INFLAMMATION- AND AGE-RELATED ALTERATIONS IN TAU EXPRESSION AND PHOSPHORYLATION

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1. Introduction

Dementia, which currently afflicts 44.4 million people worldwide, is defined by a progressive decline in cognitive function that results in the inability for an individual to complete common tasks (World Alzheimer's Report 2013). As technologies allow for longer life expectancies, the incidence of such neurodegenerative diseases are expected to increase, with 135.5 million people expected to be afflicted by 2050. In addition, Alzheimer's disease (AD) accounts for an overwhelming 50-75% of dementia (World Alzheimer's Report 2013). Many of these cases eventually culminate in patient mortality, with AD ranking as the 6th leading cause of death in the United States of America (Alzheimer's Disease Facts and Figures 2013).

The Diagnostic and Statistical Manual classifies AD into two categories. The first category, early-onset AD, applies to individuals who develop the disease before the age of 65. This form is generally caused by a number of genetic predispositions to the disease (Aging, 2011). Individuals that develop AD after the age of 65 fall into the category of sporadic-onset AD. The etiology for this form of the disease is unknown, however it accounts for over 95% percent of current cases (American Psychiatric Association, 2000). As AD progresses, increased neural degeneration leads to a significant decrease in the volume of the brain. This degradation is particularly apparent in areas of the brain associated with learning and memory (Heneka & O'Banion, 2007). Although neuronal death was thought to correlate with cognitive decline, there is evidence to support a stronger association between the loss of synapses/dendritic spines and cognitive decline (Palop & Mucke, 2009).

Dendritic spines can be seen as small protrusions from the dendrite. They play a crucial role in neuronal signaling as they have a high density of neurotransmitter receptors. As such, dendritic spines form synapses with the axons of other neurons in order to conduct intracellular signals. However, they are not permanent structures, and a decrease in the number of dendritic spines has been linked to cognitive decline that is rescued upon their reappearance (Gillani et al., 2010; Papadopoulos et al., 2006).

A potential cause for the loss of dendritic spines is the buildup of soluble forms of the amyloid-β (Aβ) peptide due to its ability to interfere with receptors at the synapse (Lacor et al., 2007; Yamin, 2009). Amyloid-β is a short peptide that results from the sequential cleavage of amyloid precursor protein (APP) by the enzymes β-secretase (extracellular-domain) and γ-secretase (intracellular-domain). Due to the ability of γ-secretase to cleave at multiple sites on APP, many different isoforms of Aβ are possible. The predominant species formed is $A\beta_{1\text{-}40}$. However, the more hydrophobic isoform, $A\beta_{1\text{-}42}$, has been incriminated in the development of AD due to its greater propensity to oligomerize (Y. Zhang, Thompson, Zhang, & Xu, 2011). The senile plaques observed during autopsy of patients with AD are due to the ability of Aβ to oligomerize and form β-sheets. These β -sheets are then able to associate and form large protein aggregates called plaques (Friedrich et al., 2010). As stated earlier, the cognitive decline associated with AD may not be the result of plaques, but rather accumulation of soluble forms of Aβ (Lacor et al., 2007).

Alzheimer's disease belongs to a large group of neurodegenerative ailments collectively known as taupathies (V. M. Lee, Goedert, & Trojanowski, 2001). Central among all taupathies is the dissociation of Tau protein from microtubules. As Tau stabilizes microtubules by binding to the microtubule's α-tubulin and β-tubulin subunits, dissociation can lead to their disintegration and disruption of their function (Gendreau & Hall, 2013; Kar, Fan, Smith, Goedert, & Amos, 2003; Morris, Maeda, Vossel, & Mucke, 2011). Microtubules function in the transport of cellular materials to distal parts of the cell. In the central nervous system (CNS), this process is crucial as axonal projections can sometimes stretch as far as a meter; a distance that is far too great to rely on simple diffusion of nutrients (Holzbaur & Scherer, 2011).These microtubules are made up of two tubulin subunits (α-tubulin and β-tubulin). Together these subunits form a heterodimer with two GTP binding sites. Through the hydrolysis of GTP into GDP, tubulin heterodimers oligomerize and form linear proto-filaments. Proto-filaments then associate laterally into a ring to produce microtubules (Holzbaur & Scherer, 2011; Lodish, Berk, Zipursky, Matsudaira, & Darnell, 2000).

The addition and removal of heterodimers from microtubules is a continuous, active process. As such, stabilizing proteins, such as Tau, exist in order to prevent the degradation of microtubules (Lodish et al., 2000). Tau is predominately found in axonal projections of the CNS (Morris et al., 2011), and as mentioned earlier, binds to and stabilizes microtubules through its microtubule binding domain (MBD) (Gendreau & Hall, 2013; Kar et al., 2003; Morris et al., 2011). Alternative splicing can lead to six main isoforms, or 12 total isoforms, that have a variable number of repeats in the MBD (Morris et al., 2011; Wei & Andreadis, 1998). In addition to the MBD being able to bind microtubules, it is also responsible for the ability of dissociated Tau to form β-sheets; a conformation that is a precursor to many taupathies (Morris et al., 2011). This is due to the PHF6 (paired helical filament; VQIVYK) and PHF6* (VQIINK) motifs, which are just a couple of many possible motifs that have been associated with the formation of PHFs, in the MBD (Morris et al., 2011; von Bergen, Barghorn, Biernat, Mandelkow, & Mandelkow, 2005). Once the normally unfolded Tau folds in β-sheets, it is capable of forming PHFs which oligomerize into neurofibrillary tangles (NFTs) – a hallmark of AD (Bancher et al., 1989; Dickey et al., 2007; Heneka & O'Banion, 2007; von Bergen et al., 2005). Although PHFs were initially visualized by electron microscopy in 1963 by Kidd, it took researchers an additional 20 years to association Tau with their formation (Grundke-Iqbal et al., 1986; Kidd, 1963; Zilka, Kovacech, Barath, Kontsekova, & Novák, 2012).

Dissociation of Tau from microtubules can occur in response to post-translational modifications, such as phosphorylation (Morris et al., 2011). Tau contains a multitude of phosphorylation sites, however not all of them contribute significantly to microtubule dissociation. Important to microtubule dissociation, and thus AD pathology, are those sites in or around the MBD (Augustinack, Schneider, Mandelkow, & Hyman, 2002; Morris et al., 2011). Phosphorylation of these sites leads to a gradual reduction in the affinity of Tau for microtubules (Biernat & Mandelkow, 1999; Gendreau & Hall, 2013). Some of these sites include Ser396, Ser235, and Thr205 (Augustinack et al., 2002; Kitazawa, Oddo, Yamasaki, Green, & LaFerla, 2005). Phosphorylation of other residues in this region (notably Ser262 and Ser356), lead to almost complete loss of Tau's affinity for microtubules (Biernat

& Mandelkow, 1999; Gendreau & Hall, 2013). Once dissociated from the microtubule, Tau is capable of becoming hyperphosphorylated; a condition that favors PHF formation (Heneka & O'Banion, 2007).

