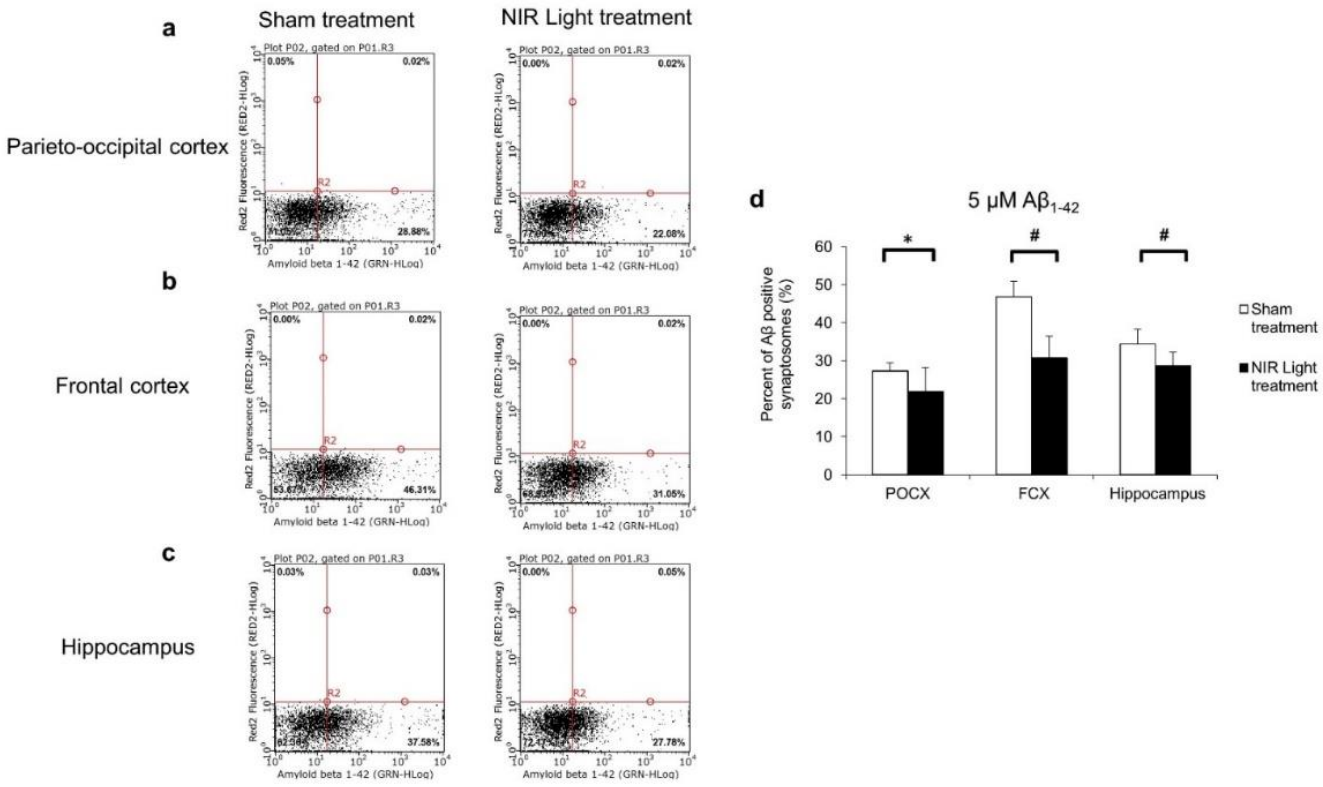


Figure 3.3. Flow cytometry analysis of *ex vivo* synaptic binding of 5 μM Aβ oligomers.
Representative flow cytometry analysis of the 5 μM Aβ oligomer binding in synaptosomes isolated from (**a**) parieto-occipital cortex (POCX) (**b**) frontal cortex (FCX) and (**c**) hippocampus in NIR light treated and sham treated wild type mice. (**d**) The results indicate a significant reduction in the percentage of Aβ positive synaptosomes in the three brain regions POCX, FCX and hippocampus of NIR light treated mice (black) compared to sham treated mice (white). (n=8; per group). Statistical significance was determined by Student's two tailed t-test analysis. Error bars represent standard deviation. *$p<0.05$; $^{\#}p<0.01$.

Decreased Aβ oligomer level in total homogenate and at the synapses in 6-month-old Tg2576 mice after treatment with NIR light.

In order to determine the effect of NIR light treatment in protecting synapses from endogenous Aβ oligomers in vivo, we investigated changes in Aβ oligomer levels in synaptosomes and total protein extracts from a well characterized mouse model of AD-like pathology, the Tg2576 mice. In the Tg2576 mouse model, oligomers begin accumulating at the age of 3-5 months and plaques are not observed until the mice are older, around 11-12 months of age (Hsiao et al., 1996; Kawarabayashi et al., 2001; Dineley et al., 2007b). NIR light treatment was started at the age of 6 months (n=7; per

group) and the mice sacrificed one month later. Levels of Aβ in the synaptosomal fractions and total homogenates from the frontal cortex, hippocampus, parieto-occipital cortex, and cerebellum were analyzed by a specific ELISA. As shown in **figure 3.4b**, there was a significant decrease in Aβ levels in the synaptosomal fractions from the four brain regions (parieto-occipital cortex, frontal cortex, hippocampus and cerebellum) in NIR light-treated mice as compared to sham animals. Western blot analysis of the synaptosomes further confirmed changes in levels of the low molecular weight Aβ oligomers **(Figure 3.4c)**. On the other hand, a significant decrease of Aβ levels in the total protein extracts was observed only in the parieto-occipital cortex **(Figure 3.4a)** of NIR-treated mice, whereas Aβ levels in the hippocampus, frontal cortex and cerebellum were unchanged, although a non-statistically significant trend toward reduction of Aβ levels in these regions was noticed.

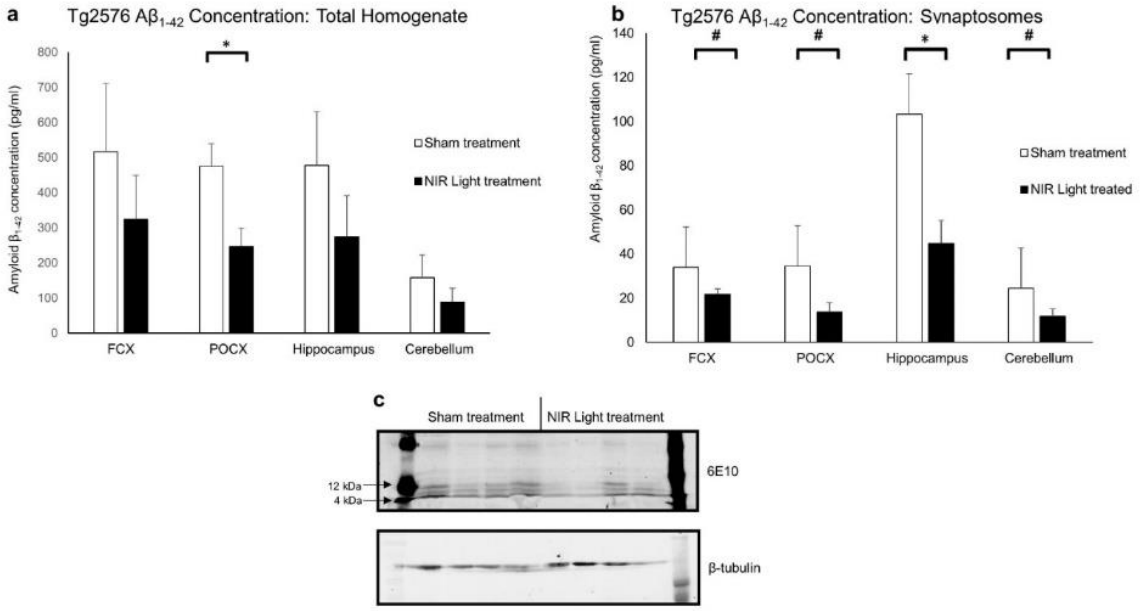


Figure 3.4. NIR light treatment decreases Aβ1-42 oligomer levels in total homogenate and at the synapses in 6-month-old Tg2576 mice.
Aβ levels in (**a**) total homogenate and (**b**) synaptosomes from four major brain regions; frontal cortex (FCX) parieto-occipital cortex (POCX), hippocampus and cerebellum of NIR light (filled columns) and sham (open columns) treated Tg2576 mice. (n=7 per group) were measured using ELISA analysis. (**a**) NIR light treated mice had decreased levels of $A\beta_{1-42}$ the total homogenate from the POCX compared to wt mice. (**b**) NIR light treated mice also had decreased levels of $A\beta_{1-42}$ in the synaptosomes isolated from all of the four brain regions assayed (POCX, FCX, hippocampus and cerebellum). (**c**) Western blot analysis was further used to determine the changes in the low molecular weight Aβ oligomers after NIR light treatment. Student's two tailed t-test was used to determine statistical significance. Error bars represent SEM. *$p< 0.05$; #$p<0.01$.

NIR light reduces Aβ oligomer-induced deficits in long term potentiation.

In order to determine whether the reduced susceptibility of synapses to Aβ oligomers would translate into functional protection, we utilized wild type mice to determine if NIR light treatment rescues the synaptic impairment of LTP that normally occurs in response to exposure to Aβ oligomers (Wang et al., 2002). As shown in figure 3.6A, we found that the magnitude of LTP in hippocampal slices from sham-treated mice was significantly reduced by exposure to 200 nM preformed Aβ oligomers. On the other hand, the magnitude of LTP in hippocampal slices from NIR light-treated animals was not affected by Aβ oligomers and remained comparable to the LTP observed in slices from both the NIR light-treated and sham animals that did not receive Aβ oligomers. In any case, the basal synaptic strength was not altered by NIR light treatment or exposure to the Aβ oligomers across our experimental groups, as determined by input-output curves **(Supplementary Fig. 3.2)**. When the last 10 minutes of LTP were averaged for each group and statistically analyzed **(Figure 3.5b)**, we confirmed a significant reduction of LTP induced by Aβ oligomers in hippocampal slices from sham-treated animals but not in slices from NIR light-treated mice that maintained a level of LTP comparable to control slices.

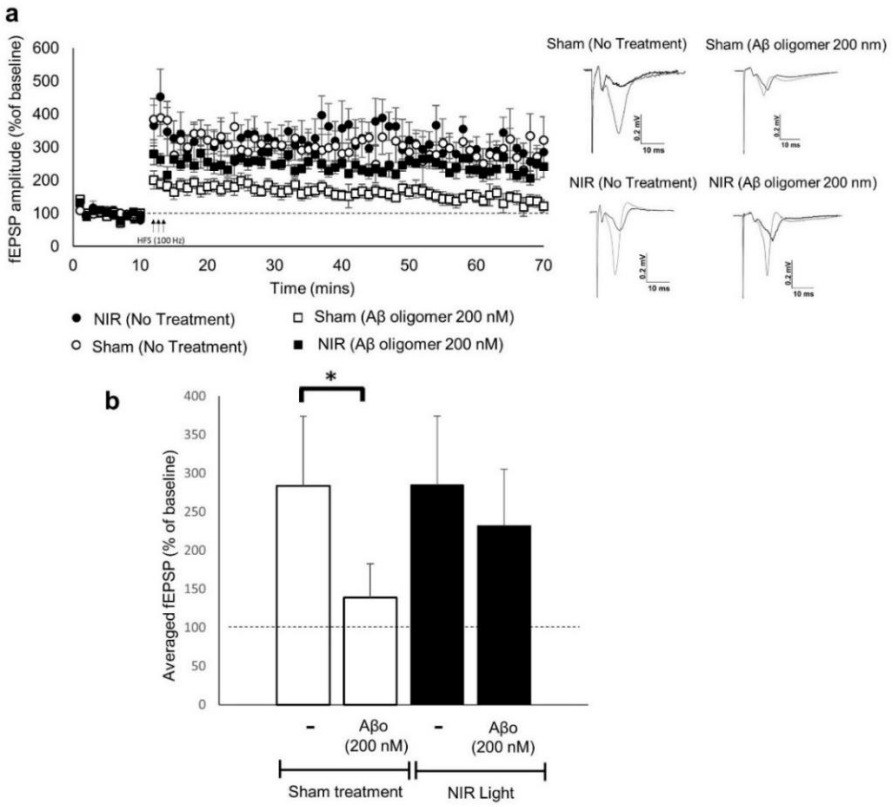


Figure 3.5. Aβ oligomer driven LTP impairment is reduced with NIR light treatment.
Schaffer collateral field recordings were performed to determine NIR light treatments impact on LTP in the presence of Aβ oligomers. (**a**) The percent of baseline in the slope of fEPSPs of NIR light treated wild type mice compared to sham treated wild type mice exposed to Aβ oligomers (n=5; per group, 2 slices per condition). (**b**) The fEPSP amplitude for the final 10 minutes (time points 50-60 minutes post high frequency stimulation) were averaged for each condition. The sham light treated groups receiving Aβ oligomers had a significant reduction in LTP. This reduction was reversed in the group that received NIR light treatment. One-way ANOVA with Dunn's post hoc analysis was used to determine statistical significance. Error bars represent ±standard error of mean. *$p<0.05$.

NIR light treatment increases synaptic mitochondrial membrane potential in both wild type and Tg2576 mice.

In these experiments, we focused on synaptic mitochondria function as one possible mechanism contributing to the observed synaptic resilience to Aβ oligomers induced by NIR light exposure. Previous studies have described the mitochondria as a key element targeted by NIR light treatment (Karu, 2010a; Begum et al., 2015; Yu et al., 2015) and reported that preserving synaptic mitochondria efficiency protects synapses from Aβ oligomers (Pitt et al., 2009; Reddy et al., 2012). We first analyzed by flow cytometry synaptosomes from wild type and Tg2576 mice treated or not with NIR light that had been labeled with MitoTracker, a mitochondrion-specific fluorescent dye whose stain intensity is directly proportional to the number of mitochondria (Presley et al., 2003). We found that there was a decreased number of synaptic mitochondria in Tg2576 mice as compared to age matched wild type mice **(Figure 3.6a, c)**. However, Tg2576 mice receiving NIR light treatment had an abundance of mitochondria at the synaptosomes comparable to wild type mice. We further investigated the mitochondrial membrane potential (reflecting overall mitochondrion health) of the synaptic mitochondria by labeling synaptosomes with the fluorescent dye MitoSense, which directly correlates with the levels of mitochondria membrane potential (Mattiasson et al., 2003). Using flow cytometry in labeled synaptosomes we observed an increase in the mitochondrial membrane potential in both the wild type and Tg2576 mice treated with NIR light as compared to sham-treated controls **(Figure 3.6b, d)**, suggesting that the NIR light treatment increases overall health of synaptic mitochondria in both wt and Tg2576 mice.

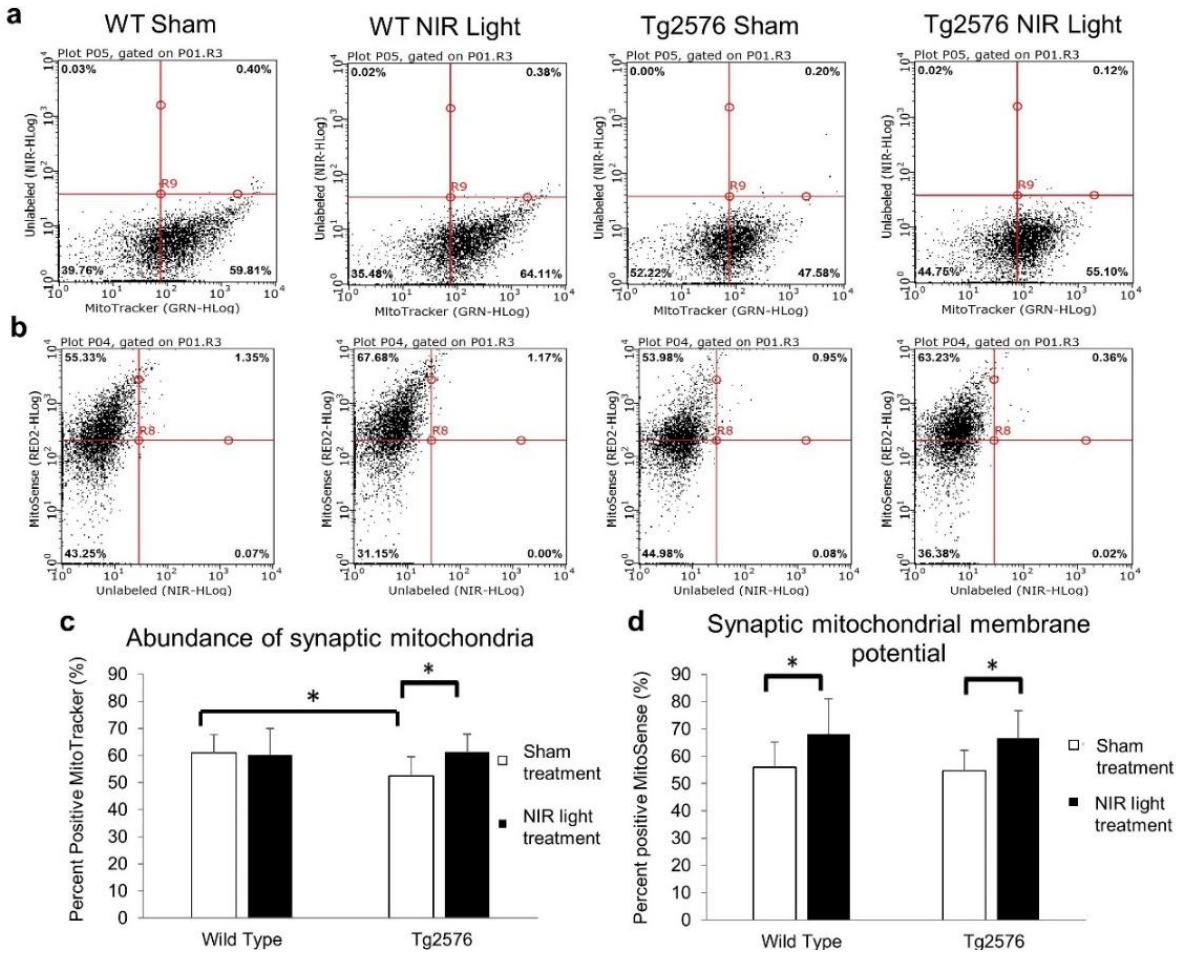


Figure 3.6. NIR light treatment increases synaptic mitochondrial membrane potential in both wild type and Tg2576 mice and rescues number of synaptic mitochondria in Tg2576 mice. Representative flow cytometry analysis of (**a**) MitoTracker (mitochondria number) positive synaptosomes and (**b**) MitoSense (mitochondria membrane potential) positive synaptosomes of the four groups including wild type and Tg2576 mice treated with NIR light or sham light treatments (n=8; per group). (**c**) The percentage of positive synaptosomes for MitoTracker is significantly reduced in Tg2576 sham treated mice (white). The Tg2576 mice receiving NIR light treatment (black) have an increase in synaptic mitochondria number resulting in comparable mitochondria numbers to wild type mice. (**d**) The percentage of positive synaptosomes for MitoSense is increased in both wild type and Tg2576 mice treated with NIR light. One-way ANOVA followed by Tukey's post hoc test was used to determine statistical significance. Error bars represent standard deviation. * $p<0.05$.

DISCUSSION

Synaptic function is an essential element in the maintenance and preservation of cognition (Terry et al., 1991; Dickson et al., 1995; Sze et al., 1997; Scheff et al., 2007; Tracy and Gan, 2017).