Phosphorylation of Tau is aided by its proline-rich domain, which includes a Src homology 3 (SH3) domain and multiple phosphorylation sites (Mayer, 2001; Reynolds et al., 2008). SH3 domains are highly conserved ~60 residue regions that are responsible for protein-protein interactions, including the binding of kinases (such as Src). (Goodsell, 1999; Mayer, 2001; McCormick, 1993).

Due to their ability to bind Src-family kinases, the SH3 domain plays a role in Tau phosphorylation. In this regard, the SH3 domain is capable of recruiting Src-family kinases to phosphorylate tyrosine residues within Tau (Mayer, 2001). However, the vast majority of residues on Tau that become phosphorylated are serine and threonine. Although Src does not play a role in phosphorylating these residues, a number of other kinases do. These include c-Abl, 5' adenosine monophosphate-activated protein kinase (AMPK), cyclin-dependent kinase-5 (Cdk5), glycogen synthase kinase-3 (GSK-3), cyclic AMP-dependent protein kinase (PKA), phosphorylase kinase (PhK), protein kinase C (PKC), Rho kinase, Alzheimer PHF-tau, etc. (Biernat & Mandelkow, 1999; Gendreau & Hall, 2013; Mayer, 2001; Morris et al., 2011). A more complete list of kinases responsible for phosphorylating Tau, including their target residues, can be found at cnr.iop.kcl.ac.uk/hangerlab/tautable (Hanger).

Conflicting reports have argued a role for Cdk5 in the phosphorylation of Tau in response to inflammation (Kitazawa et al., 2005; Sarlus et al., 2012). In the study conducted by Kitazawa et al., they showed that after 6 weeks of twice-weekly injections of lipopolysaccharide (LPS), a component of gram-negative bacteria, at a dose of 0.5mg/kg, there was a CDK5-dependent increase in phosphorylation of Tau at Ser235 and Thr205 in 3x-Tg AD and non-transgenic (WT) mice (Kitazawa et al., 2005). The 3x-Tg strain of mouse is a transgenic strain that is homozygous for three mutations. The first is in presenilin-1 (a γ-secretase subunit) and leads to the increased production of pathogenic Aβ isoforms. The second is a P301L point mutation in Tau, which predisposes it to form PHFs. Finally, human APP is introduced and overexpressed (Oddo et al., 2003). When administered in a purified form, LPS tricks the immune system into believing that there is a bacterial infection, and thus it can be considered a bacterial mimetic when administered alone (Takeuchi & Akira, 2010; Wittmann et al., 2008). Finally, they show an increase in Cdk5 activity in the brains of LPS treated mice, and that inhibition of Cdk5 significantly reduces Tau phosphorylation at Ser235 and Thr205 (Kitazawa et al., 2005).

In addition to these two residues, Cdk5 phosphorylates Tau at an additional 10 sites. As such, it plays a significant role in the phosphorylation state of Tau (Morris et al., 2011). Cdk5 belongs to a class of serine-threonine kinases known as cyclin-dependent kinases (CDKs). Unlike other members of the CDK family, Cdk5 has no known active role in the cell cycle (Dhavan & Tsai, 2001; J. Zhang et al., 2008). Instead it has been shown to have a role in post-mitotic neurons in the prevention of neuronal entry into the cell cycle, neuronal cell migration, and in the organization of neuronal cytoskeleton in the CNS (Cheung & Ip, 2012; J. Zhang et al., 2008). In order for these processes to occur Cdk5 must bind its activator, p35 (Cheung & Ip, 2012; Dhavan & Tsai, 2001; J. Zhang et al., 2008). Cdk5 is directed to the plasma membrane by p35, where it is capable of phosphorylating proteins associated with the plasma membrane (Dhavan & Tsai, 2001). P35 is a relatively unstable protein, and is prone to ubiquitin-directed proteasome degradation – thus regulating Cdk5 activity (Dhavan & Tsai, 2001; Patrick, Zhou, Kwon, Howley, & Tsai, 1998). Cdk5 is also capable of binding to a truncated form of p35, p25. P35 is cleaved into p25 by the enzyme calpain (a calcium-dependent protease) when the cell is undergoing oxidative stress; such as that seen during exposure to Aβ (Alvarez, Toro, Cáceres, & Maccioni, 1999; Dhavan & Tsai, 2001). Furthermore, Aβ neurotoxicity can be rescued by Cdk5 inhibitors, suggesting that hyperphosphorylated Tau and NFT play a major role in the eventual death of neurons exposed to Aβ (Alvarez et al., 1999). P25 localizes Cdk5 to the cytosol and nucleus, causing neurite retraction and apoptosis (Dhavan & Tsai, 2001; Patrick et al., 1999). Unsurprisingly, p25 is found at a much greater concentration in the neurons of AD patients as compared to controls (Patrick et al., 1999).

Another target of calpain is Tau (Canu et al., 1998). In addition to the MBDs and the proline-rich domain, Tau also contains a N-terminal region, which is important for microtubule spacing, as it is capable of interacting with the cell membrane (Frappier, Georgieff, Brown, & Shelanski, 1994; Morris et al., 2011). Present in PHFs is a 17kDa truncated version of Tau (Zilka et al., 2012). Immunoassays have shown that these fragments are derived from the truncation of the N-terminal region and C-terminal regions of Tau (Zilka et al., 2012).

Furthermore, these fragments disappear in the presence of calpain and caspase inhibitors, suggesting that these proteases are necessary for the cleavage of Tau (Canu et al., 1998). Not surprisingly, truncation of Tau at Asp421 by caspase3 is detrimental to neuronal survival and leads to neuronal apoptosis (Fasulo et al., 2000; Zilka et al., 2012).

Previously, researchers have linked inflammation with the onset of AD (J. W. Lee et al., 2008). One form of inflammation that is mediated by the adaptive immune system is allergies. Sarlus et al. showed that allergy-induced inflammation leads to increased phosphorylation of Tau at Ser235 and Thr205 in the hippocampus. However, they did not see a change in APP expression and failed to look into changes in the concentration of Aβ (Sarlus et al., 2012). This form of inflammation is caused due to the release of granules from mast cells IgE receptors bound to the cell recognize a previously encountered pathogen. These granules contain many pro-inflammatory molecules, including TNF-α. Through a signaling cascade, TNF-α is able to activate NF-κB-dependent cytokine release. These cytokines increase vascular permeability at the site of infection, allowing for increased immune cell extravasation, and also causes the redness, pain and swelling that is associated with inflammation.

Another mechanism for the release of cytokines exists in the innate immune system. Inflammation is a key component of the innate immune response that is induced by the recognition of conserved molecular motifs by pattern recognition receptors (PRRs) (Takeuchi & Akira, 2010). These PRRs allow for rapid recognition and response to common pathogenic epitopes. Recognition of one of these motifs leads to the secretion of cytokines that recruit the immune system to the site of infection.. A common PRR involved in the initiation of inflammation is Toll-like receptor 4 (TLR4). An important ligand for TLR4, and initiator of the innate immune response, is LPS.