Aβ oligomer preferential binding to the synaptic region disrupts synaptic transmission and contributes to the declining functionality of the synapses during the progression of AD (Lambert et al., 1998; Spires-Jones and Hyman, 2014). The main goal of the current study was to determine the ability of NIR light to mitigate the toxic binding of Aβ oligomers to the synapses, thus alleviating the resulting synaptic dysfunction. Our *ex vivo* Aβ oligomer challenge model allowed us to exclusively isolate the synaptosomes after NIR light treatment and determine differences in Aβ binding between the groups. By directly adding prepared Aβ oligomers to isolated synaptosomes, we could demonstrate acquired synaptic resistance to Aβ oligomers binding after NIR light exposure. We found that wt mice treated with NIR light have a reduction in the amount

of Aβ oligomers that bind to the synaptosomes. The results of our binding curve provided us the opportunity to characterize the binding changes that were occurring after NIR light treatment. We used Scatchard plot analysis to calculate the Aβ oligomer maximum binding capacity and affinity in the two experimental groups (NIR-treated and sham-treated). The decreased B_{max} value in the NIR light treated groups with no change in the affinity (Kd) suggests that after NIR light treatment there is a reduction in Aβ oligomer docking sites at the synapse, providing compelling evidence that a secondary mechanism of action of the NIR light treatment is contributing to the increased resilience to the toxic protein. By gating our observation to selected particle sizes, flow cytometry further allowed us to exclude nonspecific binding to nonsynaptosomal particles.

We utilized the Tg2576 mouse strain that overexpresses human APP and progressively accumulate Aβ oligomers (Hsiao et al., 1996; Kawarabayashi et al., 2001; Dineley et al., 2007b; Duffy et al., 2015) to determine whether an *in vivo* reduction of endogenous Aβ at the synapses could be observed after NIR light treatment. Previous research has shown a reduction of the Aβ plaque load after NIR light treatment in the cortex and hippocampus of APP/presenilin1 (PS1) double transgenic mice (Purushothuman et al., 2014). However, this previous study focused on overall Aβ levels in plaques and did not address the effect of NIR light on synapse vulnerability to the toxic Aβ oligomers, as we have determined here. To provide a complete understanding of the impact NIR light has on

synaptic vulnerability to Aβ, in our study Tg2576 mice were treated with NIR light and the Aβ levels were measured in both total brain protein extracts as well as synaptosomal fractions. We employed Tg2576 mice at age of 6 months when ample Aβ load, mainly in the form of oligomers, is seen and cognitive defects are prevalent, but plaques formed by large Aβ fibrils deposition are not yet present (Kawarabayashi et al., 2001; Dineley et al., 2010; Duffy et al., 2015). Therefore, Tg2576 mice at 6 months of age are an ideal model to study specifically the impact of Aβ oligomers. Our results showed a significant decrease of Aβ load in the synaptosomes from all four brain areas tested, i.e. parieto-occipital cortex, hippocampus, frontal cortex and cerebellum. However, the total brain tissue homogenates showed a significant decrease of Aβ only in the parieto-occipital cortex. The other three brain regions showed a trend of decreased levels, however, the reduction was not statistically significant. The differential impact of the NIR light among the brain regions may be attributed to higher energy exposure to superior structures or higher sensitivity to NIR light of the parietal-occipital cortex, although further experiments are needed to support either (or both) of these possibilities. The differences in reduction between the total homogenate and synaptosomes suggest that NIR light initiates a mechanism that targets specifically the synapses. Interestingly, this selective reduction of Aβ at synapse induced by the NIR light treatment is similar to what observed in the cognitively intact NDAN individuals. As we have previously described, NDAN individuals have presence of Aβ oligomers in the CNS at levels similar to demented AD patients, however, they have an absence of Aβ oligomers at the synapses, which suggests synaptic resistance to oligomer binding (Bjorklund et al., 2012). The results of our *in vivo* experiment thus suggest that NIR light treatments may effectively evoke a mechanism of synaptic resilience similar to the natural ability to resist the binding of Aβ oligomers seen in NDAN individuals with preserved cognitive integrity.

We further aimed to relate this molecular phenomenon of NIR-induced resilience with synaptic functional outcomes as an indication of preserved synaptic efficiency. The underlying deficiency of cognition in AD is the loss of synaptic function (Selkoe, 2002; Scheff et al., 2007; Shankar et al., 2007; Tracy and Gan, 2017). A well-established impairment that occurs when Aβ

oligomers bind to synapses is reduced magnitude of LTP (Chen et al., 2002) and increased long term depression (LTD) (Li et al., 2009, 2011). In the present study, we aimed to determine if NIR light reversed this observed reduction of LTP expression by Aβ oligomers. Consistent with those previous experiments, we found a significant reduction in the magnitude of LTP in wt mice brain slices incubated with 200 nM of Aβ oligomers. However, after NIR light treatment wt mice demonstrated an induction of LTP similar to the control group without Aβ oligomer exposure. This suggests that the reduction of Aβ oligomer binding that we observed following NIR light treatment in the *ex vivo* studies results in a significant rescue in synapse functionality. The electrophysiology experiment was conducted after treating the wt mice with a condensed treatment protocol. We found that mice receiving the same number of treatments (4 treatments per day for 5 days; 20 total doses of NIR light) and the same delivery of total energy as the month-long treatment over a course of 5 days showed similar reductions in the synaptic binding of Aβ oligomers (Supplementary Fig. 3.3). While there are a few studies that have investigated the total energy from NIR light needed to have a biological impact on the system (Huang et al., 2012), little is known about the persistence of NIR light's beneficial effects after the end of a complete treatment cycle. Future studies are needed to address this important issue with significant implications for the translational value of NIR treatment.

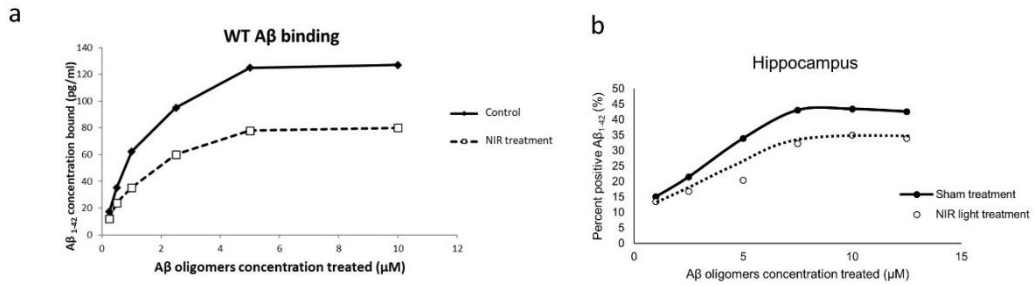
A possible mechanism that could be contributing to the protection and increased function of synapses seen in our study is increased function of the synaptic mitochondria. The 600- 1000 nm wavelength spectrum of the NIR light has been shown to optimally photostimulate cytochrome c oxidase, a key enzyme in the electron transport chain that is believed to result in an increase in ATP production (Hashmi et al., 2010; Karu, 2010a, 2010b) Cytochrome c oxidase contains 2 copper centers and 2 heme iron centers. When photostimulated, the copper centers are unable to bind nitric oxide, an inhibitor of mitochondria respiration, resulting in an increase of oxygen consumption and ATP formation (Karu, 2010a; Begum et al., 2015). Several previous studies have investigated the dysfunction of mitochondria in AD (Devi, 2006; Reddy, 2007; Marta et al., 2013). Notably, there is an impairment in the mitochondrial transport mechanisms (Calkins and Reddy, 2011) and in the

fission/fusion process of mitochondria (Wang et al., 2009; Zhang et al., 2016) specifically at the synapses (Du et al., 2010). Collectively, these impairments lead to a decrease in functional synaptic mitochondria resulting in reduced ATP supply, ultimately leaving the synapses vulnerable to toxins such as Aβ oligomers. The reported decrease in mitochondria functionality in AD may thus benefit from the mitochondria-boosting properties of NIR light. In our study, we investigated the effects of NIR light on synaptic mitochondrial membrane potential (reflecting overall mitochondrial health) and synaptic mitochondrial abundance. Consistent with the literature, we found a decrease in the abundance of synaptic mitochondria in Tg2576 mice as compared to age matched wild type mice (Wang et al., 2009) and further observed that after NIR light treatment, this deficiency in the synaptic mitochondria was rescued. On the other hand, wild type mice treated with NIR light did not have an increase in synaptic mitochondria compared to their control treatment counterparts. This suggests that under normal physiological conditions, NIR light does not induce an increase of mitochondria numbers at the synapses. However, the rescued synaptic mitochondria number that is seen in the NIR light-treated Tg2576 synapses suggests that the NIR light-induced reduction of Aβ oligomer binding contributes to the reversal of the depletion of synaptic mitochondria. Nonetheless, given the fact that the beneficial effect of NIR light in promoting synaptic resilience to Aβ oligomers was observed in both wt and Tg2576 mice, synaptic mitochondrial abundance does not appear to be a key event in this action of NIR light. On the other hand, when we examined the membrane potential of the synaptic mitochondria as an indicator of mitochondrial health after NIR light treatment, we found that both wild type and Tg2576 mice showed an increase in synaptic mitochondrial membrane potential after treatment with NIR light. Previous studies have shown a direct relationship between synaptic vulnerability to Aβ oligomer binding and the health of synaptic mitochondria (Pitt et al., 2009; Reddy et al., 2012). Furthermore, synaptic mitochondria are believed to have an integral role in the regulation of LTP induction (Weeber et al., 2002; Kimura et al., 2012). Therefore, our results suggest a mechanism of NIR light action critically involving increasing synaptic mitochondrial membrane potential, consistent with the reported increase in synaptic resilience to Aβ oligomers induced by

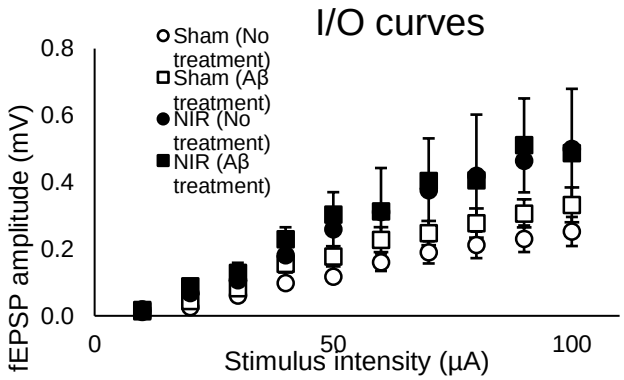
promotion of synaptic mitochondrial function/health. Taken together these results indicate that NIR light treatment increases synaptic mitochondria health, thus decreasing synaptic susceptibility to Aβ binding and consequent electrophysiological deficits in LTP expression.

Collectively, the results of our study provide valuable insight into the mechanisms that underscore the reported mitigation by NIR light of AD pathology and further introduce a previously unappreciated phenomenon of increased synaptic resilience to the disrupting action of Aβ oligomers that can be promoted by NIR light exposure.

Supplementary Figures



Supplementary Figure 3.1. ELISA and flow cytometry Aβ oligomer binding curves
Two methods were imploded in the determination of changes in the Aβ oligomer binding between NIR light treated and sham control treated wild type mice; ELISA and flow cytometry analysis. The flow cytometry analysis as described in the Methods section was used in determining the percentage of synaptosomes in our synaptosomal prep that would bind a fluorescently tagged Aβ oligomer. The ELISA method was similar, however, the Aβ oligomers were prepared without a fluorescent tag, so the analysis determined the total amount of Aβ oligomers bound in our sample. The flow cytometry method was chosen as the main focus in our current study, because of the added ability of the method to selectively analyse the synaptosomes in our prep, excluding nonspecific binding of the tagged Aβ oligomers to nonsynaptosomal particles. As shown in this figure, both methods illustrated a reduction of binding in the NIR light treated mice compared to the control sham group. Further both methods demonstrated a saturation of Aβ oligomer binding to isolated synaptosomes, thus further confirming overall validity of the *ex vivo* binding procedure used here.



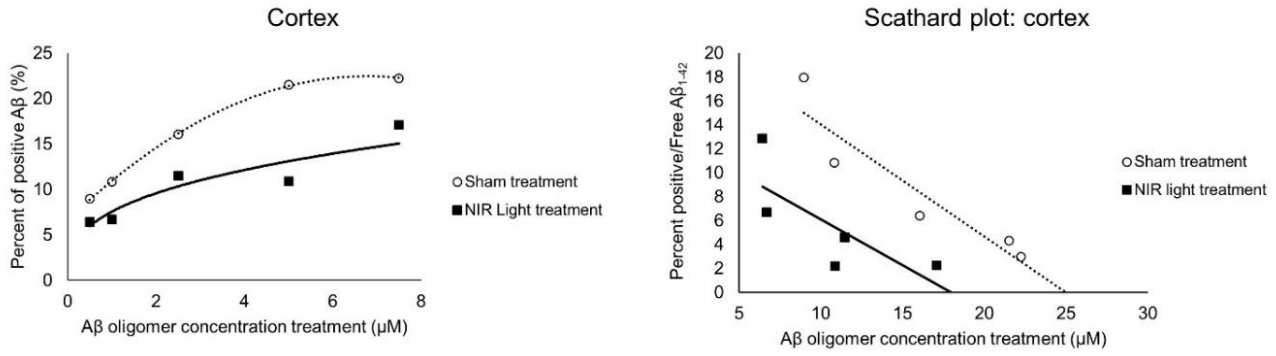
Supplementary Figure 3.2. Input/output curves for the four treatment groups.
The fEPSP amplitude (mV) obtained at increasing stimulus intensities (mA) show no significant differences in the basal synaptic strength following NIR light treatment and/or exposure to Aβ oligomers compared to sham (no treatment). n=6-8 slices from 3-6 mice; Statistical analysis was carried out using two-way ANOVA with Bonferroni post-hoc analysis, $F_{9,3}$=0.4209, P=0.9954, ns. Error bars represent standard error of mean.

MEAN (n=6)	Sham (No treatment)		Sham (Aβ treatment)		NIR (No treatment)		NIR (Aβ treatment)	
mV	Pre	Post	Pre	Post	Pre	Post	Pre	Post
10	0.012	0.034	0.018	0.060	0.020	0.056	0.014	0.081
20	0.026	0.033	0.045	0.046	0.069	0.034	0.089	0.082
30	0.061	0.077	0.083	0.083	0.107	0.140	0.129	0.167
40	0.098	0.107	0.156	0.139	0.182	0.175	0.229	0.302
50	0.117	0.140	0.178	0.163	0.260	0.258	0.303	0.308
60	0.160	0.174	0.228	0.189	0.311	0.292	0.313	0.382
70	0.190	0.215	0.248	0.226	0.376	0.360	0.404	0.455
80	0.212	0.248	0.278	0.262	0.420	0.390	0.406	0.483
90	0.231	0.262	0.307	0.273	0.464	0.424	0.512	0.550
100	0.252	0.255	0.332	0.299	0.500	0.431	0.487	0.559

SEM (n=6)	Sham (Untreated)		Sham (Abeta Treated)		NIR (Untreated)		NIR (Abeta Treated)	
mV	Pre	Post	Pre	Post	Pre	Post	Pre	Post
10	0.006	0.009	0.006	0.018	0.006	0.014	0.005	0.030
20	0.006	0.011	0.012	0.014	0.034	0.014	0.031	0.035
30	0.017	0.017	0.018	0.022	0.052	0.041	0.054	0.048
40	0.017	0.026	0.026	0.023	0.083	0.075	0.074	0.074
50	0.015	0.023	0.030	0.026	0.110	0.106	0.102	0.085
60	0.026	0.036	0.037	0.026	0.131	0.112	0.086	0.097
70	0.033	0.047	0.036	0.031	0.155	0.132	0.135	0.136
80	0.040	0.055	0.044	0.028	0.183	0.139	0.125	0.134
90	0.040	0.061	0.042	0.038	0.187	0.151	0.142	0.160
100	0.044	0.058	0.052	0.040	0.180	0.156	0.136	0.155

Supplementary Table 3.1. Table of input/output averages for the four treatment groups.

The averages of the amplitudes (mV) measured in the four treatment groups after increasing stimulus intensities. There was no change in the pre-HFS and post-HFS amplitudes for all four treatment groups. n=6-8 slices from 3-6 mice; Statistical analysis was carried out using two-way ANOVA with Bonferroni post-hoc analysis (Pre-HFS – $F_{9,3}$=0.34799, P=0.9991, ns; Post-HFS - two-way ANOVA, $F_{9,3}$=0.4209, P=0.9954, ns; Pre- vs Post- $F_{9,7}$=0.3395, P=1, ns).



Supplemental Figure 3.3. Flow cytometry analysis of condensed NIR light treatment regimen Aβ oligomer binding curve.
Pooled synaptosomes from cortex of WT mice receiving a condensed NIR light treatment schedule (20 treatments over 5 days) (black square) had a similar reduction in Aβ binding compared to sham treated mice (white circle) that was demonstrated in WT mice receiving 20 treatments over 4 weeks. Because the synaptosomes of the mice receiving a condensed schedule treatment regimen displayed similar reductions in binding, this schedule was used before performing electrophysiology experiments.

Chapter 4: The modulation of tau pathology in two transgenic mouse models by near infrared light treatment[2]

INTRODUCTION

Alzheimer's disease (AD) is the most frequent severe age-related dementia, affecting an estimated 44 million people worldwide, for which there is currently no resolving cure. The multifactorial nature of AD has contributed to the ongoing challenge of developing effective disease-modifying therapeutics. The accumulation of plaques and neurofibrillary tangles consisting of amyloid beta (Aβ) and hyperphosphorylated tau protein, respectively, are two quintessential hallmarks of AD. However, many factors such as mitochondrial dysfunction, neuroinflammation, impaired clearance mechanisms of dysfunctional proteins and synaptic retraction contribute to the disease progression (Shankar et al., 2007; Orr and Oddo, 2013; Swerdlow et al., 2014; Heneka et al., 2015). Among those factors, synaptic dysfunction is believed to underlie onset and progression of the cognitive impairment that characterized the symptomatic phase of AD (Selkoe, 2002). Small soluble intermediate aggregate forms of Aβ, oligomers, have been studied extensively as the most toxic form of Aβ which target and disrupt proper synaptic function in the earliest stages of disease development (Shankar et al., 2008; Dineley et al., 2010; Wilcox et al., 2011). Most recently, studies have found similar detrimental synaptic deficits induced by tau oligomers, the intermediate aggregate form of the other predominate dysfunctional protein in AD (Lasagna-Reeves et al., 2011; Fá et al., 2016). In addition, an intimate relationship between the synaptic toxicity of Aβ and tau has been proposed, whereby oligomers of the two amyloid proteins synergize by converging on targeted synapses (Pascoal et al., 2017; Rajmohan and Reddy, 2017). While Aβ oligomers drive the hyperphosphorylation (De Felice et al., 2008; Ittner et al., 2010; Chabrier et al., 2012), misfolding (Castillo-Carranza et al., 2015) and relocation of tau (Zempel and Mandelkow, 2014) leading to the

[2] Work published in Molecular Neurobiology: Comerota, M.M, Tumurbaatar, B., Krishnan, B., and Taglialatela, G. The modulation of tau pathology in two transgenic mouse models by near infrared light treatment. (2018). https://doi.org/10.1007/s12035-018-1248-9.

accelerated formation of toxic tau oligomers, recent evidence supports the emerging idea that the simultaneous presence of the two amyloid oligomeric proteins results in exacerbated synaptic impairments beyond the deficits inflicted by either protein alone (Spires-Jones and Hyman, 2014b; Fá et al., 2016). This complex synergistic relationship suggests that a treatment that mitigates the synaptic dysfunction induced by both Aβ and tau oligomers while inducing the synaptic clearance of both proteins would be the most effective approach to slow down the progression of AD. With this ultimate goal in mind, we previously reported that the transcranial administration of a near infrared (NIR; 600-1000 nm) light treatment results in reduced synaptic susceptibility to the binding of Aβ oligomers and resilience to the ensuing Aβ oligomer-driven synaptic dysfunction. We also found that transgenic hAPP overexpressing mice, Tg2576, treated with NIR light have reduced Aβ oligomer load at the synapses and healthier synaptic mitochondria (Comerota et al., 2017). Based on these previous results and the growing evidence that tau oligomers may also initiate a detrimental cascade on synaptic function, in the present chapter, we aimed to determine whether NIR light could promote a similar neuroprotection against the dysfunctional tau oligomer synaptic binding and synaptic accumulation of tau in two tg mouse models of human tauopathies (htau and 3xTgAD) *in vivo*. We further investigated autophagy related proteins and the chaperone inducible heat shock protein 70 (HSP70) as possible mechanisms mediating clearance of tau in response to NIR light treatment. We show that, when applied to either of these tg mouse models of human tauopathies, NIR light effectively clears toxic tau oligomers, both from the CNS parenchyma and synapse, and restores memory functions in these impaired mice. Overall, these novel results support and encourage the notion that NIR light should be further explored as a non-invasive therapeutic strategy in AD as well as related tauopathies.

RESULTS

NIR light treatment does not reduce tau oligomer binding to the synapse of wild type mice

Our previous studies established a distinct reduction of the synaptic susceptibility to Aβ oligomers in NIR light treated wild type mice (Comerota, 2017). In the current study, we aimed to determine if NIR light induces a similar reduction of synaptic vulnerability to tau oligomers. We performed an *ex vivo* tau binding study in which synaptosomes isolated from wild type mice that received either NIR light treatment or sham no light treatment (n=7, per group) were exposed to 50 nM of tau oligomers for 1 hour, as described in the Methods section. After washing unbound oligomers from the sample, the remaining levels of tau protein was measured by ELISA analysis. We found that both cortical and hippocampal synaptosomes of NIR light treated mice contained similar levels of tau compared to the sham treated mice (cortex p=0.514, hippocampus p=0.867) **(Figure 4.1)**. This suggests that NIR light treatment does not reduce the susceptibility of the synapses to the association with tau oligomers.

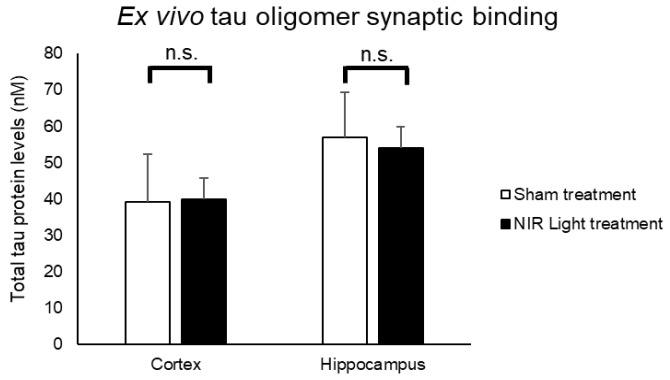


Figure 4.1. Synaptic binding of tau oligomers is not altered in NIR light treated WT mice. ELISA analysis was conducted on the ex vivo challenge of synaptosomes isolated from the cortex and hippocampus of NIR light and sham treated wild type mice with 50 nM of tau oligomers. The results indicate no change in the tau protein levels remaining in either the cortical or hippocampal synaptosomes of NIR light treated mice (filled bar) compared to sham treated mice (open bar). (n=8; per group). Statistical significance was determined by Student's two tailed t-test analysis. Error bars represent standard deviation. n.s. represents not statistically significant.

Long term potentiation deficits induced by tau oligomers is not reversed by NIR light treatment

Although we found no alterations in the susceptibility of tau oligomer binding to the synapses after NIR light treatment, we aimed to determine if NIR light provides a functional protection against tau oligomer induced synaptic dysfunction. We challenged brain slices from wild type mice that were

exposed to NIR light or sham treatment to 50 nM of tau oligomers (n=5 per treatment group, 2 slices per condition from each animal), a concentration known to impair the induction of long term potentiation (Fá et al., 2016) and measured the long term potentiation in the Schaffer collateral pathway of the hippocampus **(Figure 4.2)**. The last 10 minutes of LTP were averaged for each treatment group **(Figure 4.2b)**. Consistent with the literature, we found a statistically significant reduction in the magnitude of LTP in the slices from sham treated mice exposed to 50 nM of tau oligomers ($p=0.001$). We also found a reduction in the magnitude of LTP in slices prepared from the NIR light treated group exposed to tau oligomers compared to NIR light slices not incubated with tau oligomers ($p= 0.01$). There was no difference in the calculated LTP between the tau oligomer exposed NIR light treated group and the tau exposed sham treated group ($p>0.05$). This suggests that NIR light treatment does not protect against the tau oligomer induced LTP impairments. In all groups the basal synaptic strength was not altered, as measure by input-output curves **(Supplemental figure 4.1, Supplementary Table 4.1)**.

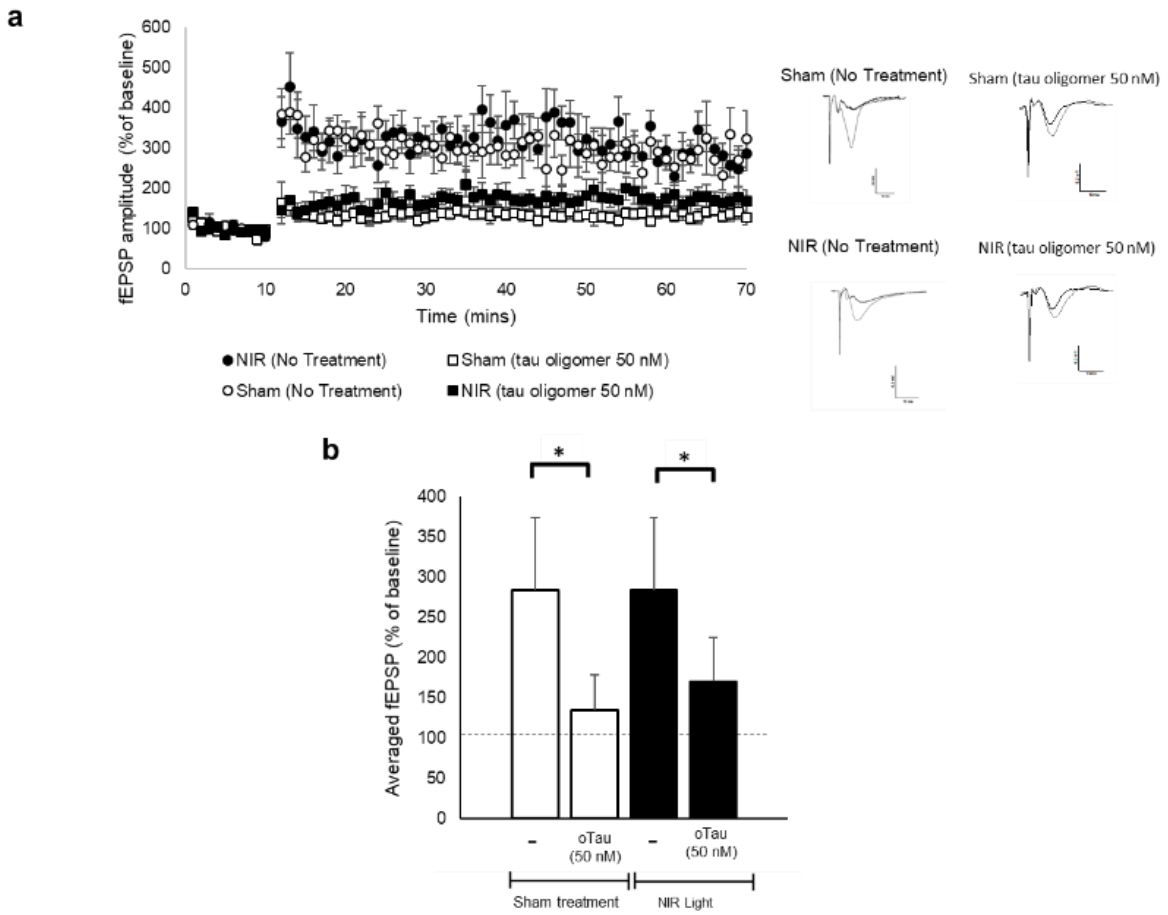


Figure 4.2. Tau oligomer induced impairment of LTP is not altered in NIR light treated mice
The long-term potentiation (LTP) was measured by Schaffer collateral field recordings to determine the impact of NIR light treatment on tau oligomer induced impairments in wild type mice. (a) The fEPSP amplitude was calculated for the four groups; NIR light treated with and without tau oligomers and sham treated with and without tau oligomers. (n=5; per group, 2 slices per condition) (b) For each group, the calculated fEPSP of the final 10 minutes of recording was averaged. The tau oligomer receiving groups had a significant reduction of fEPSP amplitude compared to the groups that did not receive tau oligomers in both sham treated and NIR light treated mice suggesting that NIR light treatment does not improve tau oligomer induced LTP impairment. One-way ANOVA with Dunn's post hoc analysis was used to determine statistical significance. Error bars represent ±standard error of mean. *$p<0.05$.

Tau pathology is reduced in cortical and hippocampal total protein extracts and synaptosomal fractions of NIR light treated 13-month-old htau mice.

We next aimed to determine if NIR light can impact endogenous tau oligomer synaptic accumulation *in vivo* by utilizing the transgenic human tau mouse model, htau. This well characterized mouse model develops aggregates of tau oligomers around 9-10 months of age (Duff et al., 2000; Andorfer et al., 2003). To ensure adequate tau accumulation prior to the start of treatment we began NIR light treatment at 12 months of age (n=7; per group). The total protein extracts and the isolated synaptosomal fractions of the cortex and hippocampus regions were analyzed by Western blot **(Figure 4.3 a-c, Figure 4.4 a-c; respectively)**, ELISA **(Figure 4.3d, Figure 4.4d; respectively)** and immunofluorescence **(Figure 4.3e, f)** to determine levels of tau oligomers and total tau. As shown in the figure, the tau oligomers were measured by densitometric analysis of Western blot bands of 110 kDa and higher **(Figure 4.3a-c)** as detected by the total tau antibody, tau5. In the cortical and hippocampal total protein extracts, we found a decrease in tau oligomers in NIR light treated htau mice compared to the sham treated mice (cortex p=0.016, hippocampus p=0.049). We further analyzed the total tau levels in the total protein extracts utilizing a tau5 ELISA analysis. As shown, **(Figure 4.3d)** there was a statistically significant decrease in the total tau in both the cortex and hippocampus regions (cortex p=0.049, hippocampus p=0.049). Finally, these results were confirmed by immunofluorescence analysis of the hippocampus and cortex regions using antibodies specific for tau oligomers, T22 and total tau, tau5. The immunofluorescence further verified a reduction of both total tau (cortex p=0.002, hippocampus p=0.032) and tau oligomers (cortex p=0.003, hippocampus p=0.045) in both regions **(Figure 4.3e, f)**. We then analyzed the levels of total and oligomeric tau in the synaptosomal fractions by Western blot and ELISA analysis to determine if the tau reduction is observed in the critically important synaptic compartment. Due to technical challenges imaging all forms of tau in the synaptosomal regions of the htau mice by Western blot, two exposure rates were taken to properly visualize monomeric tau (low exposure) and oligomeric tau (high exposure) **(Figure 4.4a, b)**. Tau oligomer levels were reduced in synaptosomal fractions of both the cortex and hippocampus of NIR light treated htau mice compared to the sham treated mice (cortex p=0.049, hippocampus p=0.049). Further, levels of total tau, as measured by tau5 ELISA, were also reduced

in the cortical and hippocampal synapses of these mice **(Figure 4.4c)** (cortex p=0.024, hippocampus p=0.003). This suggests a general, as well as, synaptic reduction of total and oligomeric tau in htau transgenic mice treated with NIR light.

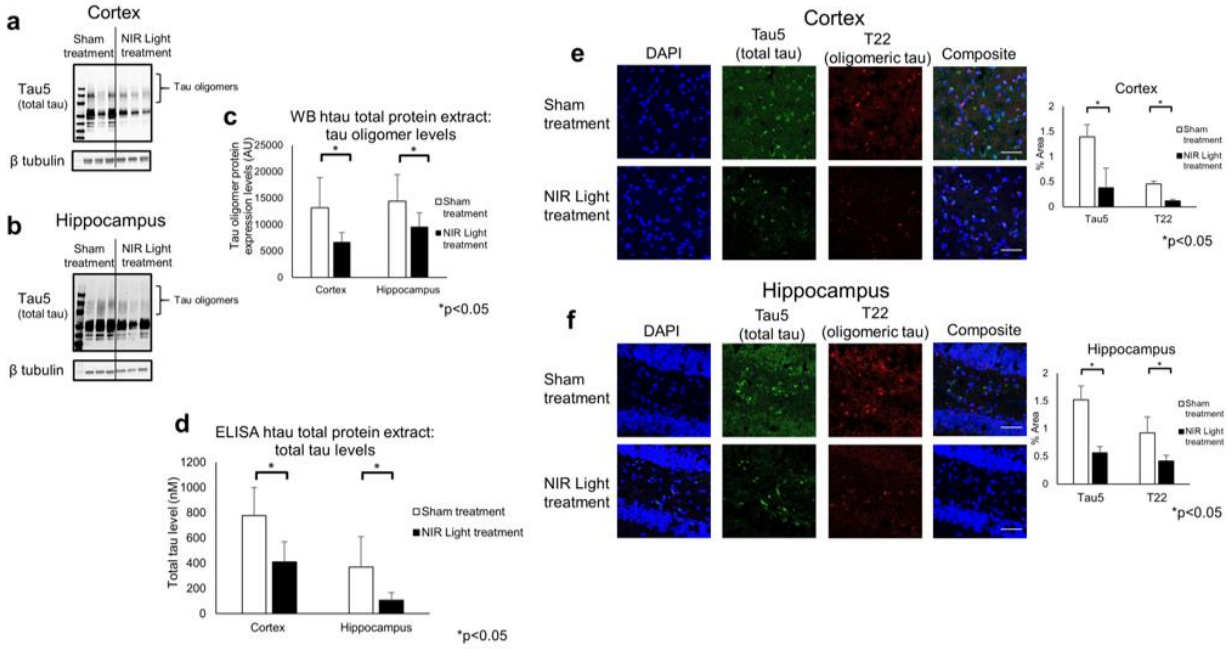


Figure 4.3. Reduced total and oligomeric tau in cortical and hippocampal total protein extract of 13-month htau mice

Western blot, ELISA and immunofluorescence analysis was used to measure total tau and oligomeric tau levels in the total protein extracts of htau mice treated with NIR light (filled bars) and htau mice receiving the sham no light treatment (open bars). Representative Western blots of total tau levels, using the Tau5 antibody, in the total protein extract of the (**a**) cortex and the (**b**) hippocampus of NIR light and sham treated htau mice. (**c**) Oligomeric tau levels were measured by calculating the densitometry of the Western blot bands greater than 110 kDa. (**d**) The total tau levels in the total protein extracts of the cortex and hippocampus, using Tau5 antibody, was also analyzed through ELISA analysis. Immunofluorescence was used to further measure the total and oligomeric tau levels. Total tau levels were measured using the total tau specific antibody, Tau5, and oligomeric tau levels were measured using the oligomer tau specific antibody, T22, in the (**e**) cortex and (**f**) hippocampus of the treated htau mice. Together, these analyses found NIR light treated htau mice have a reduction of both the total tau levels and the oligomeric tau levels in the total protein extract. This phenomenon is observed in both the cortical and hippocampal regions. Student's two tailed t-test was used to determine statistical significance. (n=6 per group). Error bars represent SD. $^{*}p<0.05$.

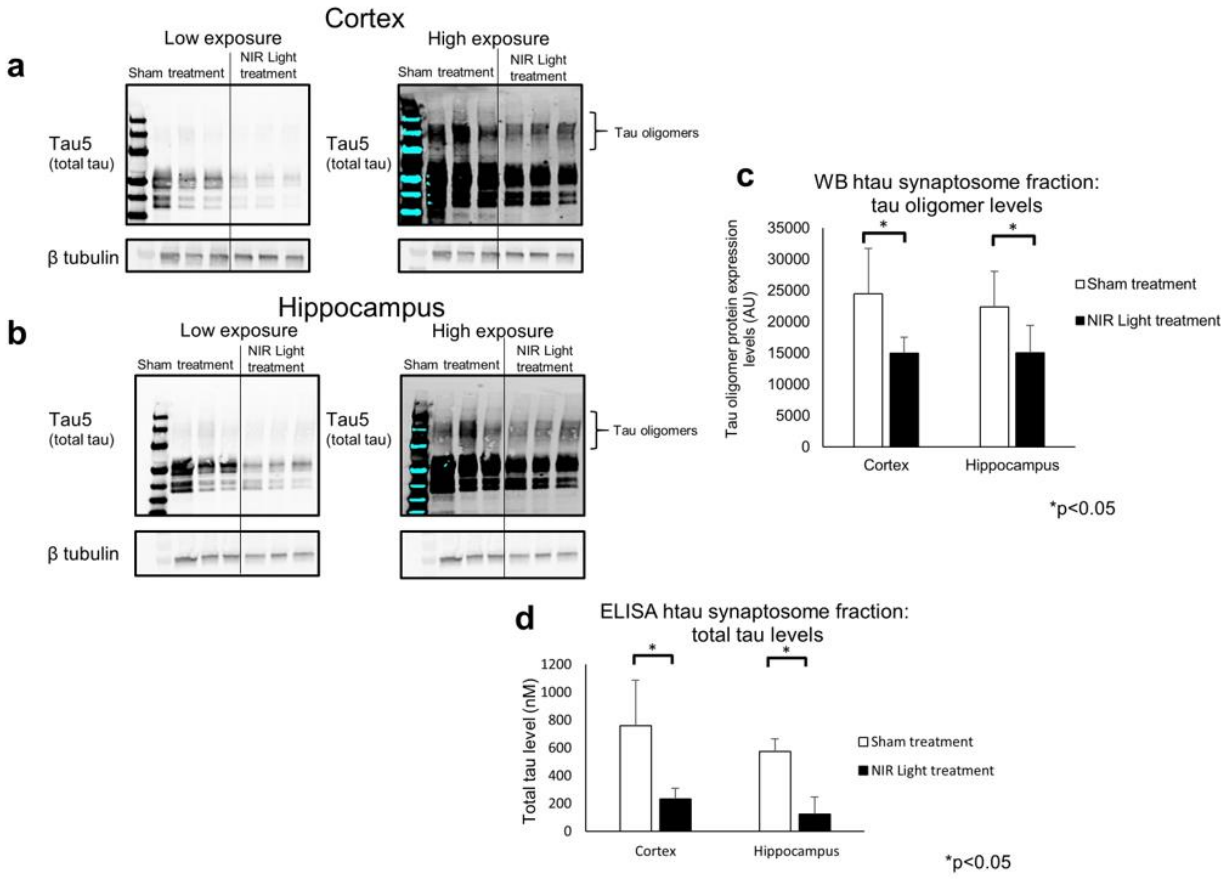


Figure 4.4. Reduced total and oligomeric tau at cortical and hippocampal synapses of 13-month htau mice.

Total and oligomeric tau was measured in the cortical and hippocampal synaptosome fractions of htau mice treated with NIR light by Western blot and ELISA analysis. Representative Western blots of the total tau levels, using the Tau5 antibody, in the (a) the cortical synaptosomes and (b) the hippocampal synaptosomes of NIR light and sham treated mice. The membranes were analyzed using different exposure rates; low exposure, to properly visualize the monomeric tau band, and high exposure, to visualize the oligomeric band of tau. The oligomeric tau band was measured in the high exposure (d) The total tau levels in the synaptosomes fractions of the cortex and hippocampus was also analyzed by ELISA analysis, using the total tau antibody, Tau5. The results suggest a reduction of total and oligomeric tau levels at the synapses in both cortical and hippocampal regions of NIR light treated htau mice. Student's two tailed t-test was used to determine statistical significance. (n=6 per group). Error bars represent SD. $^{*}p<0.05$.

Increased long term memory in NIR light treated htau mice

Due to the reduction in tau oligomers that were observed after NIR light treatment in htau mice, we next aimed to determine if such reduction would translate into a functional benefit in these mice. To determine if the cognitive function improved in htau mice treated with NIR light, we performed the novel object recognition (NOR) paradigm immediately following the last NIR light treatment. During the training phase, the mice were allowed to freely explore for 10 minutes, two identical objects placed in the testing arena. To determine long term memory, 24 hours after the training phase was completed, one of the two objects was replaced by a novel object and the mice were again allowed to freely explore the objects. Based on the propensity of mice to spend more time exploring an object they have not explored before, an extended amount of time exploring the novel object reflects memory of the familiar object. The object discrimination ratio (ODR) was calculated to determine the percentage of time spent with the novel object **(Figure 4.5)**.