Patients suffering from AD pathologies express high levels of pro-inflammatory factors in areas of the brain that are most severely affected by AD, breaking the convention that the brain is a immune-privileged site (Akiyama et al., 2000). Some of these factors include IL-1β, TNF-α and IL-6 (Akiyama et al., 2000; Blasko, Marx, Steiner, Hartmann, & Grubeck-Loebenstein, 1999; Heneka & O'Banion, 2007). Interestingly, the presence of chemokines and cytokines at these sites (especially TNF-α and IFNγ) can confound plaque formation by increasing APP cleavage into Aβ (Blasko et al., 1999). Other factors, such as IL-1β help to activate microglial cells. The binding of IL-1β to receptors on the surface of microglia can result in the secretion of other cytokines (such as TNF-α and IL-6) and formation of more IL-1β, creating a positive feedback loop (Heneka & O'Banion, 2007). The increased activity of microglial cells in the areas of the brain most affected by AD results in the increased secretion of complement components (Akiyama et al., 2000; Stalder et al., 1999). Initiation of the complement cascade is thought to trigger a cycle of increased neuronal death and increased inflammation (Akiyama et al., 2000).

Previous studies have linked injections of LPS to cognitive deficits in hippocampal dependent tasks and increases in $A\beta_{1-42}$ (J. W. Lee et al., 2008). Work from our lab agrees with these findings. We have shown that WT (C57BL/6J) mice that have received 7 consecutive days of peripheral LPS injections have significant elevations of A$\beta_{1\text{-}42}$ in the hippocampus. LPS treated animals also exhibited significant cognitive deficits in the hippocampal-dependent task, contextual fear conditioning (CFC). Furthermore, indicative of endotoxin tolerance, central cytokine levels from these animals return to baseline after 7 consecutive days of injections. This suggests that the cognitive deficit is not due to sickness behavior (Kahn et al., 2012). Our lab has also shown that the increase of A$\beta_{1\text{-}42}$ is due to peripheral cleavage of APP as demonstrated through the inhibition of γ-secretase with Imatinib (the active component of Gleevec™) (Weintraub et al., 2013). Numerous studies have reported marginal increases in cerebal spinal fluid (CSF) Imatinib concentrations given dramatic increases in plasma concentrations. This demonstrates the poor ability of Imatinib to cross the blood-brain barrier (BBB) (Kast & Focosi, 2010; le Coutre et al., 2004; Takayama, Sato, O'Brien, Ikeda, & Okamoto, 2002; Williams, den Besten, & Sherr, 2007; Wolff, Richardson, Egorin, & Ilaria, 2003). Using the CFC task we have also shown that A$\beta_{1\text{-}42}$ in the hippocampus significantly contributes to cognitive deficit (Weintraub et al., 2014).

Hippocampal lysates recovered after 7 days of injections followed by 15 days of recovery still showed elevated levels of A$\beta_{1\text{-}42}$ in LPS treated animals (Kahn et al., 2012). Interestingly, recent findings have shown that allowing the animals to exercise during this recovery period returns the levels of A$\beta_{1\text{-}42}$ to baseline (Weintraub, White et al., 2014). The exact mechanism for this added clearance is not well defined, however it could be due to the an increase in the amount of sleep mice receive (a process that has been shown to aid in A$\beta_{1\text{-}42}$ clearance) after having exercised or by increased protein turnover due to exercise (Ding, Vaynman, Souda, Whitelegge, & Gomez-Pinilla, 2006; Xie et al., 2013).

Our model looks to investigate peripheral inflammation as the initiator of AD by setting off a vicious cycle of central inflammation. Amyloid-β is a potential trigger of this inflammatory cycle due to its ability to migrate from the periphery into the CNS and bind C1q (a member of a large group of complement proteins) (Akiyama et al., 2000; Weintraub et al., 2013). This buildup of $A\beta_{1-42}$ could lead to neuronal oxidative stress triggering a Cdk5-dependent increase in the phosphorylation state of Tau (Alvarez et al., 1999; Dhavan & Tsai, 2001). As hyperphosphorylated Tau and the presence of PHFs as well as NFTs are a hallmark of AD, we hypothesized that our model could also lead to increased levels of pTau. In addition we speculated that Tau phosphorylation could potentially be a byproduct of increased levels of $A\beta_{1-42}$, leading us to investigate the concentration of pTau in the hippocampus after different temporal exposures to elevated levels of $A\beta_{1-42}$.

2. Methods

2.1 Subjects

Male and female C57BL/6J mice were utilized in all experiments. All animals were housed and treated in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Texas Christian University and in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

All subjects were housed in standard cages (12.5cm x 15cm x 25cm). Food and water were available *ad libitum*, and all experimental and control groups were on the same 12 hour light/dark schedule (lights on at 0600 and lights off at 1800).

2.2 Injections

2.2a Experiment 1: LPS inducted phosphorylation of Tau expression in young animals

To determine if peripheral inflammation leads to an elevation of pTau we set up a time course that would allow us to see pTau in the CNS after increasing days of exposure to peripheral inflammation. 2-4 month old C57BL/6J mice were divided into 8 groups. Group 1 and 2 received one injection of either LPS (n=3) or saline (n=3) respectively. Groups 3 and 4 received 3 consecutive days of once-daily LPS (n=3) or saline (n=3) injections respectively. Groups 5 and 6 received 7 consecutive days of once-daily LPS (n=3) or saline (n=3) injections respectively (Figure 1). All injections were done intraperitoneally (ip). LPS was derived from *Escherichia Coli* (Serotype: 055:B5; Sigma-Aldrich, St. Louis, Missouri). All LPS was given at a dose of 250 μg/kg.

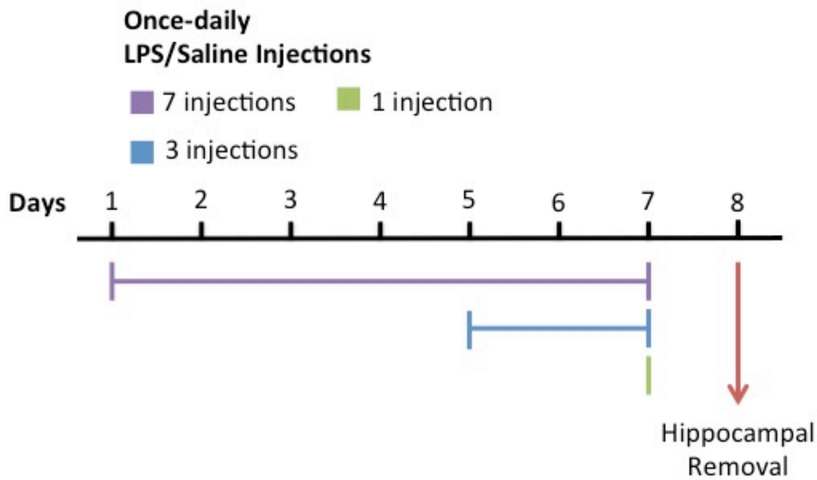

Figure 1 - Injection Timeline for Tau Expression and Phosphorylation experiment
Animals were administered 1, 3 or 7 consecutive days of LPS or saline once a day. Hippocampal tissues were collected prepared for Western blot 24 hours after the final injection.