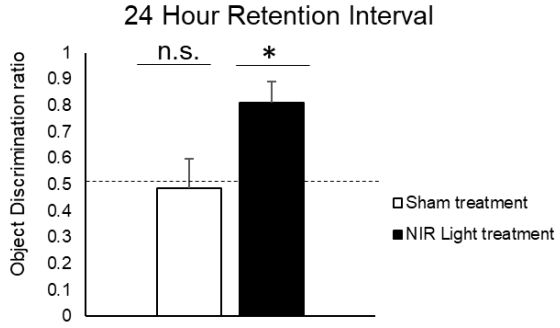


Figure 4.5. Improved memory in NIR light treat htau mice.
The novel object recognition (NOR) test was used to determine the long-term memory of htau mice that received NIR light (filled bar) or sham (open bar) treatments (n=6; per group). Object discrimination was calculated by the time spent with the novel object divided by the total time exploring the novel and familiar object. The results suggest that the memory impairment in htau mice is alleviated in htau mice receiving NIR light treatment. The object discrimination ratios of each group were analyzed by the one-sample t test to determine the statistical variation from chance (0.50). n.s. represents not statistically significant significance *$p<0.05$

We found the animals treated with NIR light had an ODR of about 0.8 (one sample t test, $p=0.0001$) of the novel object indicating an increased exploration of the novel object compared to the familiar

object. On the other hand, the htau mice receiving no treatment had an ODR of about 0.48 (one sample t test, p=0.765), indicating no difference in the exploration time between the novel and familiar object. This suggests a reduced impairment of memory in NIR light treated htau mice.

Reduced tau and Aβ pathology in the cortex and hippocampus of NIR light treated 13-month-old 3xTgAD mice

In order to determine if NIR impacts the clearance of both Aβ and tau in a combined endogenous system, we investigated changes in both Aβ and tau oligomers at the synapses and in total protein extracts of 3xTgAD mice. The 3xTgAD mice model exhibit overexpression of three AD-relevant human genes; human APP bearing the Swiss mutation, human tau with a P301L mutation and presinilin-1 with the M146V mutation (Oddo et al., 2003). These mice develop accumulations of Aβ oligomers around 4 months of age and tau fibrils around 12 months. We began the NIR light treatment at 12 months of age to ensure accumulations of both toxic proteins (n=7; per group). We first employed Western blot, ELISA and immunofluorescence analysis to measure levels of total and oligomeric tau in the total protein extracts from the cortical and hippocampal regions of 3xTgAD mice receiving NIR light treatment by **(Figure 4.6)**. As shown in the figure, the densitometry of the 110 kDa bands and higher was measured in the Western blot analysis using the tau5 antibody to determine the levels of tau oligomers **(Figure 4.6a, b)**. There was a statistically significant decrease in the tau oligomers in both brain regions of the NIR light treated mice compared to the sham treated animals (cortex p= 0.001, hippocampus p=0.047) **(Figure 4.6c)**. We further measured the total tau levels using the tau5 ELISA and found a similar decrease in the total tau levels in both brain regions of the NIR light treated mice (cortex p=0.038, hippocampus p=0.049) **(Figure 4.6d)**. We further used immunofluorescences to verify the reduction of tau oligomers, using the tau oligomer specific antibody T22, and total tau, using the total tau antibody tau5. We found a decrease of both oligomeric and total tau in the hippocampus (p=0.001, p=0.045) and cortex (p=0.007, p=0.045) of NIR light treated mice compared to the sham treated **(Figure 4.6e, f)**. We next measured the total and

oligomeric tau levels in the synaptosomal fractions of NIR light treated 3xTgAD mice by Western blot and ELISA analysis. Through Western blot analysis, we found a decrease in the oligomeric tau levels in the cortex and hippocampus regions (cortex p=0.009, hippocampus p=0.038). In addition, a significant decrease of total tau levels in the synaptosome fractions was observed in the cortex and the hippocampus (cortex p=0.039, hippocampus p=0.015) **(Figure 4.7c)** of NIR light treated mice, as measured by tau5 ELISA. Finally, we measured the levels of Aβ in the synaptosomal fractions and total protein extracts from the cortex and hippocampus synaptosomal fractions analyzed by a $A\beta_{1\text{-}42}$ specific ELISA. As shown in **Figure 4.8a**, Aβ levels was reduced in the synaptosomal fractions from the cortex and hippocampus regions of NIR light treated mice as compared to sham animals (cortex p= 0.002, hippocampus p= 0.040). However, the total protein extracts had equivalent levels of Aβ in the NIR light treated group compared to the sham treated group (cortex p= 0.495, hippocampus p= 0.145) **(Figure 4.8b)**. Collectively these results show a similar decrease in the tau and Aβ levels in the 3xTgAD mice as observed in the individual tg mouse models (tau or Aβ).

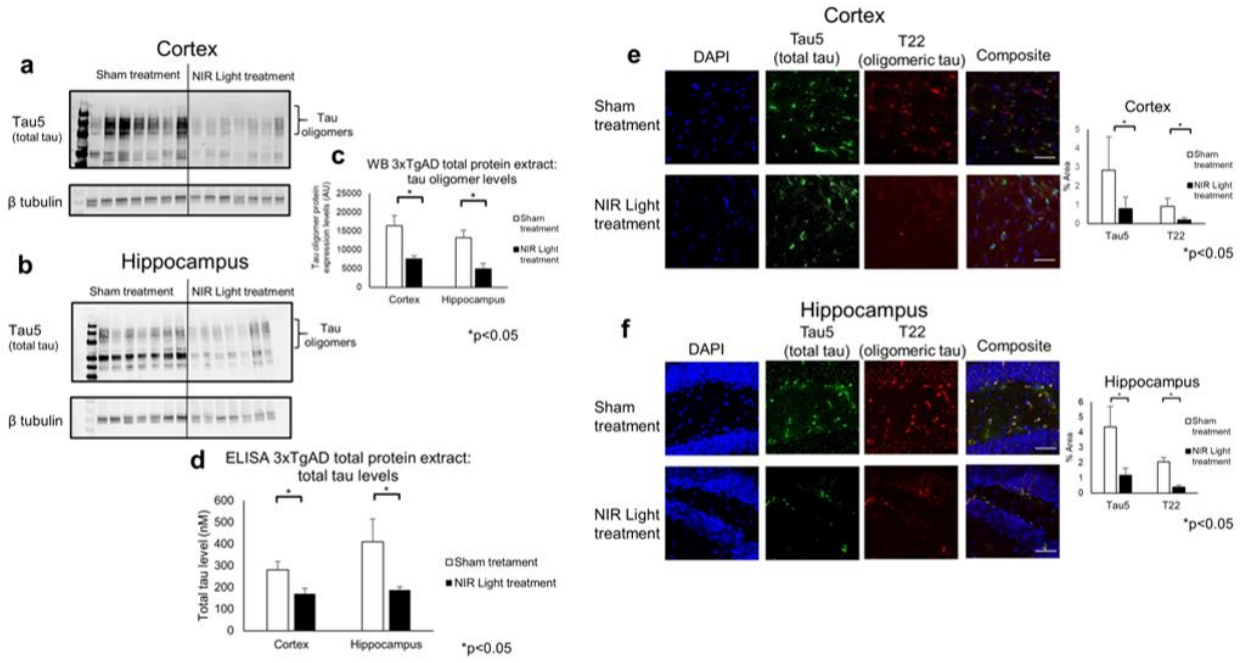


Figure 4.6. Reduced total and oligomeric tau in cortical and hippocampal total protein extracts of 13-month 3xTgAD mice.

The total protein extracts from the cortex and hippocampus of 3xTgAD mice receiving NIR light treatment (filled bars) or sham treatment (open bars) were analyzed by Western blot, ELISA and immunofluorescence analysis. Oligomeric tau levels were determined by measuring the bands displayed at 110 kDa and above on Western blots of total tau levels, using the Tau5 antibody, in the total protein extract of the (**a,c**) cortex and the (**b,c**) hippocampus of NIR light and sham treated 3xTgAD mice. (**d**) ELISA analysis using the total tau antibody, Tau5, was used to measure the levels of all forms of tau in the cortex and hippocampus. Further, immunofluorescence with the Tau5 and the tau oligomer specific antibody, T22 was used to determine the total and oligomeric tau levels in the (**e**)cortex and (**f**) hippocampus. These results found a reduction of total and oligomeric tau in the total protein extracts of the cortex and hippocampus of NIR light treated 3xTgAD mice. Student's two tailed t-test was used to determine statistical significance. (n=7 per group). Error bars represent SD. $^{*}p<0.05$.

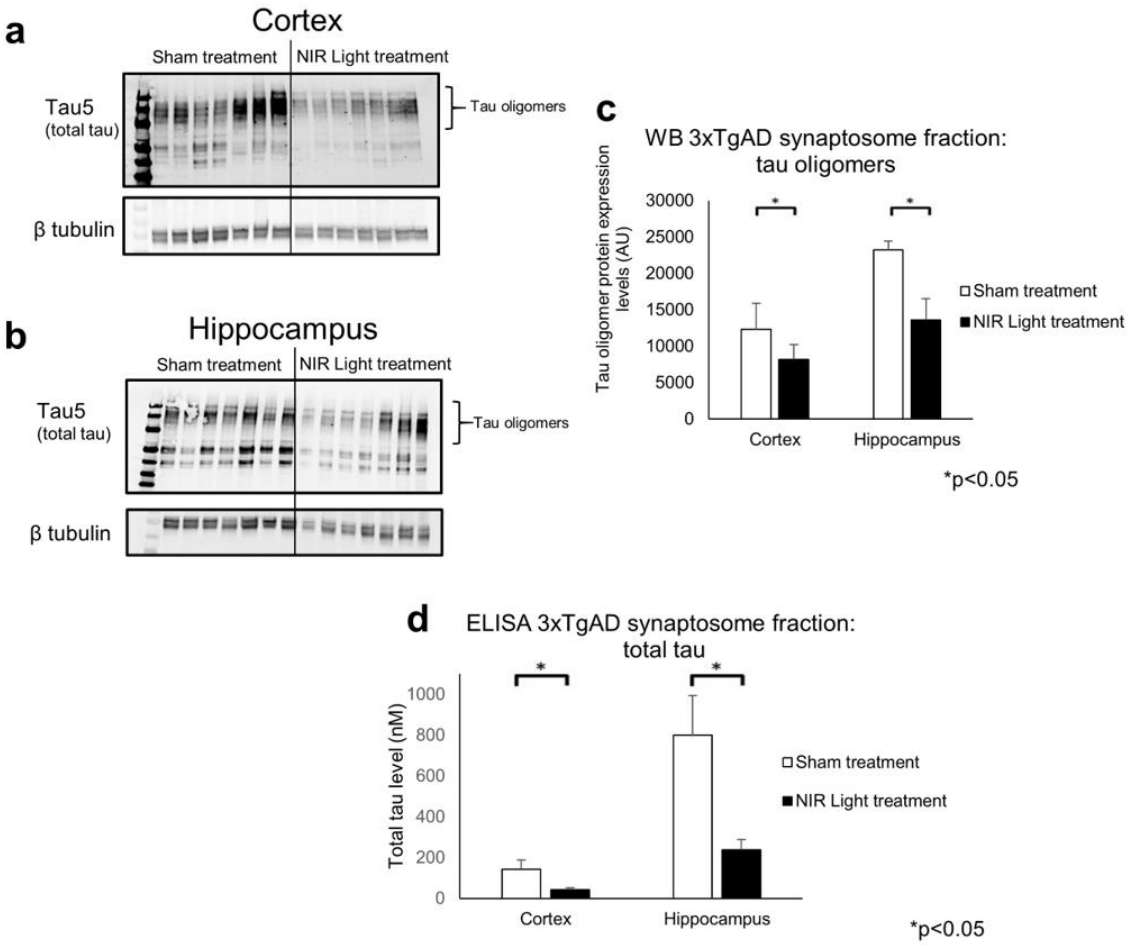


Figure 4.7. Reduced total and oligomeric tau at cortical and hippocampal synapses of 13-month 3xTgAD mice.

The total and oligomeric tau levels in the synaptosomal region of 3xTgAD mice receiving NIR light (filled bars) and sham (open bars) treatments were measured by Western blot and ELISA analysis. Representative Western blots of total tau levels, Tau5 antibody, in the synaptosomes of the (**a**) cortex and the (**b**) hippocampus of NIR light and sham treated 3xTgAD mice. (**c**) Western blot bands greater than 110 kDa were measured to determine the levels of the oligomeric form of tau. (**d**) The total tau levels in the total protein extracts of the cortex and hippocampus was also analyzed through tau5 ELISA analysis. The NIR light treated 3xTgAD mice had reduced levels of both total tau and oligomeric tau in cortical and hippocampal synaptosomes. Student's two tailed t-test was used to determine statistical significance. (n=7 per group). Error bars represent SD. $^{*}p<0.05$.

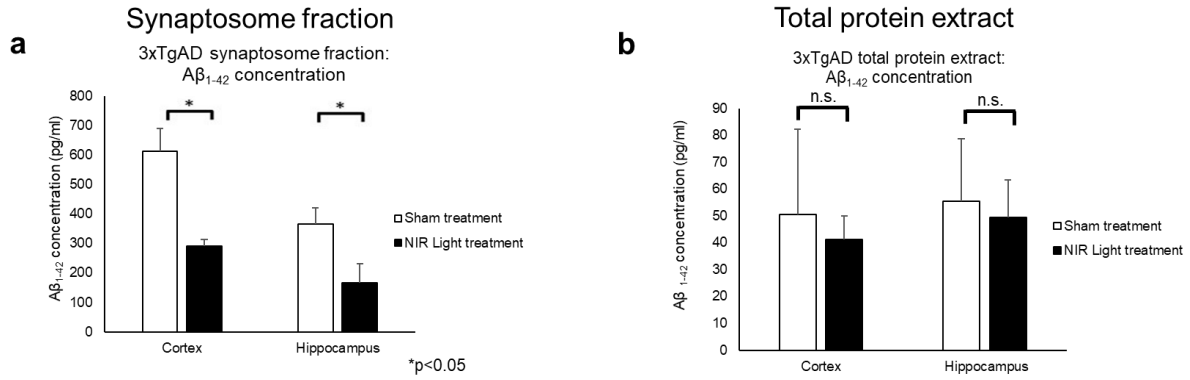


Figure 4.8. Amyloid beta levels are decreased in the synapses of 3xTgAD NIR light treated mice Aβ levels in (**a**) synaptosome fraction and (**b**) the total protein extract from the cortex and of NIR light (filled columns) and sham (open columns) treated 3xTgAD mice (n=7; per group) were measured using ELISA analysis. (**a**) NIR light treated mice had decreased levels of $A\beta_{1-42}$ in the synaptosome fractions from both the cortex and hippocampus. (**b**) However, there was no change in the levels of $A\beta_{1-42}$ in the total protein extracts of either region. Student's two tailed t-test was used to determine statistical significance. Error bars represent standard deviation. *p< 0.05; n.s. represents not statistically significant.

Increased inducible HSP70 in the synapses of NIR light treated 3xTgAD, htau and wild type mice

The results of our experiments suggested that NIR light initiates the reduction of both the total tau and the oligomeric form of tau. To investigate a potential mechanism for this clearance, we measured heat shock protein 70 (HSP70) levels in wild type, 3xTgAD and htau mice after NIR light treatment. We elected to investigate HSP70 because previous studies have described the intimate relationship between upregulation of inducible HSP70 and the reduction of tau (Jinwal et al., 2013). We utilized Western blot analysis to measure the protein levels of inducible HSP70 and the constitutive form of HSP70, HSC70, in the synaptosomal fraction as well as in the total protein extract from brains of mice treated with NIR light. We found that in all three animal models there was an increase of HSP70 levels in the synaptosomal fractions **(Figure 4.9a-c)** ((a)p=0.049 (b) p=0.034 (c) p=0.029) but no change in the total protein extract **(Figure 4.9d-f)** ((d) p=0.651 (e) p=0.127 (f) p=0.844). On the other hand, there was no change in the levels of constitutively active HSC70 in either the total protein or the synaptosome fractions of the NIR light treated mice ((a) p=0.664 (b)

p=0.143 (c) p=0.268 (d) p=0.401 (e) p=0.084 (f) p=0.275). These results indicate a selective increase of the inducible HSP70 at the synapse in NIR light treated mice.

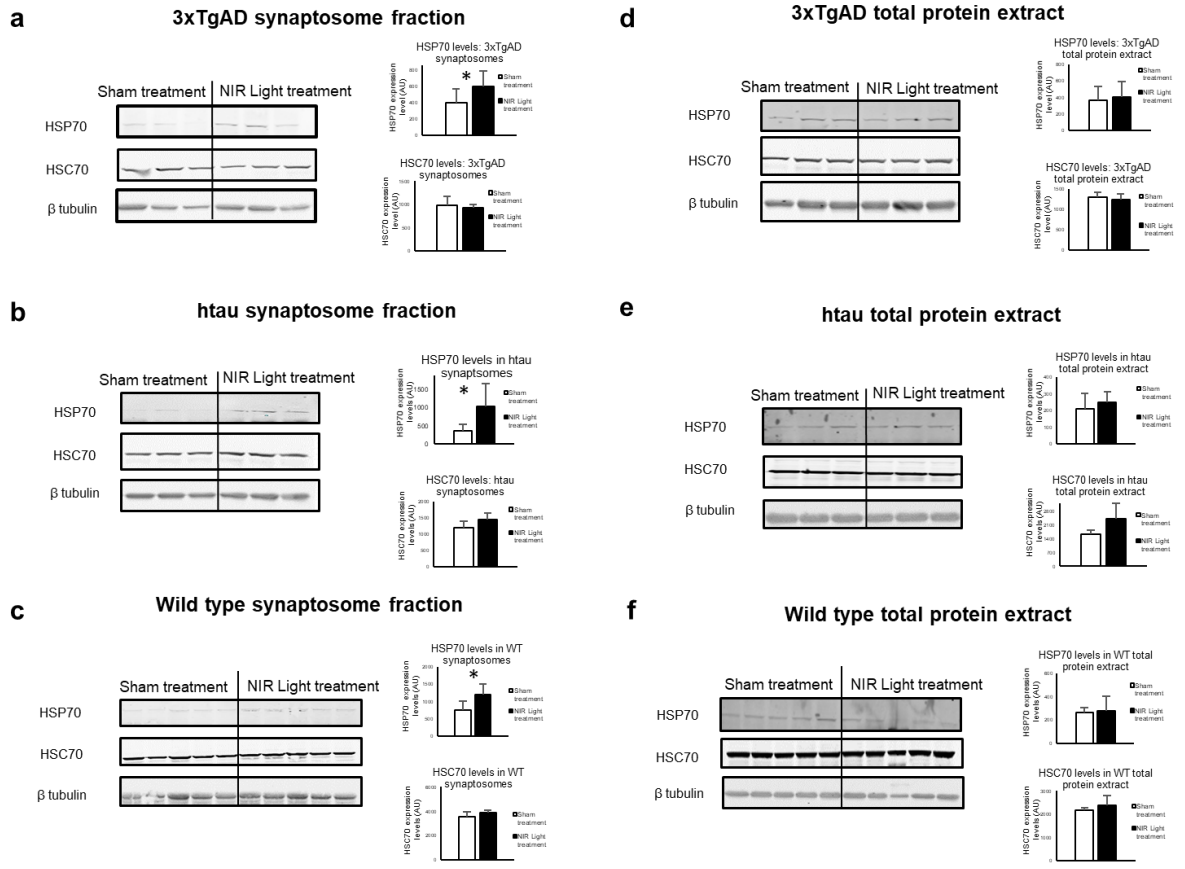


Figure 4.9. HSP70 is increased in the synaptosomal fractions but not in the total protein extracts.
Representative Western blot analysis of inducible HSP70 (iHSP70) and HSC70 protein levels in 3xTgAD, htau and wild type mice hippocampal synaptosome fractions and total protein extract. The synaptosome fractions of NIR light treated (filled bars) (**a**) 3xTgAD (**b**) htau (**c**) wild type mice had increased levels of the inducible HSP70 protein but no changes in HSC70 protein expression levels compared to sham treated groups (open bars). The total protein extracts of (**d**) 3xTgAD (**e**) htau (**f**) wild type mice had no change in either inducible HSP70 levels or HSC70 levels between the NIR light treated and the sham no light treated groups. These results suggest a synaptic specific increase in inducible HSP70 in NIR light treated mice. Statistical significance was determined by Student's two tailed t-test analysis. Error bars represent standard deviation. *p<0.05.

Increased induction of autophagy in NIR light treated 3xTgAD.

To further gain an understanding of the contributing mechanisms to the NIR light induced reduction of tau in the transgenic mice models, we measured the levels of autophagy related proteins. In hippocampal total protein extracts, we measured the protein levels, as well as, the expression of mRNA of LC3A and B, and Atg5, proteins key in the autophagy initiation. We found that the levels

of LC3B is increased (p=0.001) in the NIR light treated mice (n=8) compared to sham treated mice (n=10) while the levels of LC3A remained unchanged (0.992) between the treatment groups **(Figure 4.10a)**. The increased ratio between LC3B and LC3A (p=0.008) in the NIR light treated group suggests the increased promotion of autophagosome formation. The protein expression levels of Atg5 showed a trend of an increase in NIR light treated mice but not statistically significant (data not shown). A comparison of Atg5 mRNA expression between these NIR light treated and sham treated mice showed a statistically significant increase (p= 0.044) in the NIR light treated 3xTgAD **(Figure 4.10b)**. These results suggest NIR light treated 3xTgAD mice have increased expression of proteins that contribute to the induction of autophagy.

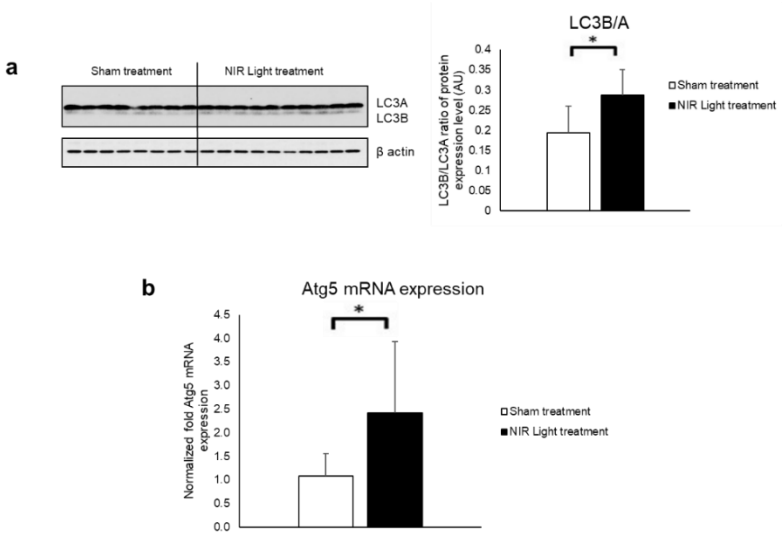


Figure 4.10. Upregulation of autophagy markers in total protein extracts after NIR light treatment

Levels of autophagy markers in the total protein extracts of NIR light (filled bars) and sham treated (open bars) 3xTgAD mice were determined by Western blot analysis and PCR. (**a**) Representative Western blot of LC3A and LC3B protein expression levels showed an increase ratio of LC3B/A in the NIR light treated group. (**b**) An increase of Atg5 mRNA expression in NIR light treated 3xTgAD mice was measured by PCR. These results suggest autophagy is initiated in NIR light treated 3xTgAD mice. Statistical significance was determined by Student's two tailed t-test analysis. Error bars represent standard deviation. *p<0.05.

DISCUSSION

The impairment of synaptic function is a key event that initiates and propagates the progressive cognitive decline that is associated with Alzheimer's disease (AD) (Terry et al., 1991; Selkoe, 2002). The oligomeric form of the microtubule associated protein tau is emerging as a key contributor to the disruption of synaptic function in AD (Lasagna-Reeves et al., 2011; Tai et al., 2014; Fontaine et al., 2017). The main goal of our study was to determine if NIR light induces neuroprotection against the synaptic association and accumulation of the toxic tau oligomers, as well as the subsequent oligomer-driven dysfunction. We utilized an *ex vivo* binding challenge to determine if synaptosomes isolated from NIR light-treated wild type (wt) mice displayed an altered affinity to tau oligomers. The results showed an equivalent tau association to the synapses isolated from the NIR light treated group compared to the sham treated mice. We further measured the long term potentiation (LTP) in the Schaffer collateral pathway of the hippocampus after the application of tau oligomers to brain slices prepared from these NIR light treated wt mice that has previously been shown to induce an impairment of LTP induction (Fá et al., 2016). As in the case of the binding challenge experiments, the NIR light treated group had similar LTP impairment after incubation with tau oligomers as observed in the sham treated group exposed to tau oligomers. Contrary to what was previously observed in an Aβ challenge system, these results suggest that NIR light does not induce mechanisms that reduce the synaptic vulnerability to and functional impact of tau oligomers. The specificity of the NIR light-induced neuroprotection could suggest that tau and Aβ oligomers act on the synapses in differing ways. While Aβ oligomers are known to have multiple synaptic binding partners such as mGluR5 and α7-nicotinic acetylcholine receptors among others (Parri et al., 2011; Um et al., 2013), the mechanisms by which extracellular tau oligomers associate with the synapses remain elusive. Previous studies have implied that the similar structures of Aβ and tau oligomers contribute to similar mechanisms of toxicity (Kayed, 2003b). However, our combined studies suggest that NIR light alters mechanisms that exclusively contribute to Aβ oligomer synaptic association.

Although a change in the association of tau oligomers to the synapses was not observed in our *ex vivo* synaptic challenge system, we next aimed to determine if the administration of NIR light

treatments in two mouse models of tauopathies, htau (accumulation of tau) and 3xTgAD (accumulation of Aβ and tau), would mediate the synaptic accumulation of tau oligomers. Because of the complex relationship between Aβ and tau with extensive evidence that these proteins can influence the pathology of each other, it is critical to examine the proteins in a combined mouse model as well as in simpler mouse models where Aβ or tau are singly overexpressed. Previous research has found a general reduction of hyperphosphorylated tau in the Parkinsonian mouse model K369I upon exposure to NIR light (Purushothuman et al., 2014). However, whether this phenomenon would extend to the synaptic accumulation of tau oligomers (a major determinant of tau neurotoxicity) remained unexplored. In a normal state, tau is localized to the axonal region of neurons with low levels in the synaptic regions contributing to the stability of protein scaffolding. However, in AD tau relocalizes to the somatodendritic region. The elevated levels of tau oligomers in the synapses is believed to contribute to the interference of synaptic function (Delacourte et al., 1990; Zempel and Mandelkow, 2014; Li and Götz, 2017). The htau mice, a mouse that expresses a human MAPT transgene and knockout of mouse MAPT, served as a model that exclusively expresses the accumulation of tau protein (Andorfer et al., 2003). Our results showed not only a reduction of tau oligomer levels at the synapses in NIR light treated animals but also in the cortical and hippocampal total protein extract. We further found that total tau levels, which include monomers as well as all aggregated tau specie, are decreased in both the synapses and the total protein extract. Unlike our previous results in which NIR induces a selective reduction of Aβ oligomers at the synapses in the human amyloid precursor protein (APP) overexpressing mouse model, Tg2576, (Comerota et al., 2017), the tau reduction in htau mice is thus not occurring specifically at synapses but rather globally. Nonetheless, this molecular phenomenon of overall reduced tau oligomers translates into a functional benefit as illustrated by the observed memory improvement in the novel object recognition (NOR) test on htau mice that received NIR light treatment. This mouse model is known to have memory deficits as measured by NOR at 10 months of age (Castillo-Carranza et al., 2014). The NIR light treated htau displayed an improved performance compared to the sham treated htau mice, as measured

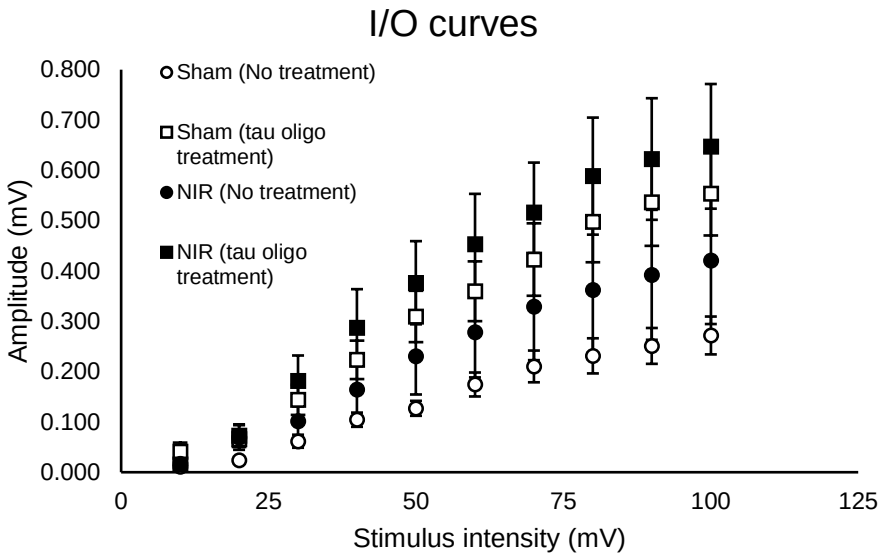
by the increased time spent with the novel object. This test indicates that the NIR light treatment ameliorated the cognitive impairment typically displayed by htau mice at this age. Collectively, our data show that NIR light treatment reduced the tau pathology and the corresponding memory deficits in htau mice.

The second mouse model we utilized, 3xTgAD, exhibits overexpression of three genes associated with familial AD; presenilin 1, human tau with the P301L mutation and human APP with the Swedish mutation. These mice display deposits of both aggregated Aβ and tau by 12 months of age, serving as a model for the simultaneous presence of these toxic proteins as observed in AD (Oddo et al., 2003). We found that both total and oligomeric tau was reduced in the synaptic compartment and in the total protein extract in 3xTgAD mice, whereas the Aβ was reduced exclusively at the synapses. This is similar to what we observed here in the htau mice, as well as we previously shown in the Tg2576 mice (Comerota et al., 2017). Our results thus suggest that the NIR light-induced mitigation of the individual amyloid proteins is sufficient and equally effective when the two amyloid proteins coexist in a system, supporting NIR light as a promising treatment for AD.

While the primary mechanisms of action of the NIR light (600-1000 nm) in stimulating bioenergy output and efficiency of mitochondria has been well established, the secondary mechanisms that lead to neuroprotection remains poorly understood (Karu, 2010a). The systemic reduction of both total and oligomeric tau in NIR light-treated htau mice that we report here suggests that NIR light initiates mechanisms that contribute to the regulation and the clearance of tau. In the current study we investigated the chaperone heat shock protein 70 and the induction of autophagy as two known pathways that have been reported to promote the degradation of dysfunctional tau (Orr and Oddo, 2013; Evgen'ev et al., 2017). The heat shock protein 70 (HSP70) family of proteins are chaperones that are involved in the refolding and shuttling of dysfunctional proteins to degradation pathways (Leak, 2014). There is extensive literature describing the intimate relationship between inducible HSP70 and the clearance of the toxic tau proteins. Particularly, evidence demonstrates the reduction of aggregated tau after stimulating the activity of or overexpressing endogenous inducible

HSP70 or following administration of exogenous inducible HSP70 (Young et al., 2016; Evgen'ev et al., 2017; Kundel et al., 2018). On the other hand, an opposite correlation exists between tau and heat shock cognate 70 (HSC70), a constitutively expressed member of the HSP70 family, whereby HSC70 overexpression slows the clearance of misfolded tau deposition (Jinwal et al., 2013). The observed exclusive synaptic increase of inducible HSP70 and unchanged levels of HSC70 after NIR light treatment that we observed in all animal models surveyed here suggests specificity of the phenomenon. Such increase could indicate a specific induction of HSP70 in neurons or possibly reflect the relocalization of inducible HSP70 to the synapses as a means of protecting the compartment from future insults. We further investigated the autophagy pathway, one of the pathways that HSP70 has been shown to shuttle dysfunctional tau to degeneration pathways (Demand et al., 2001). The increase in expression levels of the autophagy related protein Atg5 and the raised ratio between LC3A and LC3B in the total protein extracts in NIR treated 3xTgAD mice suggests an increased induction of autophagy as reflected by increased production of autophagosomes. Atg5 is associated with the elongation of the autophagosomal membrane and the LC3B protein is necessary for the formation and closure of the autophagosome (Friedman et al., 2015). The increased expression of these proteins thus demonstrates the increased availability of important machinery involved in the degradation of dysfunctional tau in NIR light treated animals, providing the opportunity for the increased clearance of the toxic protein. Together, these results provide insight into chaperone-mediated clearance and induced autophagy mechanisms that may be contributing to NIR light-induced reduction of tau.

In conclusion, this study provided valuable evidence of the beneficial effects of NIR light treatments on the reduction of tau pathology and related cognitive dysfunction. The novel demonstration of this reduction of toxic tau species in the htau mice, combined with the decrease of synaptic Aβ pathology in the Aβ/tau co-expressing 3xTgAD mice, further support the effectiveness of NIR light as a non-invasive treatment to reduce AD-related neuropathology and encourages its future clinical development.



Supplementary Figure 4.1. Input/output curves for the four treatment groups.
The fEPSP amplitude (mV) obtained at increasing stimulus intensities (mA) show no significant differences in the basal synaptic strength following NIR light treatment and/or exposure to Aβ oligomers compared to sham (no treatment). n=6-8 slices from 3-6 mice; Statistical analysis was carried out using two-way ANOVA with Bonferroni post-hoc analysis, ns. Error bars represent standard error of mean.

MEAN (n=6)	Sham (No treatment)		Sham (tau oligo treatment)		NIR (No treatment)		NIR (tau oligo treatment)	
mV	Pre	Post	Pre	Post	Pre	Post	Pre	Post
10	0.011	0.034	0.041	0.100	0.017	0.059	0.016	0.080
20	0.024	0.033	0.066	0.051	0.069	0.038	0.073	0.069
30	0.062	0.077	0.144	0.151	0.102	0.130	0.181	0.187
40	0.104	0.115	0.223	0.226	0.164	0.167	0.287	0.276
50	0.127	0.150	0.310	0.285	0.230	0.236	0.377	0.334
60	0.174	0.194	0.360	0.342	0.278	0.266	0.453	0.388
70	0.210	0.239	0.423	0.384	0.329	0.319	0.516	0.478
80	0.231	0.280	0.498	0.440	0.362	0.342	0.588	0.487
90	0.251	0.298	0.537	0.463	0.392	0.366	0.622	0.495
100	0.272	0.289	0.554	0.501	0.421	0.379	0.648	0.511

SEM (n=6)	Sham (Untreated)		Sham (tau oligo Treated)		NIR (Untreated)		NIR (tau oligo Treated)	
mV	Pre	Post	Pre	Post	Pre	Post	Pre	Post
10	0.004	0.007	0.018	0.029	0.004	0.013	0.006	0.011
20	0.005	0.008	0.016	0.014	0.024	0.013	0.022	0.035
30	0.013	0.013	0.030	0.048	0.038	0.032	0.051	0.056
40	0.014	0.021	0.038	0.062	0.058	0.053	0.076	0.085
50	0.015	0.019	0.051	0.078	0.076	0.075	0.083	0.085
60	0.024	0.033	0.059	0.083	0.090	0.079	0.100	0.101
70	0.032	0.042	0.072	0.091	0.106	0.095	0.099	0.126
80	0.035	0.052	0.081	0.101	0.125	0.100	0.116	0.106
90	0.036	0.057	0.087	0.106	0.129	0.108	0.121	0.112
100	0.038	0.055	0.083	0.119	0.127	0.112	0.124	0.117

Supplementary Table 4.1. Table of input/output averages for the four treatment groups.

The averages of the amplitudes (mV) measured in the four treatment groups after increasing stimulus intensities. There was no change in the pre-HFS and post-HFS amplitudes for all four treatment groups. n=6-8 slices from 3-6 mice; Statistical analysis was carried out using two-way ANOVA with Bonferroni post-hoc analysis.

Chapter 5: Conclusions and Future Directions

CONCLUSIONS

The experiments in this study provided novel evidence that toxic oligomers of both Aβ and tau are reduced at the synapse after NIR light treatments in both transgenic animal models overexpressing individual amyloids (i.e., htau mice for tau and Tg3576 mice for Aβ) and co-expressing tau and Aβ (i.e., the 3xTgAD mice). This provides evidence that NIR light induces a direct reduction of each individual protein that is further seen when Aβ and tau pathology co-exists in a transgenic animal system closely mimicking the clinical scenario of AD. An interesting component of this phenomenon is the selective reduction of Aβ oligomers at the synapses with no change in Aβ levels in the total protein extracts, that we observed in both the Tg2576 and 3xTgAD mouse models. Furthermore, we found that synapses from wt mice treated with NIR light were resistant to the binding of Aβ oligomers, overall suggesting that the NIR light treatment specifically made synapses less vulnerable to endogenous or exogenous Aβ synaptotoxic species. By contrast, tau oligomer levels were equally reduced at the synapses and in total protein extracts in NIR light-treated htau and 3xTgAD mice, and NIR light afforded no specific synaptic protection against the functional disruption brought about by exogenously applied tau oligomers. The reduction of synaptic Aβ oligomers is similar to the phenomenon observed in the cognitively intact NDAN individuals. These individuals have reduced Aβ oligomer levels at the synapses but substantial levels of Aβ in the total protein extract comparable to demented AD humans. The tau oligomer levels in NDAN individuals is still under investigation, however, preliminary observations suggest that synaptic tau oligomers are also reduced. It seems therefore that NIR light in our animal model systems is inducing similar mechanisms that serve as neuroprotection in NDAN individuals. Nonetheless, further investigation needs to be completed to discern the exact mechanisms that could be contributing to the reduction of the synaptic Aβ and tau oligomers.

Using an established *ex vivo* system involving isolated brain synaptosomes we investigated the binding of exogenous amyloid oligomers to the synapses to determine a direct modulation of this key event by NIR light. While we found a reduction of Aβ oligomer binding to the synapses of wild type mice treated with NIR light treated mice, no change in the binding of tau oligomers was observed. This could possibly be due to different mechanisms by which tau oligomers and Aβ oligomers associate with the synapses. Such differential mechanisms need to be further investigated to fully characterize the binding profile of the two amyloid proteins to the synapses and the mechanisms that contribute to the selective reduction of their association, an event that holds high interest as a viable therapeutic target in AD. From a functional point of view, we further observed a similar differential impact of NIR light on the detrimental suppression of synaptic plasticity brought about by exogenously-applied Aβ and tau oligomers in brain slices from NIR light-treated wild type mice.

Collectively these results demonstrate that NIR light, while reducing levels of both Aβ and tau oligomers, selectively induces synaptic resistance to Aβ oligomers and the resulting impairment of synaptic function. The difference in reduction patterns of the levels of Aβ and tau oligomers and the selective protection against Aβ oligomer synaptic binding thus implies that different mechanisms of clearance or protection against each amyloid protein is being induced by NIR light.

To gain a greater understanding of the mechanisms that may be contributing to the beneficial effects of NIR light, we investigated several mechanisms associated with synaptic protection against Aβ oligomers and the clearance of tau oligomers. We found an increase in synaptic mitochondria efficiency in NIR light treated wild type mice, a phenomenon that has previously been shown to correlate with decreased Aβ synaptic binding. Further, we found an upregulation of autophagy markers and chaperone proteins associated with shuttling tau to degradation pathways. These results provide novel evidence of mechanisms directly induced by NIR light that may contribute to neuroprotection and the slowing of the progression of AD.