2.2b Experiment 2: $A\beta_{1\text{-}42}$ exposure related induction of Tau phosphorylation

Previous studies have indicated that exposure of neurons to $A\beta_{1\text{-}42}$ leads to Tau phosphorylation (Alvarez et al., 1999). As our lab has shown that $A\beta_{1\text{-}42}$ remains elevated even after 14 days of rest, we decided to see if this prolonged exposure leads to an increase in pTau. Young 2-4 month old C57BL/6J mice were broken into 2 groups. Group 1 received 7 consecutive days of once-daily ip saline (n=3) injections whereas group 2 received 7 consecutive days of once-daily ip LPS (n=3) injections. LPS was derived from *Escherichia Coli* (Serotype: 055:B5; Sigma-Aldrich, St. Louis, Missouri). All LPS was given at a dose of 250 μg/kg. Mice were allowed to recover in their cages for 15 days.

2.2c Experiment 3: Age-related discrepancies in $A\beta_{1\text{-}42}$ concentrations in the CNS

As previously mentioned, the presence of Aβ can lead to the phosphorylation of Tau (Alvarez et al., 1999). As such, we sought to find another model in which there may be increased levels of Aβ. Since AD is a disease of the aged population, we first determined whether age plays a role in $A\beta_{1-42}$ buildup in the CNS of mice. C57BL/6J mice were divided into 6 groups. Groups 1 and 2 contained young mice (2-4 months) that received 7 consecutive days of once-daily ip LPS (n=7) or saline (n=8) injections respectively. Groups 3 and 4 contained middle-aged mice (14-16 months) that received 7 consecutive days of once-daily ip LPS (n=7) or saline (n=7) injections respectively. Groups 5 and 6 contained aged mice (24-26 months) that received 7 consecutive days of once-daily ip LPS (n=6) or saline (n=6) injections respectively (Table 1). LPS was derived from *Escherichia Coli* (Serotype: 055:B5; Sigma-Aldrich, St. Louis, Missouri). All LPS was given at a dose of 125 μg/kg.

	Age (months)	Treatment	n
Group 1	2-4	LPS	7
Group 2	2-4	Saline	8
Group 3	14-16	LPS	7
Group 4	14-16	Saline	7
Group 5	24-26	LPS	6
Group 6	24-26	Saline	6

Table 1 – List of groups, their age and their treatment for use in comparing age-related differences in hippocampal $A\beta_{1-42}$ levels.

2.2d Experiment 4: Age related discrepancies in Tau and pTau

As AD is considered a disease of the aged population, we sought to determine if age plays a role in Tau expression and phosphorylation. To determine if aged mice follow the same inflammation-induced changes in Tau as young mice, C57BL/6J mice were divided into 2 groups each containing mice between 24-26 months of age. Group 1 received 7 consecutive days of once-daily ip LPS (n=3), whereas group 2 received 7 consecutive days of once-daily ip saline (n=3). LPS was derived from

Escherichia Coli (Serotype: 055:B5; Sigma-Aldrich, St. Louis, Missouri). All LPS was given at a dose of 125 μg/kg.

In order to determine if there is an age related difference in Tau expression and phosphorylation, C57BL/6J mice were divided into 2 groups. Group 1 (n=3) contained aged mice (24-26 months) and group 2 (n=3) contained young mice (2-4 months).

2.3 Tissue Collection and Preparation

At the end of each experimental time point mice were humanely euthanized in accordance with IACUC-approved methods 24 hours after the last injection. Hippocampal tissue samples were then removed and homogenized with cell lysis buffer (PRO-PREP Boca Scientific, Boca Raton, FL), which contains protease inhibitors. Samples were then snap-frozen at -80^{O}C. Lysates were allowed to thaw and then centrifuged at 13,300 rpm for 20 minutes followed by another 10 minutes at 13,300 rpm. The clear lysate was then removed and the pellet discarded.

2.4 DC Protein Assay

The DC protein assay working reagent is used with detergent based buffers in order to quantify the total protein that is present in a sample. Samples were diluted 1:4 with PRO-PREP and 5μL of each sample were loaded onto a 96-well plate. The protein standard curve was made by preparing dilutions of 0.2 mg/mL, 0.4 mg/mL, 0.8 mg/mL and 1.52mg/mL. 5μL of each standard was added to the 96-well plate. Solution A' was created by mixing 20μL of reagent S to every 1mL of reagent A. 25μL of A' was then added to each well containing sample or standard. 200μL of reagent B was then added to each well. After 15 minutes, the optical density (OD) of each well was read at 750nm using the BMG LabTech FLUOstar Omega plate reader (Cary, NC).

2.5 Western Blotting

Samples were diluted to a working concentration of 1μg/μL with sample buffer. Proteins were then denatured by boiling at 100^{O}C for 5 minutes in the presence of β-mercaptoethanol (Sigma, St. Louis, MO) present in the sample buffer, which reduces disulfide bonds. Samples were then separated based on size by loading 15μL of sample into a 10% sodium dodecyl sulfate (SDS) polyacrylamide gel. All Western blot hardware (gel cassettes, gel box and transfer cells) were purchased from Bio-Rad (Hercules, CA). 200V was applied across the gel for 50 minutes. The gels were removed and equilibrated in Towbin transfer buffer for 20 minutes. Proteins were then transferred (Bio-Rad Trans-blot SD, Bio-Rad, Hercules, CA) onto polyvinylidene fluoride (PVDF) or nitrocellulose membranes (Millipore, Billerica, MA). 0.15 amps per gel were applied for 40 minutes during the transfer. The current was conducted through filter papers (Kaysville, UT) soaked in Towbin transfer buffer.

After the transfer, membranes were blocked in 5% blotting-grade blocker (Bio-Rad, Hercules, CA) in phosphate buffered saline with 0.3% Tween®20 (PBST) for 2 hours. Primary antibodies, goat polyclonal IgG anti-Tau, rabbit polyclonal IgG anti-pTau (Ser235) and rabbit IgG anti-pTau (Thr205) were mixed at a 1:200 dilution in PBST. Goat polyclonal IgG anti-GADPH was mixed at a 1:400 dilution in PBST. Primary antibodies were applied to membranes and allowed to incubate overnight at $4^{O}C$. All primary antibodies were purchased from Santa Cruz Biotechnology, Santa Cruz, CA.

The next day the primary antibodies were poured off and the membranes were washed 3 times for 5 minutes and 3 times for 15minuntes in PBST. This procedure was performed for all subsequent washes. Peroxidase-conjugated AffiniPure Donkey anti-Goat IgG secondary antibodies were then applied to blots containing Tau and GADPH antibodies at a 1:5000 dilution in PBST. Biotin-SP-conjugated AffiniPure Donkey anti-rabbit IgG was applied at a 1:20,000 dilution in PBST to blots probing for p-Tau. All secondary antibodies were purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). All blots were allowed to incubate at $27^{O}C$ for 2 hours. Secondary antibody was then discarded and another wash was performed. Blots probing for pTau were then incubated with Peroxidase-conjugated Streptavidin (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) at a 1:20,000 dilution in PBST for 1 hour at $27^{O}C$. This was then discarded and another wash was performed.

All blots were placed on transparencies and coated with the detection reagent, SuperSignal West Pico Chemiluminescent substrate (Thermo Scientific, Waltham, MA). The resulting chemiluminescence was then imaged using a Syngene G:Box (Syngene, Frederick, MD). Densimetric analysis was then performed using GeneTools software (Syngene, Frederick, MD). GADPH was used as a loading control.