In conclusion, my research has contributed critical missing information to support the development of NIR light as a plausible efficient therapy for AD. The noninvasive aspect of its administration also opens the possibility of treating patients prior to the development of the clinical manifestation of symptoms and LED devices also serve as affordable and convenient mode of treatment expected to gain rapid access to the clinical evaluation.

FUTURE DIRECTIONS

While the depth of studies aimed at understanding biological mechanisms induced by NIR light is continuously growing, much is left to be understood. There are several other events that have been proposed as driving the onset and clinical progression of AD, such as neuroinflammation, oxidative damages and reduced neurogenesis, that can be further investigated after NIR light treatment to fully determine the comprehensive extent of its expected benefits. The current study demonstrated NIR light-induced synaptic resilience to Aβ oligomer binding and protection from its induced toxicity in wild type mice, a phenomenon that appears to mimic what was originally observed in the NDAN individuals that resist the onset of dementia despite the presence of extensive AD-like neuropathology. The current studies also suggested increased synaptic mitochondrial efficiency as a means contributing to this reduction of Aβ synaptic binding. However, further studies can be conducted to determine changes in the proteomic signature of the synapse after NIR light treatment that contributes to the reduction of Aβ oligomer association with the synapse. Notably, further investigation of the changes that occur after NIR light-driven increased synaptic mitochondria health could lead to novel targets that induce synaptic protection against toxic amyloid oligomers.

The main goal of future research should focus on the translation of NIR light treatments to humans. One of the greatest obstacles of translation of light treatments to humans is administering a sufficient amount of light energy through the human skull and maximizing penetration depths to reach deep brain structures such as the hippocampus. Determining alternative routes of administration or more efficient NIR light sources such as nano-pulsed laser devices and if similar beneficial biological

mechanisms as reported here are initiated by such novel administration strategies will be ideal in the implementation of treatments in a clinical setting. Among the alternative routes of administration that can be investigated is implantation of optical fibers to deliver NIR light specifically to selective brain areas that are targeted early in AD such as the locus coeruleus and the hippocampus, which could reveal a novel approach to stop disease onset and/or progression. Indeed, the use of optic fibers in AD mouse models can also open the possibly of understanding the spread of AD pathology throughout the brain. Early administration of NIR light can be used to demonstrate the cessation of pathology spreading by inducing clearance in select regions of the brain. Although surgically implanting a fiber diminish the noninvasive nature of the treatment, similar probes are used in methods of deep brain stimulation in Parkinson's disease.

The studies presented in this dissertation provide a strong foundation to the understanding of the mechanisms by which NIR light induces the protection of synapses from the toxicity of both Aβ and tau oligomers. It is my expectation that the continued research of this treatment option will further the pursuit of NIR light treatments as a viable means of disease-modifying intervention in AD.

Bibliography

Ali YO, Allen HM, Yu L, Li-Kroeger D, Bakhshizadehmahmoudi D, Hatcher A, McCabe C, Xu J, Bjorklund N, Taglialatela G, Bennett DA, De Jager PL, Shulman JM, Bellen HJ, Lu H-C (2016) NMNAT2:HSP90 Complex Mediates Proteostasis in Proteinopathies Barres BA, ed. PLOS Biology 14:e1002472.

Alldred MJ, Duff KE, Ginsberg SD (2012) Microarray analysis of CA1 pyramidal neurons in a mouse model of tauopathy reveals progressive synaptic dysfunction. Neurobiology of Disease 45:751–762.

Alzheimer A, Stelzmann RA, Schnitzlein HN, Murtagh FR (1995) An English translation of Alzheimer's 1907 paper, "Uber eine eigenartige Erkankung der Hirnrinde." Clin Anat 8:429–431.

Andorfer C, Kress Y, Espinoza M, de Silva R, Tucker KL, Barde Y-A, Duff K, Davies P (2003) Hyperphosphorylation and aggregation of tau in mice expressing normal human tau isoforms. J Neurochem 86:582–590.

Barakos J, Sperling R, Salloway S, Jack C, Gass A, Fiebach JB, Tampieri D, Melançon D, Miaux Y, Rippon G, Black R, Lu Y, Brashear HR, Arrighi HM, Morris KA, Grundman M (2013)

MR Imaging Features of Amyloid-Related Imaging Abnormalities. American Journal of Neuroradiology 34:1958–1965.

Begum R, Calaza K, Kam JH, Salt TE, Hogg C, Jeffery G (2015) Near-infrared light increases ATP, extends lifespan and improves mobility in aged Drosophila melanogaster. Biology Letters 11:20150073–20150073.

Berman MH, Halper JP, Nichols TW, H J, Lundy A, Huang JH (2017) Photobiomodulation with Near Infrared Light Helmet in a Pilot, Placebo Controlled Clinical Trial in Dementia Patients Testing Memory and Cognition. Journal of Neurology and Neuroscience 08 Available at: http://www.jneuro.com/neurology-neuroscience/photobiomodulation-with-near-infrared-light-helmet-in-a-pilot-placebo-controlled-clinical-trial-in-dementia-patients-testing-memor.php?aid=18528 [Accessed April 15, 2018].

Binder LI, Frankfurter A, Rebhun LI (1985) The distribution of tau in the mammalian central nervous system. J Cell Biol 101:1371–1378.

Bjorklund NL, Reese LC, Sadagoparamanujam VM, Ghirardi V, Woltjer RL, Taglialatela G (2012) Absence of amyloid β oligomers at the postsynapse and regulated synaptic Zn 2+ in cognitively intact aged individuals with Alzheimer's disease neuropathology. Molecular neurodegeneration 7:23.

Bobba A, Amadoro G, Valenti D, Corsetti V, Lassandro R, Atlante A (2013) Mitochondrial respiratory chain Complexes I and IV are impaired by β-amyloid via direct interaction and through Complex I-dependent ROS production, respectively. Mitochondrion 13:298–311.

Bomfim TR, Forny-Germano L, Sathler LB, Brito-Moreira J, Houzel J-C, Decker H, Silverman MA, Kazi H, Melo HM, McClean PL, Holscher C, Arnold SE, Talbot K, Klein WL, Munoz DP, Ferreira ST, De Felice FG (2012) An anti-diabetes agent protects the mouse brain from defective insulin signaling caused by Alzheimer's disease–associated Aβ oligomers. Journal of Clinical Investigation 122:1339–1353.

Bond M, Rogers G, Peters J, Anderson R, Hoyle M, Miners A, Moxham T, Davis S, Thokala P, Wailoo A, Jeffreys M, Hyde C (2012) The effectiveness and cost-effectiveness of donepezil, galantamine, rivastigmine and memantine for the treatment of Alzheimer's disease (review of Technology Appraisal No. 111): a systematic review and economic model. Health Technology Assessment 16 Available at: https://www.journalslibrary.nihr.ac.uk/hta/hta16210/ [Accessed April 10, 2018].

Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. Acta Neuropathologica 82:239–259.

Brion JP, Flament-Durand J, Dustin P (1986) Alzheimer's disease and tau proteins. Lancet 2:1098.

Calkins MJ, Reddy PH (2011) Assessment of newly synthesized mitochondrial DNA using BrdU labeling in primary neurons from Alzheimer's disease mice: Implications for impaired mitochondrial biogenesis and synaptic damage. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1812:1182–1189.

Casey DA, Antimisiaris D, O'Brien J (2010) Drugs for Alzheimer's disease: are they effective? P T 35:208–211.

Castillo-Carranza DL, Gerson JE, Sengupta U, Guerrero-Muñoz MJ, Lasagna-Reeves CA, Kayed R (2014) Specific Targeting of Tau Oligomers in Htau Mice Prevents Cognitive Impairment and Tau Toxicity Following Injection with Brain-Derived Tau Oligomeric Seeds. Journal of Alzheimer's Disease 40:S97–S111.

Castillo-Carranza DL, Guerrero-Muñoz MJ, Sengupta U, Hernandez C, Barrett ADT, Dineley K, Kayed R (2015) Tau immunotherapy modulates both pathological tau and upstream amyloid pathology in an Alzheimer's disease mouse model. J Neurosci 35:4857–4868.

Chabrier MA, Blurton-Jones M, Agazaryan AA, Nerhus JL, Martinez-Coria H, LaFerla FM (2012) Soluble A Promotes Wild-Type Tau Pathology In Vivo. Journal of Neuroscience 32:17345–17350.

Chang W-D, Wu J-H, Jiang J-A, Yeh C-Y, Tsai C-T (2008) Carpal Tunnel Syndrome Treated with a Diode Laser: A Controlled Treatment of the Transverse Carpal Ligament. Photomedicine and Laser Surgery 26:551–557.

Chen Q-S, Wei W-Z, Shimahara T, Xie C-W (2002) Alzheimer Amyloid β-Peptide Inhibits the Late Phase of Long-Term Potentiation through Calcineurin-Dependent Mechanisms in the Hippocampal Dentate Gyrus. Neurobiology of Learning and Memory 77:354–371.

Chow RT, Johnson MI, Lopes-Martins RA, Bjordal JM (2009) Efficacy of low-level laser therapy in the management of neck pain: a systematic review and meta-analysis of randomised placebo or active-treatment controlled trials. The Lancet 374:1897–1908.

Cissé M, Halabisky B, Harris J, Devidze N, Dubal DB, Sun B, Orr A, Lotz G, Kim DH, Hamto P, Ho K, Yu G-Q, Mucke L (2011) Reversing EphB2 depletion rescues cognitive functions in Alzheimer model. Nature 469:47–52.

Citron M et al. (1997) Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid β-protein in both transfected cells and transgenic mice. Nature Medicine 3:67–72.

Citron M, Oltersdorf T, Haass C, McConlogue L, Hung AY, Seubert P, Vigo-Pelfrey C, Lieberburg I, Selkoe DJ (1992) Mutation of the β-amyloid precursor protein in familial Alzheimer's disease increases β-protein production. Nature 360:672–674.

Colovic MB, Krstic DZ, Lazarevic-Pasti TD, Bondzic AM, Vasic VM (2013) Acetylcholinesterase Inhibitors: Pharmacology and Toxicology. Current Neuropharmacology 11:315–335.

Comerota MM, Krishnan B, Taglialatela G (2017) Near infrared light decreases synaptic vulnerability to amyloid beta oligomers. Scientific Reports 7 Available at: http://www.nature.com/articles/s41598-017-15357-x [Accessed January 8, 2018].

da Luz Eltchechem C, Salgado ASI, Zângaro RA, da Silva Pereira MC, Kerppers II, da Silva LA, Parreira RB (2017) Transcranial LED therapy on amyloid-β toxin 25–35 in the hippocampal region of rats. Lasers in Medical Science 32:749–756.

De Felice FG, Wu D, Lambert MP, Fernandez SJ, Velasco PT, Lacor PN, Bigio EH, Jerecic J, Acton PJ, Shughrue PJ, Chen-Dodson E, Kinney GG, Klein WL (2008) Alzheimer's disease-type neuronal tau hyperphosphorylation induced by Aβ oligomers. Neurobiology of Aging 29:1334–1347.

De Taboada L, Yu, Jin, El-Amouri S, Gattoni-Celli S, Richieri S, McCarthy T, Streeter J, Kindy MS (2011) Transcranial Laser Therapy Attenuates Amyloid-β Peptide Neuropathology in Amyloid-β Protein Precursor Transgenic Mice. Journal of Alzheimer's Disease:521–535.

Delacourte A, Flament S, Dibe EM, Hublau P, Sablonnière B, Hémon B, Shérrer V, Défossez A (1990) Pathological proteins Tau 64 and 69 are specifically expressed in the somatodendritic domain of the degenerating cortical neurons during Alzheimer's disease. Demonstration with a panel of antibodies against Tau proteins. Acta Neuropathol 80:111–117.

Demand J, Alberti S, Patterson C, Höhfeld J (2001) Cooperation of a ubiquitin domain protein and an E3 ubiquitin ligase during chaperone/proteasome coupling. Curr Biol 11:1569–1577.

Devi L (2006) Accumulation of Amyloid Precursor Protein in the Mitochondrial Import Channels of Human Alzheimer's Disease Brain Is Associated with Mitochondrial Dysfunction. Journal of Neuroscience 26:9057–9068.

Dickson DW, Crystal HA, Bevona C, Honer W, Vincent I, Davies P (1995) Correlations of synaptic and pathological markers with cognition of the elderly. Neurobiol Aging 16:285–298; discussion 298-304.

Dineley KT, Hogan D, Zhang W-R, Taglialatela G (2007a) Acute inhibition of calcineurin restores associative learning and memory in Tg2576 APP transgenic mice. Neurobiol Learn Mem 88:217–224.

Dineley KT, Hogan D, Zhang W-R, Taglialatela G (2007b) Acute inhibition of calcineurin restores associative learning and memory in Tg2576 APP transgenic mice. Neurobiology of learning and memory 88:217–224.

Dineley KT, Kayed R, Neugebauer V, Fu Y, Zhang W, Reese LC, Taglialatela G (2010) Amyloid-β oligomers impair fear conditioned memory in a calcineurin-dependent fashion in mice. Journal of Neuroscience Research:n/a-n/a.

Doody RS, Raman R, Farlow M, Iwatsubo T, Vellas B, Joffe S, Kieburtz K, He F, Sun X, Thomas RG, Aisen PS, Siemers E, Sethuraman G, Mohs R (2013) A Phase 3 Trial of Semagacestat for Treatment of Alzheimer's Disease. New England Journal of Medicine 369:341–350.

Doody RS, Thomas RG, Farlow M, Iwatsubo T, Vellas B, Joffe S, Kieburtz K, Raman R, Sun X, Aisen PS, Siemers E, Liu-Seifert H, Mohs R (2014) Phase 3 Trials of Solanezumab for Mild-to-Moderate Alzheimer's Disease. New England Journal of Medicine 370:311–321.

Du H, Guo L, Yan S, Sosunov AA, McKhann GM, Yan SS (2010) Early deficits in synaptic mitochondria in an Alzheimer's disease mouse model. Proceedings of the National Academy of Sciences 107:18670–18675.

Duan R, Zhu L, Liu TC-Y, Li Y, Liu J, Jiao J, Xu X, Yao L, Liu S (2003) Light emitting diode irradiation protect against the amyloid beta 25-35 induced apoptosis of PC12 cell in vitro. Lasers in Surgery and Medicine 33:199–203.

DuBoff B, Götz J, Feany MB (2012) Tau Promotes Neurodegeneration via DRP1 Mislocalization In Vivo. Neuron 75:618–632.

Duff K, Knight H, Refolo LM, Sanders S, Yu X, Picciano M, Malester B, Hutton M, Adamson J, Goedert M, Burki K, Davies P (2000) Characterization of pathology in transgenic mice over-expressing human genomic and cDNA tau transgenes. Neurobiol Dis 7:87–98.

Duffy ÁM, Morales-Corraliza J, Bermudez-Hernandez KM, Schaner MJ, Magagna-Poveda A, Mathews PM, Scharfman HE (2015) Entorhinal cortical defects in Tg2576 mice are present as early as 2–4 months of age. Neurobiology of Aging 36:134–148.

Duggett NA, Chazot PL (2014) Low-Intensity Light Therapy (1068 nm) Protects CAD Neuroblastoma Cells from Î²-Amyloid-Mediated Cell Death. Biology and Medicine 01 Available at: https://www.omicsonline.com/open-access/lowintensity-light-therapy-nm-protects-cad-neuroblastoma-cells-from-amyloidmediated-cell-death-0974-8369-s1-1000003.php?aid=31009 [Accessed March 24, 2018].

Eckert A, Schmitt K, Götz J (2011) Mitochondrial dysfunction - the beginning of the end in Alzheimer's disease? Separate and synergistic modes of tau and amyloid-β toxicity. Alzheimer's Research & Therapy 3:15.

Eckman CB, Mehta ND, Crook R, Perez-tur J, Prihar G, Pfeiffer E, Graff-Radford N, Hinder P, Yager D, Zenk B, Refolo LM, Prada CM, Younkin SG, Hutton M, Hardy J (1997) A new pathogenic mutation in the APP gene (I716V) increases the relative proportion of A beta 42(43). Hum Mol Genet 6:2087–2089.

Erten-Lyons D, Woltjer RL, Dodge H, Nixon R, Vorobik R, Calvert JF, Leahy M, Montine T, Kaye J (2009) Factors associated with resistance to dementia despite high Alzheimer disease pathology. Neurology 72:354–360.

Espinoza M, de Silva R, Dickson DW, Davies P (2008) Differential incorporation of tau isoforms in Alzheimer's disease. J Alzheimers Dis 14:1–16.

Evgen'ev MB, Krasnov GS, Nesterova IV, Garbuz DG, Karpov VL, Morozov AV, Snezhkina AV, Samokhin AN, Sergeev A, Kulikov AM, Bobkova NV (2017) Molecular Mechanisms Underlying Neuroprotective Effect of Intranasal Administration of Human Hsp70 in Mouse Model of Alzheimer's Disease. Journal of Alzheimer's Disease 59:1415–1426.

Fá M et al. (2016) Extracellular Tau Oligomers Produce An Immediate Impairment of LTP and Memory. Scientific Reports 6 Available at: http://www.nature.com/articles/srep19393 [Accessed January 27, 2018].

Farfara D, Tuby H, Trudler D, Doron-Mandel E, Maltz L, Vassar RJ, Frenkel D, Oron U (2015) Low-Level Laser Therapy Ameliorates Disease Progression in a Mouse Model of Alzheimer's Disease. Journal of Molecular Neuroscience 55:430–436.

Fontaine SN, Nation G, Meier SE, Abisambra JF (2017) TAU OLIGOMERS MEDIATE RIBOSOMAL DYSFUNCTION AT THE SYNAPSE. Alzheimer's & Dementia 13:P603.

Franklin W, Taglialatela G (2016) A method to determine insulin responsiveness in synaptosomes isolated from frozen brain tissue. J Neurosci Methods 261:128–134.

Friedman LG, Qureshi YH, Yu WH (2015) Promoting Autophagic Clearance: Viable Therapeutic Targets in Alzheimer's Disease. Neurotherapeutics 12:94–108.

Gerson J, Castillo-Carranza DL, Sengupta U, Bodani R, Prough DS, DeWitt DS, Hawkins BE, Kayed R (2016) Tau Oligomers Derived from Traumatic Brain Injury Cause Cognitive Impairment and Accelerate Onset of Pathology in Htau Mice. Journal of Neurotrauma 33:2034–2043.

Glenner GG, Wong CW (1984) Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun 120:885–890.

Gordon MN, Holcomb LA, Jantzen PT, DiCarlo G, Wilcock D, Boyett KW, Connor K, Melachrino J, O'Callaghan JP, Morgan D (2002) Time Course of the Development of Alzheimer-like Pathology in the Doubly Transgenic PS1+APP Mouse. Experimental Neurology 173:183–195.

Gredilla R, Weissman L, Yang J-L, Bohr VA, Stevnsner T (2012) Mitochondrial base excision repair in mouse synaptosomes during normal aging and in a model of Alzheimer's disease. Neurobiology of Aging 33:694–707.

Grillo SL, Duggett NA, Ennaceur A, Chazot PL (2013) Non-invasive infra-red therapy (1072nm) reduces β-amyloid protein levels in the brain of an Alzheimer's disease mouse model, TASTPM. Journal of Photochemistry and Photobiology B: Biology 123:13–22.

Grudzien A, Shaw P, Weintraub S, Bigio E, Mash DC, Mesulam MM (2007) Locus coeruleus neurofibrillary degeneration in aging, mild cognitive impairment and early Alzheimer's disease. Neurobiology of Aging 28:327–335.

Grundke-Iqbal I, Iqbal K, Quinlan M, Tung YC, Zaidi MS, Wisniewski HM (1986) Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. J Biol Chem 261:6084–6089.

Gylys KH, Fein JA, Yang F, Cole GM (2004) Enrichment of presynaptic and postsynaptic markers by size-based gating analysis of synaptosome preparations from rat and human cortex. Cytometry 60A:90–96.

Haass C (2004) Take five—BACE and the γ-secretase quartet conduct Alzheimer's amyloid β-peptide generation. The EMBO Journal 23:483–488.

Haass C, Lemere CA, Capell A, Citron M, Seubert P, Schenk D, Lannfelt L, Selkoe DJ (1995) The Swedish mutation causes early-onset Alzheimer's disease by beta-secretase cleavage within the secretory pathway. Nat Med 1:1291–1296.

Hanger DP, Byers HL, Wray S, Leung K-Y, Saxton MJ, Seereeram A, Reynolds CH, Ward MA, Anderton BH (2007) Novel Phosphorylation Sites in Tau from Alzheimer Brain Support a Role for Casein Kinase 1 in Disease Pathogenesis. Journal of Biological Chemistry 282:23645–23654.

Hardingham GE, Bading H (2010) Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. Nature Reviews Neuroscience 11:682–696.

Hardy JA, Higgins GA (1992) Alzheimer's disease: the amyloid cascade hypothesis. Science 256:184–185.

Hashmi JT, Huang Y-Y, Osmani BZ, Sharma SK, Naeser MA, Hamblin MR (2010) Role of Low-Level Laser Therapy in Neurorehabilitation. PM&R 2:S292–S305.

Hawkins BE, Krishnamurthy S, Castillo-Carranza DL, Sengupta U, Prough DS, Jackson GR, DeWitt DS, Kayed R (2013) Rapid Accumulation of Endogenous Tau Oligomers in a Rat Model of Traumatic Brain Injury: POSSIBLE LINK BETWEEN TRAUMATIC BRAIN INJURY AND SPORADIC TAUOPATHIES. Journal of Biological Chemistry 288:17042–17050.

Hebert LE, Scherr PA, Bienias JL, Bennett DA, Evans DA (2003) Alzheimer disease in the US population: prevalence estimates using the 2000 census. Arch Neurol 60:1119–1122.

Hebert LE, Weuve J, Scherr PA, Evans DA (2013) Alzheimer disease in the United States (2010-2050) estimated using the 2010 census. Neurology 80:1778–1783.

Heneka MT et al. (2015) Neuroinflammation in Alzheimer's disease. The Lancet Neurology 14:388–405.

Honig LS et al. (2018) Trial of Solanezumab for Mild Dementia Due to Alzheimer's Disease. New England Journal of Medicine 378:321–330.

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

Huang Y-Y, Chen AC-H, Carroll JD, Hamblin MR (2009) Biphasic Dose Response in Low Level Light Therapy. Dose-Response 7:dose-response.0.

Huang Y-Y, Gupta A, Vecchio D, Arce VJB de, Huang S-F, Xuan W, Hamblin MR (2012) Transcranial low level laser (light) therapy for traumatic brain injury. Journal of Biophotonics 5:827–837.

Ittner LM, Fath T, Ke YD, Bi M, van Eersel J, Li KM, Gunning P, Gotz J (2008) Parkinsonism and impaired axonal transport in a mouse model of frontotemporal dementia. Proceedings of the National Academy of Sciences 105:15997–16002.

Ittner LM, Ke YD, Delerue F, Bi M, Gladbach A, van Eersel J, Wölfing H, Chieng BC, Christie MJ, Napier IA, Eckert A, Staufenbiel M, Hardeman E, Götz J (2010) Dendritic Function of Tau Mediates Amyloid-β Toxicity in Alzheimer's Disease Mouse Models. Cell 142:387–397.

Jinwal UK et al. (2013) Imbalance of Hsp70 family variants fosters tau accumulation. The FASEB Journal 27:1450–1459.

Johnston JA, Cowburn RF, Norgren S, Wiehager B, Venizelos N, Winblad B, Vigo-Pelfrey C, Schenk D, Lannfelt L, O'Neill C (1994) Increased beta-amyloid release and levels of amyloid precursor protein (APP) in fibroblast cell lines from family members with the Swedish Alzheimer's disease APP670/671 mutation. FEBS Lett 354:274–278.

Johnstone DM, el Massri N, Moro C, Spana S, Wang XS, Torres N, Chabrol C, De Jaeger X, Reinhart F, Purushothuman S, Benabid A-L, Stone J, Mitrofanis J (2014) Indirect application of near infrared light induces neuroprotection in a mouse model of parkinsonism – An abscopal neuroprotective effect. Neuroscience 274:93–101.

Jung JH, An K, Kwon OB, Kim H, Kim J-H (2011) Pathway-specific alteration of synaptic plasticity in Tg2576 mice. Molecules and Cells 32:197–201.

Kang J, Lemaire H-G, Unterbeck A, Salbaum JM, Masters CL, Grzeschik K-H, Multhaup G, Beyreuther K, Müller-Hill B (1987) The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature 325:733–736.

Karu T (2010a) Mitochondrial Mechanisms of Photobiomodulation in Context of New Data About Multiple Roles of ATP. Photomedicine and Laser Surgery 28:159–160.

Karu TI (2010b) Multiple roles of cytochrome c oxidase in mammalian cells under action of red and IR-A radiation. IUBMB Life 62:607–610.

Karu TI, Kolyakov SF (2005) Exact Action Spectra for Cellular Responses Relevant to Phototherapy. Photomedicine and Laser Surgery 23:355–361.

Katzman R (1976) The Prevalence and Malignancy of Alzheimer Disease: A Major Killer. Archives of Neurology 33:217.

Kavirajan H, Schneider LS (2007) Efficacy and adverse effects of cholinesterase inhibitors and memantine in vascular dementia: a meta-analysis of randomised controlled trials. The Lancet Neurology 6:782–792.

Kawarabayashi T, Younkin LH, Saido TC, Shoji M, Ashe KH, Younkin SG (2001) Age-dependent changes in brain, CSF, and plasma amyloid (beta) protein in the Tg2576 transgenic mouse model of Alzheimer's disease. J Neurosci 21:372–381.

Kayed R (2003) Common Structure of Soluble Amyloid Oligomers Implies Common Mechanism of Pathogenesis. Science 300:486–489.

Kelley AS, McGarry K, Gorges R, Skinner JS (2015) The burden of health care costs for patients with dementia in the last 5 years of life. Ann Intern Med 163:729–736.

Kelly SC, He B, Perez SE, Ginsberg SD, Mufson EJ, Counts SE (2017) Locus coeruleus cellular and molecular pathology during the progression of Alzheimer's disease. Acta Neuropathologica Communications 5 Available at: http://actaneurocomms.biomedcentral.com/articles/10.1186/s40478-017-0411-2 [Accessed April 9, 2018].

Kimura R, Ma L-Y, Wu C, Turner D, Shen J-X, Ellsworth K, Wakui M, Maalouf M, Wu J (2012) Acute Exposure to the Mitochondrial Complex I Toxin Rotenone Impairs Synaptic Long-Term Potentiation in Rat Hippocampal Slices: Rotenone Impairs LTP in Hippocampal Slices. CNS Neuroscience & Therapeutics 18:641–646.

Kish SJ, Bergeron C, Rajput A, Dozic S, Mastrogiacomo F, Chang LJ, Wilson JM, DiStefano LM, Nobrega JN (1992) Brain cytochrome oxidase in Alzheimer's disease. J Neurochem 59:776–779.

Koffie RM, Meyer-Luehmann M, Hashimoto T, Adams KW, Mielke ML, Garcia-Alloza M, Micheva KD, Smith SJ, Kim ML, Lee VM, Hyman BT, Spires-Jones TL (2009) Oligomeric amyloid associates with postsynaptic densities and correlates with excitatory synapse loss near senile plaques. Proceedings of the National Academy of Sciences 106:4012–4017.

Kok K-S, Loke Y, Southgate J (2015) Upper gastrointestinal bleed associated with cholinesterase inhibitor use. BMJ Case Reports:bcr2015211859.

Kopeikina KJ, Wegmann S, Pitstick R, Carlson GA, Bacskai BJ, Betensky RA, Hyman BT, Spires-Jones TL (2013) Tau Causes Synapse Loss without Disrupting Calcium Homeostasis in the rTg4510 Model of Tauopathy Dickey CA, ed. PLoS ONE 8:e80834.

Kuhn P-H, Wang H, Dislich B, Colombo A, Zeitschel U, Ellwart JW, Kremmer E, Roßner S, Lichtenthaler SF (2010) ADAM10 is the physiologically relevant, constitutive α-secretase of the amyloid precursor protein in primary neurons. The EMBO Journal 29:3020–3032.

Kundel F, De S, Flagmeier P, Horrocks MH, Kjaergaard M, Shammas SL, Jackson SE, Dobson CM, Klenerman D (2018) Hsp70 Inhibits the Nucleation and Elongation of Tau and Sequesters Tau Aggregates with High Affinity. ACS Chemical Biology Available at: http://pubs.acs.org/doi/10.1021/acschembio.7b01039 [Accessed March 14, 2018].

Lacor PN (2004) Synaptic Targeting by Alzheimer's-Related Amyloid Oligomers. Journal of Neuroscience 24:10191–10200.

Lai AY, McLaurin J (2011) Mechanisms of Amyloid-Beta Peptide Uptake by Neurons: The Role of Lipid Rafts and Lipid Raft-Associated Proteins. International Journal of Alzheimer's Disease 2011:1–11.

Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, Wals P, Zhang C, Finch CE, Krafft GA, Klein WL (1998) Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. Proc Natl Acad Sci USA 95:6448–6453.

Lapchak PA, Boitano PD, Butte PV, Fisher DJ, Hölscher T, Ley EJ, Nuño M, Voie AH, Rajput PS (2015) Transcranial Near-Infrared Laser Transmission (NILT) Profiles (800 nm): Systematic Comparison in Four Common Research Species Hamblin M, ed. PLOS ONE 10:e0127580.

Lasagna-Reeves CA, Castillo-Carranza DL, Guerrero-Muñoz MJ, Jackson GR, Kayed R (2010) Preparation and Characterization of Neurotoxic Tau Oligomers. Biochemistry 49:10039–10041.

Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Clos AL, Jackson GR, Kayed R (2011) Tau oligomers impair memory and induce synaptic and mitochondrial dysfunction in wild-type mice. Molecular Neurodegeneration 6:39.

Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Guerrero-Munoz MJ, Kiritoshi T, Neugebauer V, Jackson GR, Kayed R (2012a) Alzheimer brain-derived tau oligomers propagate pathology from endogenous tau. Scientific Reports 2 Available at: http://www.nature.com/articles/srep00700 [Accessed April 7, 2018].

Lasagna-Reeves CA, Castillo-Carranza DL, Sengupta U, Sarmiento J, Troncoso J, Jackson GR, Kayed R (2012b) Identification of oligomers at early stages of tau aggregation in Alzheimer's disease. The FASEB Journal 26:1946–1959.

Laurén J, Gimbel DA, Nygaard HB, Gilbert JW, Strittmatter SM (2009) Cellular prion protein mediates impairment of synaptic plasticity by amyloid-β oligomers. Nature 457:1128–1132.

Leak RK (2014) Heat shock proteins in neurodegenerative disorders and aging. Journal of Cell Communication and Signaling 8:293–310.

Lee S, Kim W, Li Z, Hall GF (2012) Accumulation of Vesicle-Associated Human Tau in Distal Dendrites Drives Degeneration and Tau Secretion in an *In Situ* Cellular Tauopathy Model. International Journal of Alzheimer's Disease 2012:1–16.

Li C, Götz J (2017) Somatodendritic accumulation of Tau in Alzheimer's disease is promoted by Fyn-mediated local protein translation. The EMBO Journal 36:3120–3138.

Li S, Hong S, Shepardson NE, Walsh DM, Shankar GM, Selkoe D (2009) Soluble Oligomers of Amyloid β Protein Facilitate Hippocampal Long-Term Depression by Disrupting Neuronal Glutamate Uptake. Neuron 62:788–801.

Li S, Jin M, Koeglsperger T, Shepardson NE, Shankar GM, Selkoe DJ (2011) Soluble A Oligomers Inhibit Long-Term Potentiation through a Mechanism Involving Excessive Activation of Extrasynaptic NR2B-Containing NMDA Receptors. Journal of Neuroscience 31:6627–6638.

Liang J, Liu L, Xing D (2012) Photobiomodulation by low-power laser irradiation attenuates Aβ-induced cell apoptosis through the Akt/GSK3β/β-catenin pathway. Free Radical Biology and Medicine 53:1459–1467.

Lin Y-S, Huang M-H, Chai C-Y (2006) Effects of helium–neon laser on the mucopolysaccharide induction in experimental osteoarthritic cartilage. Osteoarthritis and Cartilage 14:377–383.

Linnane AW, Marzuki S, Ozawa T, Tanaka M (1989) Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. Lancet 1:642–645.

Liu F, Li B, Tung E-J, Grundke-Iqbal I, Iqbal K, Gong C-X (2007) Site-specific effects of tau phosphorylation on its microtubule assembly activity and self-aggregation: Site-specific effects of tau phosphorylation. European Journal of Neuroscience 26:3429–3436.

Lu Y, Wang R, Dong Y, Tucker D, Zhao N, Ahmed ME, Zhu L, Liu TC-Y, Cohen RM, Zhang Q (2017) Low-level laser therapy for beta amyloid toxicity in rat hippocampus. Neurobiology of Aging 49:165–182.

M. Vargas L, Leal N, Estrada LD, González A, Serrano F, Araya K, Gysling K, Inestrosa NC, Pasquale EB, Alvarez AR (2014) EphA4 Activation of c-Abl Mediates Synaptic Loss and LTP Blockade Caused by Amyloid-β Oligomers Arendt T, ed. PLoS ONE 9:e92309.

Manczak M, Reddy PH (2012) Abnormal interaction of VDAC1 with amyloid beta and phosphorylated tau causes mitochondrial dysfunction in Alzheimer's disease. Human Molecular Genetics 21:5131–5146.

Marta B, Belinda G, Tiziana C, Moreno S, Francesco T, Costanza B, Giorgio A, Patrizia F (2013) Early Selective Vulnerability of Synapses and Synaptic Mitochondria in the Hippocampal CA1 Region of the Tg2576 Mouse Model of Alzheimer's Disease. Journal of Alzheimer's Disease:887–896.

Masliah E, Mallory M, Alford M, DeTeresa R, Hansen LA, McKeel DW, Morris JC (2001) Altered expression of synaptic proteins occurs early during progression of Alzheimer's disease. Neurology 56:127–129.

Mattiasson G, Friberg H, Hansson M, Elmér E, Wieloch T (2003) Flow cytometric analysis of mitochondria from CA1 and CA3 regions of rat hippocampus reveals differences in permeability transition pore activation. J Neurochem 87:532–544.

Maurer I, Zierz, Stephan, Moller, Hans-Jurgen (2000) A selective defect of cytochrome c oxidase is present in brain of Alzheimer disease patients. Neurobiology of Aging 21:455–462.

Melov S, Adlard PA, Morten K, Johnson F, Golden TR, Hinerfeld D, Schilling B, Mavros C, Masters CL, Volitakis I, Li Q-X, Laughton K, Hubbard A, Cherny RA, Gibson B, Bush AI (2007) Mitochondrial Oxidative Stress Causes Hyperphosphorylation of Tau Khoury JE, ed. PLoS ONE 2:e536.

Meng C, He Z, Xing D (2013) Low-Level Laser Therapy Rescues Dendrite Atrophy via Upregulating BDNF Expression: Implications for Alzheimer's Disease. Journal of Neuroscience 33:13505–13517.

Mester E, Spiry T, Szende B, Tota JG (1971) Effect of laser rays on wound healing. Am J Surg 122:532–535.

Mester E, Szende B, Spiry T, Scher A (1972) Stimulation of wound healing by laser rays. Acta Chir Acad Sci Hung 13:315–324.

Mitchell UH, Mack GL (2013) Low-Level Laser Treatment with Near-Infrared Light Increases Venous Nitric Oxide Levels Acutely: A Single-Blind, Randomized Clinical Trial of Efficacy. American Journal of Physical Medicine & Rehabilitation 92:151–156.

Morris RG, Salmon DP (2007) The Centennial of Alzheimer's Disease and the Publication of "ÜBer Eine Eigenartige Erkankung Der Hirnrinde" By AlÖis Alzheimer. Cortex 43:821–825.

Muratore CR, Rice HC, Srikanth P, Callahan DG, Shin T, Benjamin LNP, Walsh DM, Selkoe DJ, Young-Pearse TL (2014) The familial Alzheimer's disease APPV717I mutation alters APP processing and Tau expression in iPSC-derived neurons. Human Molecular Genetics 23:3523–3536.

Nguyen PV, Marin L, Atwood HL (1997) Synaptic Physiology and Mitochondrial Function in Crayfish Tonic and Phasic Motor Neurons. Journal of Neurophysiology 78:281–294.

Oakley H, Cole SL, Logan S, Maus E, Shao P, Craft J, Guillozet-Bongaarts A, Ohno M, Disterhoft J, Van Eldik L, Berry R, Vassar R (2006) Intraneuronal beta-Amyloid Aggregates, Neurodegeneration, and Neuron Loss in Transgenic Mice with Five Familial Alzheimer's Disease Mutations: Potential Factors in Amyloid Plaque Formation. Journal of Neuroscience 26:10129–10140.

Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kayed R, Metherate R, Mattson MP, Akbari Y, LaFerla FM (2003) Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron 39:409–421.

Oron A, Oron U, Chen J, Eilam A, Zhang C, Sadeh M, Lampl Y, Streeter J, DeTaboada L, Chopp M (2006) Low-Level Laser Therapy Applied Transcranially to Rats After Induction of Stroke Significantly Reduces Long-Term Neurological Deficits. Stroke 37:2620–2624.

Orr ME, Oddo S (2013) Autophagic/lysosomal dysfunction in Alzheimer's disease. Alzheimer's Research & Therapy 5:53.

Parker WD, Mahr NJ, Filley CM, Parks JK, Hughes D, Young DA, Cullum CM (1994a) Reduced platelet cytochrome c oxidase activity in Alzheimer's disease. Neurology 44:1086–1090.

Parker WD, Parks J, Filley CM, Kleinschmidt-DeMasters BK (1994b) Electron transport chain defects in Alzheimer's disease brain. Neurology 44:1090–1096.

Parri HR, Hernandez CM, Dineley KT (2011) Research update: Alpha7 nicotinic acetylcholine receptor mechanisms in Alzheimer's disease. Biochemical Pharmacology 82:931–942.

Pascoal TA, Mathotaarachchi S, Shin M, Benedet AL, Mohades S, Wang S, Beaudry T, Kang MS, Soucy J-P, Labbe A, Gauthier S, Rosa-Neto P (2017) Synergistic interaction between amyloid and tau predicts the progression to dementia. Alzheimer's & Dementia 13:644–653.

Pedersen WA, Kloczewiak MA, Blusztajn JK (1996) Amyloid beta-protein reduces acetylcholine synthesis in a cell line derived from cholinergic neurons of the basal forebrain. Proc Natl Acad Sci USA 93:8068–8071.

Pham E, Crews L, Ubhi K, Hansen L, Adame A, Cartier A, Salmon D, Galasko D, Michael S, Savas JN, Yates JR, Glabe C, Masliah E (2010) Progressive accumulation of amyloid-β oligomers in Alzheimer's disease and in amyloid precursor protein transgenic mice is accompanied by selective alterations in synaptic scaffold proteins: Aβ oligomers and synaptic proteins in AD. FEBS Journal 277:3051–3067.

Pitt J, Roth W, Lacor P, Smith AB, Blankenship M, Velasco P, De Felice F, Breslin P, Klein WL (2009) Alzheimer's-associated Aβ oligomers show altered structure, immunoreactivity and synaptotoxicity with low doses of oleocanthal. Toxicology and Applied Pharmacology 240:189–197.

Plassman BL, Langa KM, Fisher GG, Heeringa SG, Weir DR, Ofstedal MB, Burke JR, Hurd MD, Potter GG, Rodgers WL, Steffens DC, Willis RJ, Wallace RB (2007) Prevalence of dementia in the United States: the aging, demographics, and memory study. Neuroepidemiology 29:125–132.

Presley AD, Fuller KM, Arriaga EA (2003) MitoTracker Green labeling of mitochondrial proteins and their subsequent analysis by capillary electrophoresis with laser-induced fluorescence detection. J Chromatogr B Analyt Technol Biomed Life Sci 793:141–150.