2.6 ELISA

To detect levels of Aβ, we utilized a Colorimetric BetaMark™ x-42 Elisa Kit (Covance Reasearch Products, Dedham, Massachusetts). In preparation for this ELISA, a 1X wash buffer, working incubation buffer and standard intermediates were prepared. Standard Diluent was then used to reconstitute Aβ standard which was used to prepare the standard curve. Samples were diluted 2:1 in working incubation buffer. This contains HRP-labeled detection antibody. In duplicate, 100μL of sample or standards were placed into a 96-well plate coated in capture antibody. This was then incubated overnight at 2-8 degrees Celsius. The next day, 5 washes with 1X wash buffer were performed. 200μL of the HRP substrate, TMB, was added to all wells. After a 45 minute incubation in the dark at room temperature the plate was read at an OD of 620nm.

2.7 Statistical Analysis

For Experiment 5 we used a 2(LPS, Saline) X 3(young, middle, old) analysis of variance (ANOVA). For all other experiments we used 1(time point) X 2(LPS, Saline) ANOVA. To determine where which groups were significantly different, Fisher's PLSD post-hocs were used.

3. Results

To investigate the effect of inflammation on Tau expression in the hippocampus, 6 groups of C56BL/6J mice were used. Groups 1 and 2 received 1 day of LPS or saline ip injections respectively. Groups 3 and 4 received 3 days of once-daily LPS or saline ip injections respectively. Groups 5 and 6 received 7 days of once-daily LPS or saline ip injections respectively. 24 hours after the final injection,

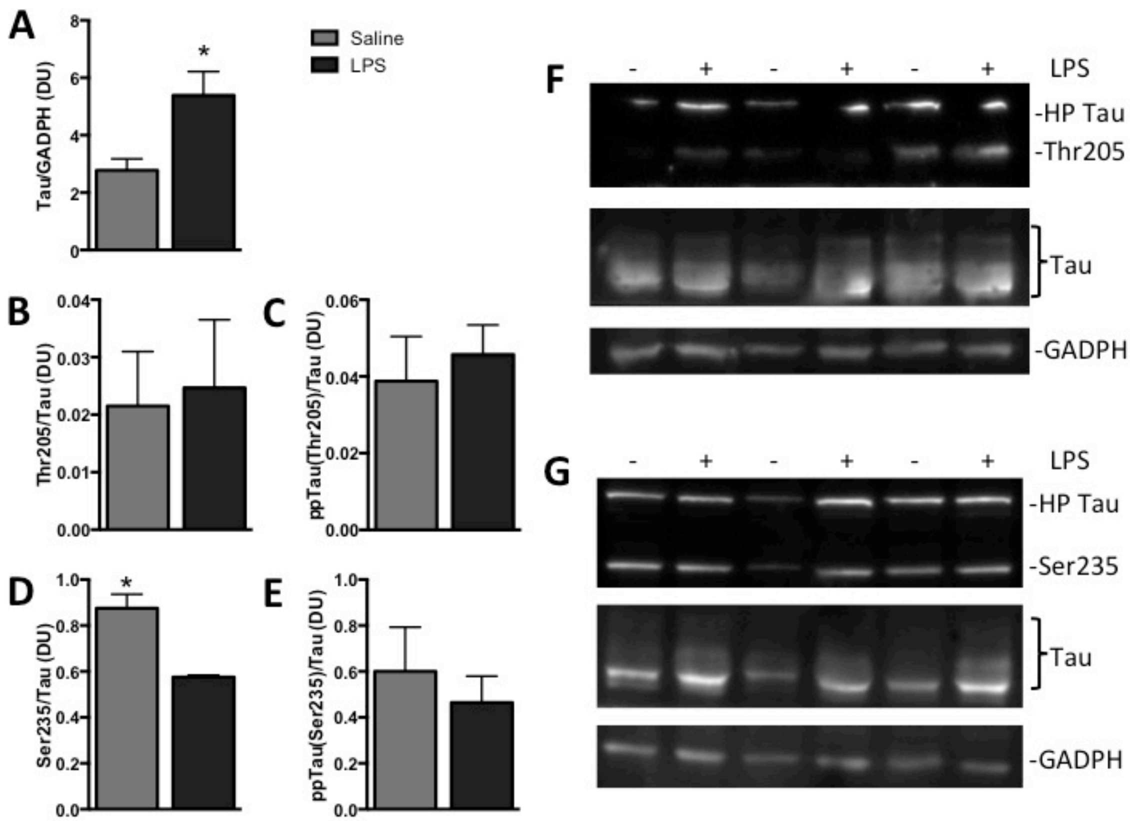

Figure 2 – 7 consecutive days of peripheral inflammation effects Tau expression and pTau in the hippocampus
2-6 month old C57BL/6J mice received 7 days of once-daily injections of either LPS (n=3) or saline (n=3). Western blot analysis of hippocampal lysates revealed **A**) a significant increase in Tau expression in LPS treated mice ($p<0.05$); **B**) no change in pTau (Thr205) ($p>0.05$) or **C)** ppTau (Thr205) ($p>0.05$); and **D)** a significant increase in pTau (Ser235) in saline treated animals ($p<0.05$), but **E)** no increase in ppTau (Ser235) ($p>0.05$). Representative blots for **F)** pTau (Thr205) and **G)** pTau (Ser235). (ppTau – hyperphosphorylated Tau; DU – densitometry units).

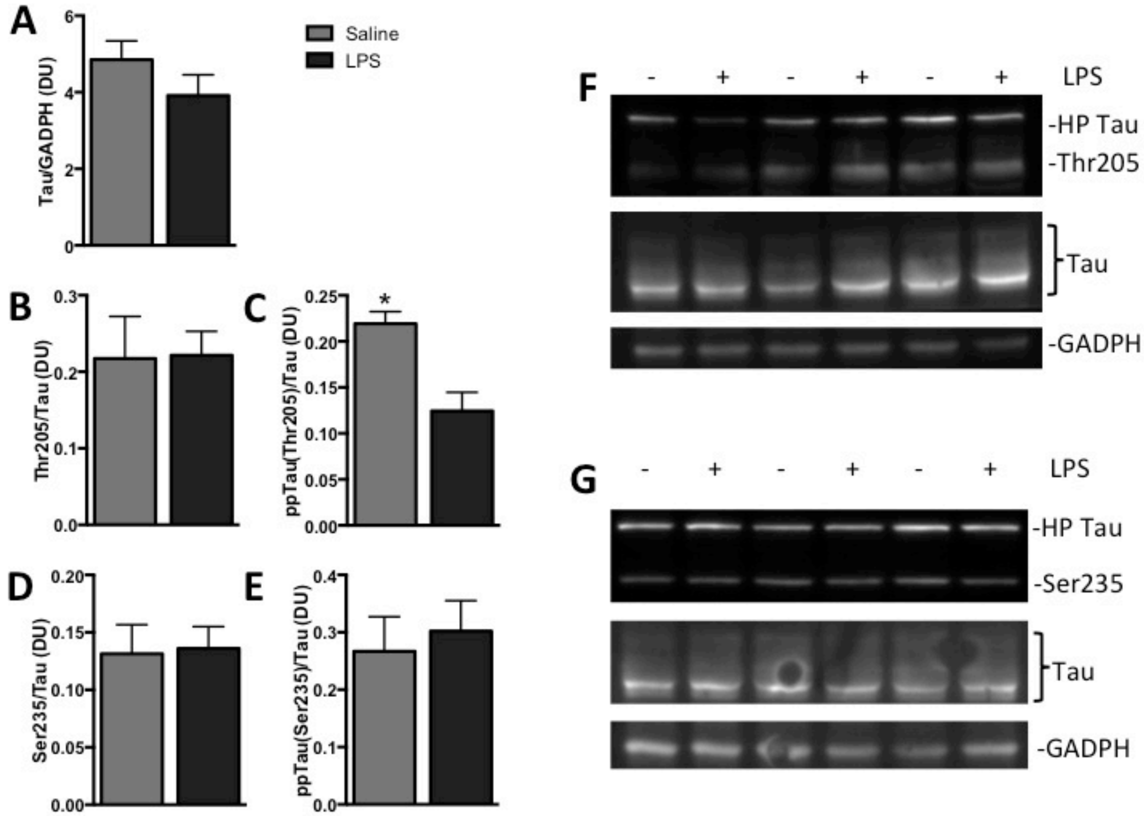

Figure 3 – 1 day of peripheral inflammation does not effect Tau expression and pTau in the hippocampus
2-6 month old C57BL/6J mice received 1 injection of either LPS (n=3) or saline (n=3). Western blot analysis of hippocampal lysates revealed **A**) no significant increase in Tau expression ($p>0.05$); **B**) no change in pTau (Thr205) ($p>0.05$) but **C)** there was an increase in ppTau (Thr205) in saline treated animals ($p<0.05$). **D)** There was no significant increase in pTau (Ser235) ($p>0.05$), and **E)** no increase in ppTau (Ser235) ($p>0.05$). Representative blots for **F)** pTau (Thr205) and **G)** pTau (Ser235). (ppTau – hyperphosphorylated Tau; DU – densitometry units).

hippocampal lysates were analyzed for Tau levels by Western blotting. There was a significant increase in Tau in animals treated with 7 days of LPS over those treated with 7 days of saline (Figure 2A,F,G; $p<0.05$). There was no difference in Tau between LPS and saline treated animals given 1 injection (Figure 3A,F,G; $p>0.05$) or 3 injections (Figure 4A,F,G; $p>0.05$).

Samples were then probed for Tau phosphorylated at Thr205. As can be seen in Figure 2F,G pTau antibodies yield two bands. The lower band corresponds to a

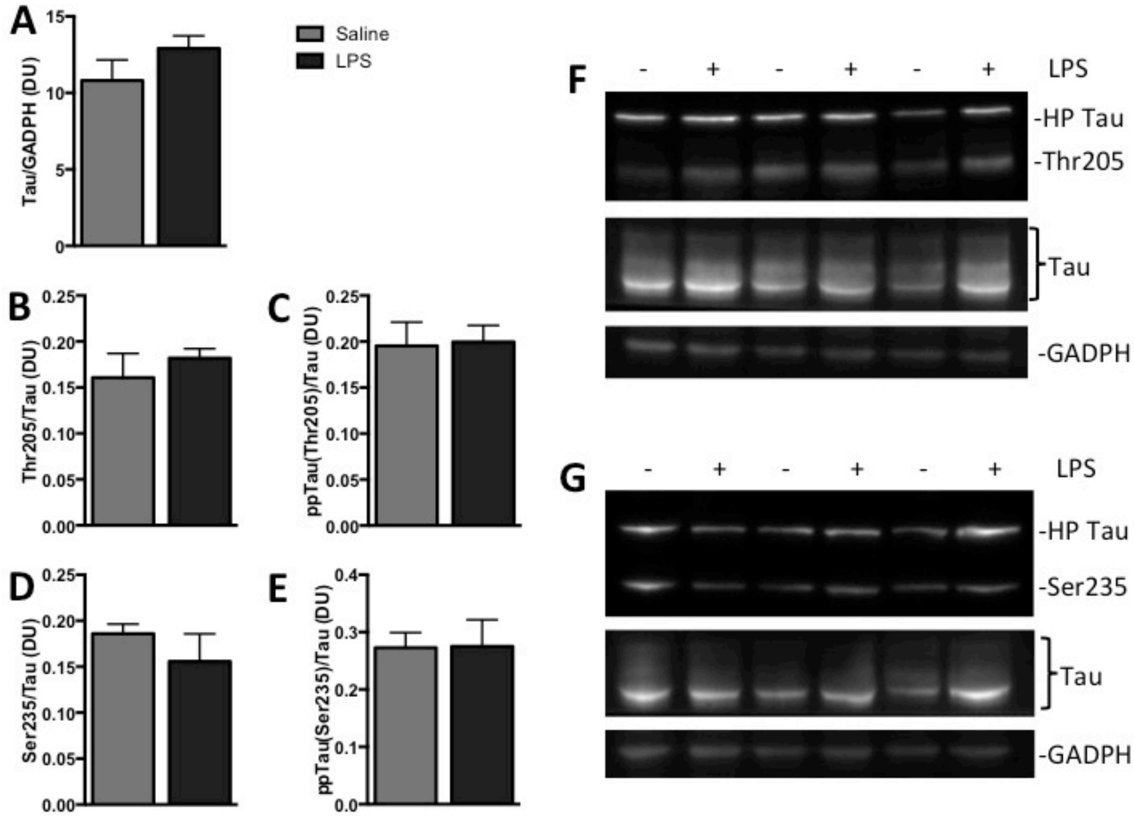

Figure 4 – 3 days of peripheral inflammation does not effect Tau expression and pTau in the hippocampus
2-6 month old C57BL/6J mice received 3 once-daily injections of either LPS (n=3) or saline (n=3). Western blot analysis of hippocampal lysates revealed **A**) no significant increase in Tau expression ($p>0.05$); **B**) no change in pTau (Thr205) ($p>0.05$); **C)** no difference in ppTau (Thr205) ($p>0.05$); **D)** no significant increase in pTau (Ser235) ($p>0.05$); and **E)** no increase in ppTau (Ser235) ($p>0.05$). Representative blots for **F)** pTau (Thr205) and **G)** pTau (Ser235). (ppTau – hyperphosphorylated Tau; DU – densitometry units).

less phosphorylated version of Tau (herein referred to as pTau), whereas the upper band corresponds to a hyperphosphorylated version of Tau (herein referred to as ppTau). Again there was no difference in pTau (Thr205) between LPS and saline treated animals given 1 injection (Figure 3B,F; $p>0.05$) or 3 injections (Figure 4B,F; $p>0.05$). Accordingly, there was also no change in ppTau (Thr205) after 3 injections (Figure 4C,F; $p>0.05$) Oddly, however, there was a significant difference in ppTau (Thr205) between animals treated with 1 day of LPS or 1 day of