Purushothuman S, Johnstone DM, Nandasena C, Eersel J van, Ittner LM, Mitrofanis J, Stone J (2015) Near infrared light mitigates cerebellar pathology in transgenic mouse models of dementia. Neuroscience Letters 591:155–159.

Purushothuman S, Johnstone DM, Nandasena C, Mitrofanis J, Stone J (2014) Photobiomodulation with near infrared light mitigates Alzheimer's disease-related pathology in cerebral cortex – evidence from two transgenic mouse models. Alzheimer's Research & Therapy 6:2.

Puzzo D, Lee L, Palmeri A, Calabrese G, Arancio O (2014) Behavioral assays with mouse models of Alzheimer's disease: Practical considerations and guidelines. Biochemical Pharmacology 88:450–467.

Radde R, Bolmont T, Kaeser SA, Coomaraswamy J, Lindau D, Stoltze L, Calhoun ME, Jäggi F, Wolburg H, Gengler S, Haass C, Ghetti B, Czech C, Hölscher C, Mathews PM, Jucker M (2006) Aβ42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology. EMBO reports 7:940–946.

Rajmohan R, Reddy PH (2017) Amyloid-Beta and Phosphorylated Tau Accumulations Cause Abnormalities at Synapses of Alzheimer's disease Neurons. Journal of Alzheimer's Disease 57:975–999.

Reddy PH (2007) Mitochondrial Dysfunction in Aging and Alzheimer's Disease: Strategies to Protect Neurons. Antioxidants & Redox Signaling 9:1647–1658.

Reddy PH, Manczak M, Yin X (2017) Mitochondria-Division Inhibitor 1 Protects Against Amyloid-β induced Mitochondrial Fragmentation and Synaptic Damage in Alzheimer's Disease. Journal of Alzheimer's Disease 58:147–162.

Reddy PH, Tripathi R, Troung Q, Tirumala K, Reddy TP, Anekonda V, Shirendeb UP, Calkins MJ, Reddy AP, Mao P, Manczak M (2012) Abnormal mitochondrial dynamics and synaptic degeneration as early events in Alzheimer's disease: Implications to mitochondria-targeted antioxidant therapeutics. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1822:639–649.

Reese LC, Zhang W, Dineley KT, Kayed R, Taglialatela G (2008) Selective induction of calcineurin activity and signaling by oligomeric amyloid beta. Aging Cell 7:824–835.

Reitz C, Mayeux R (2014) Alzheimer disease: Epidemiology, diagnostic criteria, risk factors and biomarkers. Biochemical Pharmacology 88:640–651.

Resende R, Ferreiro E, Pereira C, Resende de Oliveira C (2008) Neurotoxic effect of oligomeric and fibrillar species of amyloid-beta peptide 1-42: Involvement of endoplasmic reticulum calcium release in oligomer-induced cell death. Neuroscience 155:725–737.

Rhein V, Song X, Wiesner A, Ittner LM, Baysang G, Meier F, Ozmen L, Bluethmann H, Drose S, Brandt U, Savaskan E, Czech C, Gotz J, Eckert A (2009) Amyloid- and tau synergistically impair the oxidative phosphorylation system in triple transgenic Alzheimer's disease mice. Proceedings of the National Academy of Sciences 106:20057–20062.

Robinson JL, Molina-Porcel L, Corrada MM, Raible K, Lee EB, Lee VM-Y, Kawas CH, Trojanowski JQ (2014) Perforant path synaptic loss correlates with cognitive impairment and Alzheimer's disease in the oldest-old. Brain 137:2578–2587.

Roks G, Dermaut B, Heutink P, Julliams A, Backhovens H, Van de Broeck M, Serneels S, Hofman A, Van Broeckhoven C, van Duijn CM, Cruts M (1999) Mutation screening of the tau gene in patients with early-onset Alzheimer's disease. Neurosci Lett 277:137–139.

Röskam S, Neff F, Schwarting R, Bacher M, Dodel R (2010) APP transgenic mice: The effect of active and passive immunotherapy in cognitive tasks. Neuroscience & Biobehavioral Reviews 34:487–499.

Rozenstein-Tsalkovich L, Grigoriadis N, Lourbopoulos A, Nousiopoulou E, Kassis I, Abramsky O, Karussis D, Rosenmann H (2013) Repeated immunization of mice with phosphorylated-tau peptides causes neuroinflammation. Experimental Neurology 248:451–456.

Salloway S et al. (2014) Two Phase 3 Trials of Bapineuzumab in Mild-to-Moderate Alzheimer's Disease. New England Journal of Medicine 370:322–333.

Saltmarche AE, Naeser MA, Ho KF, Hamblin MR, Lim L (2017) Significant Improvement in Cognition in Mild to Moderately Severe Dementia Cases Treated with Transcranial Plus Intranasal Photobiomodulation: Case Series Report. Photomedicine and Laser Surgery 35:432–441.

Scheff SW, Price DA, Schmitt FA, DeKosky ST, Mufson EJ (2007) Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment. Neurology 68:1501–1508.

Scheuner D et al. (1996) Secreted amyloid β–protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nature Medicine 2:864–870.

Scott I, Youle RJ (2010) Mitochondrial fission and fusion. Essays In Biochemistry 47:85–98.

Selkoe DJ (2002) Alzheimer's Disease Is a Synaptic Failure. Science 298:789–791.

Sengupta A, Kabat J, Novak M, Wu Q, Grundke-Iqbal I, Iqbal K (1998) Phosphorylation of Tau at Both Thr 231 and Ser 262 Is Required for Maximal Inhibition of Its Binding to Microtubules. Archives of Biochemistry and Biophysics 357:299–309.

Serra-Batiste M, Ninot-Pedrosa M, Bayoumi M, Gairí M, Maglia G, Carulla N (2016) Aβ42 assembles into specific β-barrel pore-forming oligomers in membrane-mimicking environments. Proceedings of the National Academy of Sciences 113:10866–10871.

Sesaki H, Jensen RE (1999) Division versus fusion: Dnm1p and Fzo1p antagonistically regulate mitochondrial shape. J Cell Biol 147:699–706.

Sevigny J et al. (2016) The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. Nature 537:50–56.

Shankar GM, Bloodgood BL, Townsend M, Walsh DM, Selkoe DJ, Sabatini BL (2007) Natural Oligomers of the Alzheimer Amyloid- Protein Induce Reversible Synapse Loss by Modulating an NMDA-Type Glutamate Receptor-Dependent Signaling Pathway. Journal of Neuroscience 27:2866–2875.

Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, Selkoe DJ (2008) Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nature Medicine 14:837–842.

Sheehan JP, Swerdlow RH, Miller SW, Davis RE, Parks JK, Parker WD, Tuttle JB (1997) Calcium homeostasis and reactive oxygen species production in cells transformed by mitochondria from individuals with sporadic Alzheimer's disease. J Neurosci 17:4612–4622.

Sisodia SS (1992) Beta-amyloid precursor protein cleavage by a membrane-bound protease. Proc Natl Acad Sci USA 89:6075–6079.

Sommer AP, Bieschke J, Friedrich RP, Zhu D, Wanker EE, Fecht HJ, Mereles D, Hunstein W (2012) 670 nm laser light and EGCG complementarily reduce amyloid-β aggregates in

human neuroblastoma cells: basis for treatment of Alzheimer's disease? Photomed Laser Surg 30:54–60.

Song L, Lu SX, Ouyang X, Melchor J, Lee J, Terracina G, Wang X, Hyde L, Hess JF, Parker EM, Zhang L (2015) Analysis of tau post-translational modifications in rTg4510 mice, a model of tau pathology. Molecular Neurodegeneration 10 Available at: http://www.molecularneurodegeneration.com/content/10/1/14 [Accessed April 7, 2018].

Spina S, Schonhaut DR, Boeve BF, Seeley WW, Ossenkoppele R, O'Neil JP, Lazaris A, Rosen HJ, Boxer AL, Perry DC, Miller BL, Dickson DW, Parisi JE, Jagust WJ, Murray ME, Rabinovici GD (2017) Frontotemporal dementia with the V337M *MAPT* mutation: Tau-PET and pathology correlations. Neurology 88:758–766.

Spires-Jones TL, Hyman BT (2014) The intersection of amyloid beta and tau at synapses in Alzheimer's disease. Neuron 82:756–771.

Swerdlow RH, Burns JM, Khan SM (2010) The Alzheimer's Disease Mitochondrial Cascade Hypothesis Zhu X, Beal MF, Wang X, Perry G, Smith MA, eds. Journal of Alzheimer's Disease 20:S265–S279.

Swerdlow RH, Burns JM, Khan SM (2014) The Alzheimer's disease mitochondrial cascade hypothesis: Progress and perspectives. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1842:1219–1231.

Swerdlow RH, Parks JK, Cassarino DS, Maguire DJ, Maguire RS, Bennett JP, Davis RE, Parker WD (1997) Cybrids in Alzheimer's disease: a cellular model of the disease? Neurology 49:918–925.

Sze CI, Troncoso JC, Kawas C, Mouton P, Price DL, Martin LJ (1997) Loss of the presynaptic vesicle protein synaptophysin in hippocampus correlates with cognitive decline in Alzheimer disease. J Neuropathol Exp Neurol 56:933–944.

Tai H-C, Wang BY, Serrano-Pozo A, Frosch MP, Spires-Jones TL, Hyman BT (2014) Frequent and symmetric deposition of misfolded tau oligomers within presynaptic and postsynaptic terminals in Alzheimer's disease. Acta Neuropathologica Communications 2 Available at: http://actaneurocomms.biomedcentral.com/articles/10.1186/s40478-014-0146-2 [Accessed March 6, 2018].

Takeuchi T, Duszkiewicz AJ, Morris RGM (2013) The synaptic plasticity and memory hypothesis: encoding, storage and persistence. Philosophical Transactions of the Royal Society B: Biological Sciences 369:20130288–20130288.

Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R (1991) Physical basis of cognitive alterations in alzheimer's disease: Synapse loss is the major correlate of cognitive impairment. Annals of Neurology 30:572–580.

Thal DR, Rüb U, Orantes M, Braak H (2002) Phases of Aβ-deposition in the human brain and its relevance for the development of AD. Neurology 58:1791–1800.

Tiphlova O, Karu T (1991) Action of low-intensity laser radiation on Escherichia coli. Crit Rev Biomed Eng 18:387–412.

Tracy TE, Gan L (2017) Acetylated tau in Alzheimer's disease: An instigator of synaptic dysfunction underlying memory loss: Increased levels of acetylated tau blocks the postsynaptic signaling required for plasticity and promotes memory deficits associated with tauopathy. BioEssays 39:1600224.

Tu S, Okamoto S, Lipton SA, Xu H (2014) Oligomeric Aβ-induced synaptic dysfunction in Alzheimer's disease. Molecular Neurodegeneration 9:48.

Um JW, Kaufman AC, Kostylev M, Heiss JK, Stagi M, Takahashi H, Kerrisk ME, Vortmeyer A, Wisniewski T, Koleske AJ, Gunther EC, Nygaard HB, Strittmatter SM (2013) Metabotropic Glutamate Receptor 5 Is a Coreceptor for Alzheimer Aβ Oligomer Bound to Cellular Prion Protein. Neuron 79:887–902.

Um JW, Nygaard HB, Heiss JK, Kostylev MA, Stagi M, Vortmeyer A, Wisniewski T, Gunther EC, Strittmatter SM (2012) Alzheimer amyloid-β oligomer bound to postsynaptic prion protein activates Fyn to impair neurons. Nature Neuroscience 15:1227–1235.

Usenovic M, Niroomand S, Drolet RE, Yao L, Gaspar RC, Hatcher NG, Schachter J, Renger JJ, Parmentier-Batteur S (2015) Internalized Tau Oligomers Cause Neurodegeneration by Inducing Accumulation of Pathogenic Tau in Human Neurons Derived from Induced Pluripotent Stem Cells. Journal of Neuroscience 35:14234–14250.

Vargas L, Leal N, Estrada LD, González A, Serrano F, Araya K, Gysling K, Inestrosa NC, Pasquale EB, Alvarez AR (2014) EphA4 Activation of c-Abl Mediates Synaptic Loss and LTP Blockade Caused by Amyloid-β Oligomers Arendt T, ed. PLoS ONE 9:e92309.

Velazquez R, Tran A, Ishimwe E, Denner L, Dave N, Oddo S, Dineley KT (2017) Central insulin dysregulation and energy dyshomeostasis in two mouse models of Alzheimer's disease. Neurobiology of Aging 58:1–13.

Versijpt J (2014) Effectiveness and Cost-Effectiveness of the Pharmacological Treatment of Alzheimer's Disease and Vascular Dementia. Journal of Alzheimer's Disease:S19–S25.

von Bergen M, Barghorn S, Biernat J, Mandelkow E-M, Mandelkow E (2005) Tau aggregation is driven by a transition from random coil to beta sheet structure. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1739:158–166.

Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ (2002) Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo. Nature 416:535–539.

Wang H-W, Pasternak JF, Kuo H, Ristic H, Lambert MP, Chromy B, Viola KL, Klein WL, Stine WB, Krafft GA, Trommer BL (2002) Soluble oligomers of beta amyloid (1-42) inhibit long-term potentiation but not long-term depression in rat dentate gyrus. Brain Res 924:133–140.

Wang H-Y, Lee DHS, D'Andrea MR, Peterson PA, Shank RP, Reitz AB (2000) β-Amyloid 1–42 Binds to α7 Nicotinic Acetylcholine Receptor with High Affinity: IMPLICATIONS FOR ALZHEIMER'S DISEASE PATHOLOGY. Journal of Biological Chemistry 275:5626–5632.

Wang J-Z, Grundke-Iqbal I, Iqbal K (1996) Glycosylation of microtubule–associated protein tau: An abnormal posttranslational modification in Alzheimer's disease. Nature Medicine 2:871–875.

Wang J-Z, Xia Y-Y, Grundke-Iqbal I, Iqbal K (2013) Abnormal Hyperphosphorylation of Tau: Sites, Regulation, and Molecular Mechanism of Neurofibrillary Degeneration. Journal of Alzheimer's Disease:S123–S139.

Wang X, Su B, Lee H -g., Li X, Perry G, Smith MA, Zhu X (2009) Impaired Balance of Mitochondrial Fission and Fusion in Alzheimer's Disease. Journal of Neuroscience 29:9090–9103.

Wang Y, Fu X, Li D, Wang K, Wang X, Li Y, Sun B, Yang X, Zheng Z, Cho N (2016) Cyanidin suppresses amyloid beta-induced neurotoxicity by inhibiting reactive oxygen species-mediated DNA damage and apoptosis in PC12 cells. Neural Regeneration Research 11:795.

Weeber EJ, Levy M, Sampson MJ, Anflous K, Armstrong DL, Brown SE, Sweatt JD, Craigen WJ (2002) The Role of Mitochondrial Porins and the Permeability Transition Pore in Learning and Synaptic Plasticity. Journal of Biological Chemistry 277:18891–18897.

Wilcox KC, Lacor PN, Pitt J, Klein WL (2011) Aβ Oligomer-Induced Synapse Degeneration in Alzheimer's Disease. Cellular and Molecular Neurobiology 31:939–948.

Wolf A, Bauer B, Abner EL, Ashkenazy-Frolinger T, Hartz AMS (2016) A Comprehensive Behavioral Test Battery to Assess Learning and Memory in 129S6/Tg2576 Mice Reddy H, ed. PLOS ONE 11:e0147733.

Xia P, Chen H -s. V, Zhang D, Lipton SA (2010) Memantine Preferentially Blocks Extrasynaptic over Synaptic NMDA Receptor Currents in Hippocampal Autapses. Journal of Neuroscience 30:11246–11250.

Xuan W, Agrawal T, Huang L, Gupta GK, Hamblin MR (2015) Low-level laser therapy for traumatic brain injury in mice increases brain derived neurotrophic factor (BDNF) and synaptogenesis. Journal of Biophotonics 8:502–511.

Xuan W, Vatansever F, Huang L, Hamblin MR (2014) Transcranial low-level laser therapy enhances learning, memory, and neuroprogenitor cells after traumatic brain injury in mice. Journal of biomedical optics 19:108003–108003.

Yang X, Askarova S, Sheng W, Chen JK, Sun AY, Sun GY, Yao G, Lee JC-M (2010) Low energy laser light (632.8 nm) suppresses amyloid-β peptide-induced oxidative and inflammatory responses in astrocytes. Neuroscience 171:859–868.

Yiannopoulou KG, Papageorgiou SG (2013) Current and future treatments for Alzheimer's disease. Therapeutic advances in neurological disorders 6:19–33.

Young ZT, Rauch JN, Assimon VA, Jinwal UK, Ahn M, Li X, Dunyak BM, Ahmad A, Carlson GA, Srinivasan SR, Zuiderweg ERP, Dickey CA, Gestwicki JE (2016) Stabilizing the Hsp70-Tau Complex Promotes Turnover in Models of Tauopathy. Cell Chemical Biology 23:992–1001.

Yu Z, Liu N, Zhao J, Li Y, McCarthy TJ, Tedford CE, Lo EH, Wang X (2015) Near infrared radiation rescues mitochondrial dysfunction in cortical neurons after oxygen-glucose deprivation. Metabolic Brain Disease 30:491–496.

Zempel H, Luedtke J, Kumar Y, Biernat J, Dawson H, Mandelkow E, Mandelkow E-M (2013) Amyloid-β oligomers induce synaptic damage via Tau-dependent microtubule severing by TTLL6 and spastin: Aβ causes microtubule severing by spastin via Tau. The EMBO Journal 32:2920–2937.

Zempel H, Mandelkow E (2014) Lost after translation: missorting of Tau protein and consequences for Alzheimer disease. Trends in Neurosciences 37:721–732.

Zempel H, Thies E, Mandelkow E, Mandelkow E-M (2010) Abeta oligomers cause localized Ca(2+) elevation, missorting of endogenous Tau into dendrites, Tau phosphorylation, and destruction of microtubules and spines. J Neurosci 30:11938–11950.

Zhang L, Trushin S, Christensen TA, Bachmeier BV, Gateno B, Schroeder A, Yao J, Itoh K, Sesaki H, Poon WW, Gylys KH, Patterson ER, Parisi JE, Diaz Brinton R, Salisbury JL, Trushina E (2016) Altered brain energetics induces mitochondrial fission arrest in Alzheimer's Disease. Scientific Reports 6 Available at: http://www.nature.com/articles/srep18725 [Accessed August 18, 2017].

Zheng W, Tsai M-Y, Wolynes PG (2017) Comparing the Aggregation Free Energy Landscapes of Amyloid Beta(1–42) and Amyloid Beta(1–40). Journal of the American Chemical Society 139:16666–16676.

Zheng W-H, Bastianetto S, Mennicken F, Ma W, Kar S (2002) Amyloid beta peptide induces tau phosphorylation and loss of cholinergic neurons in rat primary septal cultures. Neuroscience 115:201–211.

Zhou L, McInnes J, Wierda K, Holt M, Herrmann AG, Jackson RJ, Wang Y-C, Swerts J, Beyens J, Miskiewicz K, Vilain S, Dewachter I, Moechars D, De Strooper B, Spires-Jones TL, De Wit J, Verstreken P (2017) Tau association with synaptic vesicles causes presynaptic dysfunction. Nature Communications 8:15295.

Zolochevska O, Taglialatela G (2016) Non-Demented Individuals with Alzheimer's Disease Neuropathology: Resistance to Cognitive Decline May Reveal New Treatment Strategies. Curr Pharm Des 22:4063–4068.

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Publications

1. **Comerota M. M.**, Krishnan, B. and Taglialatela, G. Near infrared light decreases synaptic vulnerability to amyloid beta oligomers. Scientific Reports. 7, 15012 (2017). doi:10.1038/s41598-017-15357-x

2. **Comerota, M.M.**, Tumurbaatar, B., Krishnan, B., and Taglialatela, G. The modulation of tau pathology in two transgenic mouse models by near infrared light treatment. Molecular Neurobiology. (2018). https://doi.org/10.1007/s12035-018-1248-9

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