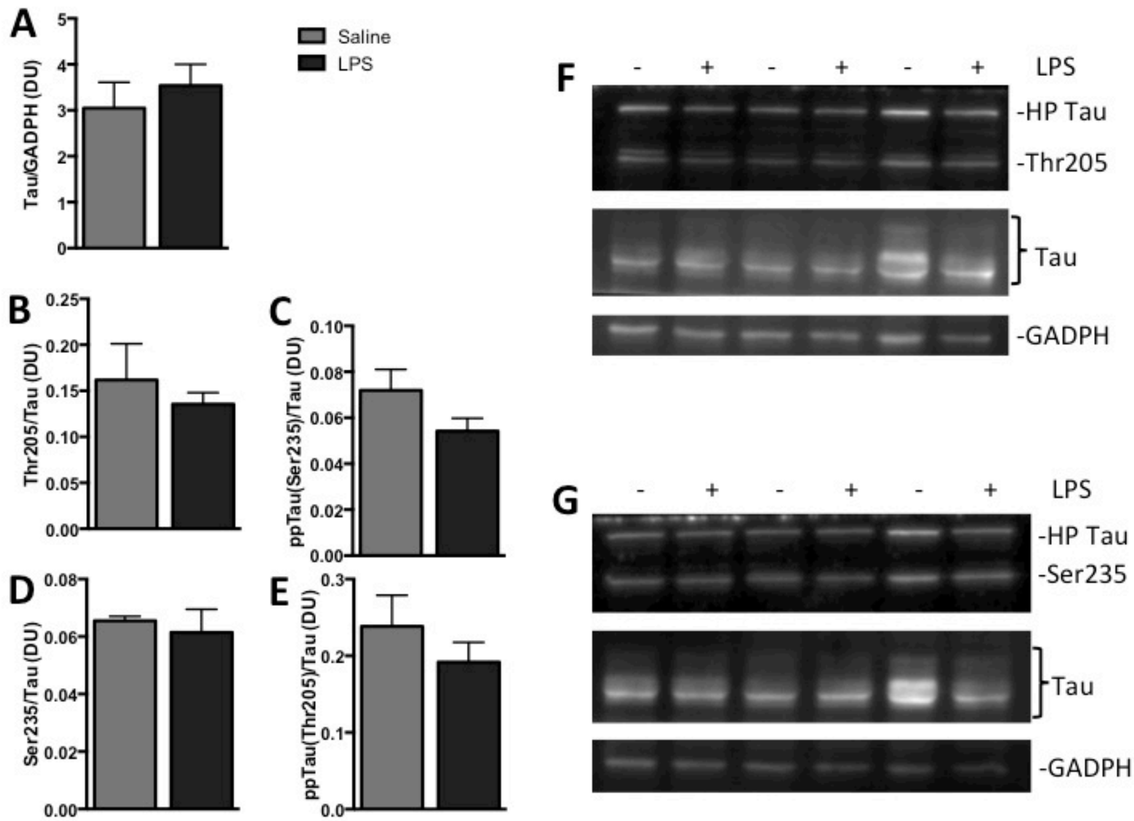

Figure 5 – 15 days of neuronal exposure to A$\beta_{1\text{-}42}$ does not effect Tau expression or phosphorylation
After receiving 7 days of once-daily injections of either LPS (n=3) or saline (n=3), 2-6 month old C57BL/6J mice were allowed to recover in their cages for 15 days. Western blot analysis of hippocampal lysates revealed **A**) no significant increase in Tau expression (p>0.05); **B**) no change in pTau (Thr205) (p>0.05); **C)** no difference in ppTau (Thr205) (p>0.05); **D)** no significant increase in pTau (Ser235) (p>0.05); and **E)** no increase in ppTau (Ser235) (p>0.05). Representative blots for **F)** pTau (Thr205) and **G)** pTau (Ser235). (ppTau – hyperphosphorylated Tau; DU – densitometry units).

saline (Figure 3C,F; p<0.05). Samples were then probed for Tau phosphorylated at Ser235 and no difference was seen in animals given 1 injection (Figure 3D,G; p>0.05) or 3 injections (Figure 4D,G; p>0.05) of either LPS or saline. Furthermore, no difference was seen in ppTau (Ser235) in animals given 1 injection (Figure 3E,G; p>0.05) or 3 injections (Figure 4E,G; p>0.05) of either LPS or saline.

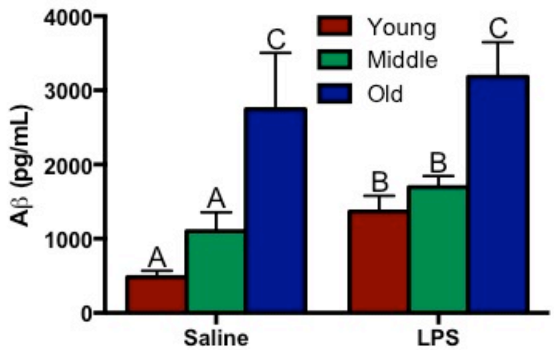

Figure 6 – Comparison of LPS-induced hippocampal Abeta levels in young, middle age, and old mice
After receiving 7 days of once-daily injections of either LPS or saline, hippocampi were recovered from young (2-4 months), middle-aged (14-16 months) and old (24-26 months) and ran in duplicate on an $A\beta_{1\text{-}42}$ ELISA. There was a significant increase in the concentration of $A\beta_{1\text{-}42}$ in young mice given LPS over saline ($p<0.01$.). Middle-aged mice also showed this same increase in $A\beta_{1\text{-}42}$ ($p<0.05$). There was no change in $A\beta_{1\text{-}42}$ between LPS and saline treated aged animals ($p>0.05$). $A\beta_{1\text{-}42}$ was a significantly elevated in older mice treated with saline ($p<0.0001$) and LPS ($p<0.001$) compared to younger and middle aged mice.

Notably, there was a significant increase in pTau (Ser235) in animals given 7 injections of saline over LPS (Figure2D,G; $p<0.05$). This change was not accompanied by an increase in ppTau (Ser235) (Figure 2E,G; $p>0.05$) between LPS and saline treated animals. In addition, there was no change in pTau species phosphorylated at Thr205 after 7 days of either LPS or Saline injections (Figure 2B,C,F; $p>0.05$).

To determine if prolonged exposure to $A\beta_{1\text{-}42}$ leads to increased phosphorylation of Tau we allowed mice to recover for 14 days in their cage after receiving their 7th injection. As shown in a previous study, after 7 consecutive days of LPS injections, $A\beta_{1\text{-}42}$ remains significantly elevated for over 15 days (Kahn et al., 2012). Densitometry analysis showed no significant difference in Tau between saline and LPS treated groups after allowing the mice time to recover (Figure 5A,F,G; $p>0.05$). Samples were then probed for pTau (Ser235) and pTau (Thr205). No difference was seen between LPS and saline treated animals in pTau or ppTau

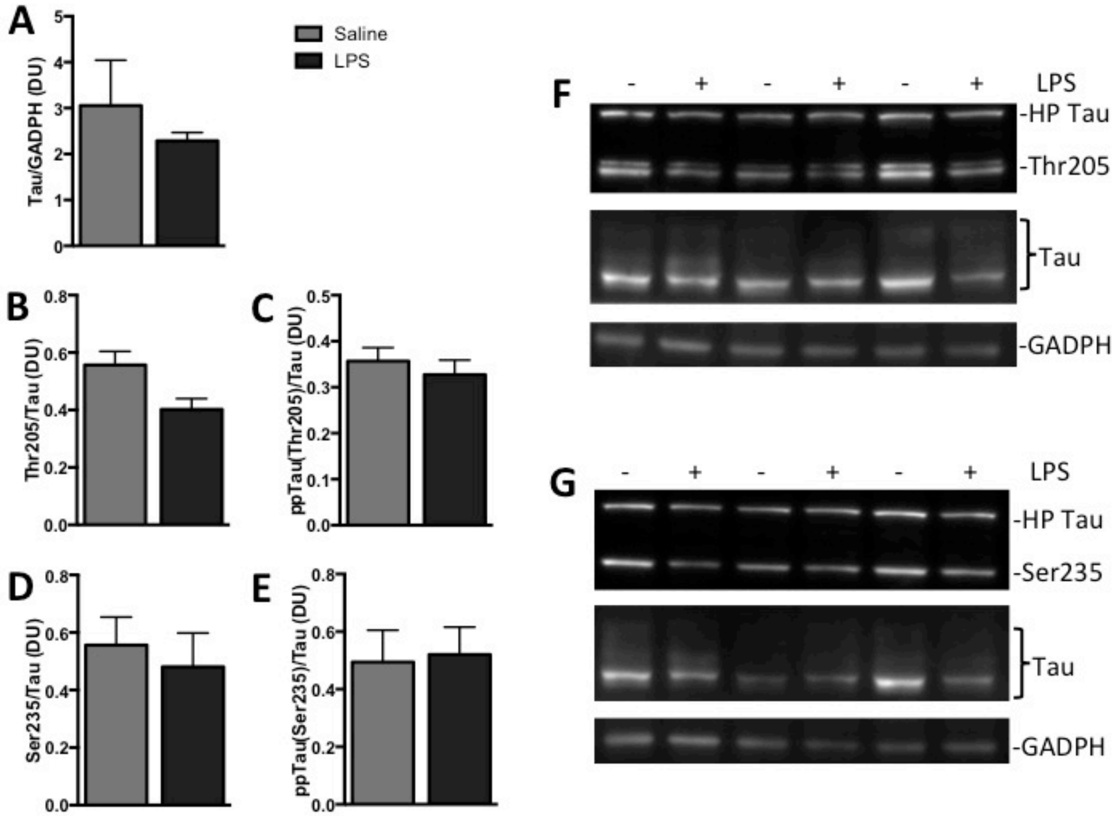

Figure 7 – Tau expression and phosphorylation are not effected by peripheral inflammation in old mice

24-26 month old C57BL/6J mice received 7 once-daily injections of either LPS (n=3) or saline (n=3). Western blot analysis of hippocampal lysates revealed **A**) no significant increase in Tau expression ($p>0.05$); **B**) no change in pTau (Thr205) ($p>0.05$); **C)** no difference in ppTau (Thr205) ($p>0.05$); **D)** no significant increase in pTau (Ser235) ($p>0.05$); and **E)** no increase in ppTau (Ser235) ($p>0.05$). Representative blots for **F)** pTau (Thr205) and **G)** pTau (Ser235). (ppTau – hyperphosphorylated Tau; DU – densitometry units).

(Thr205) (Figure 5B,C,F; $P>0.05$). Also, no difference was seen in pTau or ppTau (Ser235) (Figure 5D,E,G; $P>0.05$) between LPS and saline groups.

Previous studies have shown a correlation between neuronal exposure to $A\beta_{1-42}$ and increased levels of pTau (Alvarez et al., 1999). As such, we set out to investigate if aging plays a role in a buildup in $A\beta_{1-42}$ in the CNS. $A\beta_{1-42}$ levels are significantly elevated in young ($p<0.01$) and middle ($p<0.05$) aged mice who received LPS over those that received saline (Figure 6). This increase was not seen

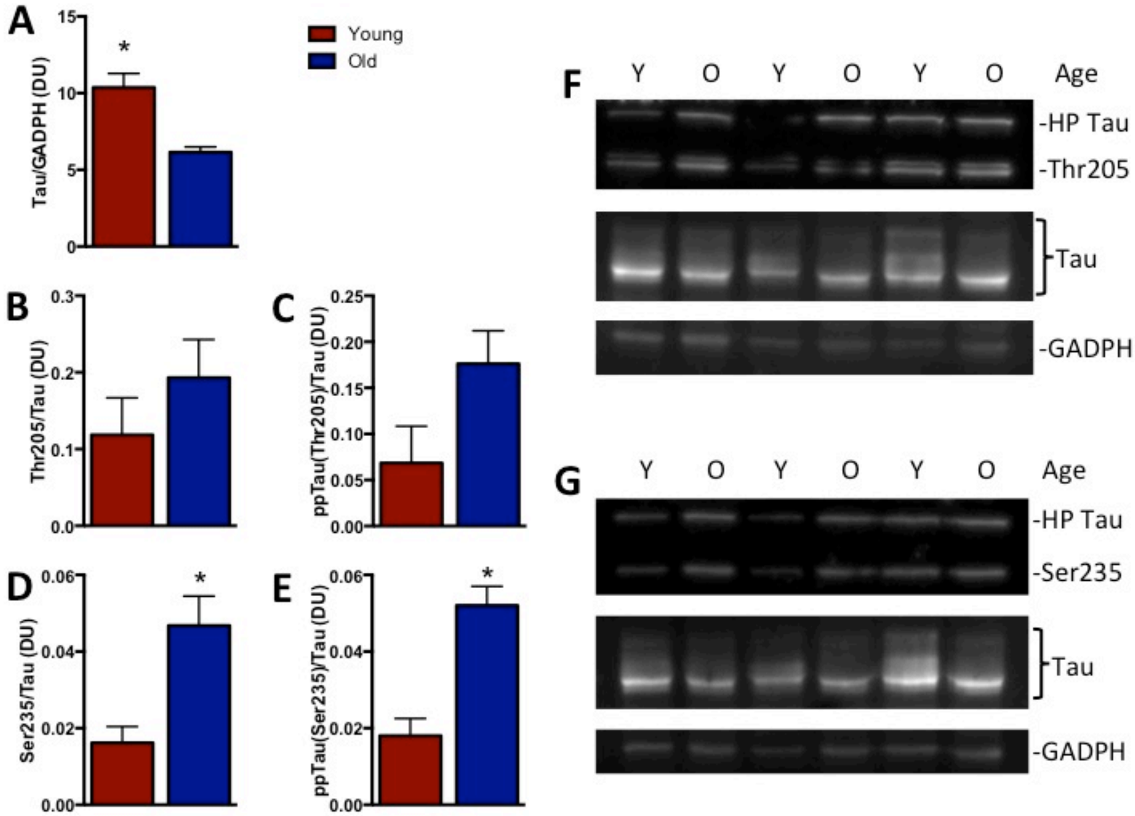

Figure 8 – Age related discrepancies in Tau expression and phosphorylation Hippocampal lysates from young (2-6 month; n=3) and old (24-26 month; n=3) mice were recovered and compared for the presence of Tau and pTau species via Western blot. **A**) There was a significant increase in Tau expression in young mice ($p<0.01$); **B**) no change in pTau (Thr205) ($p>0.05$) or **C)** ppTau (Thr205) ($p>0.05$); and **D)** a significant increase in both pTau (Ser235) ($p<0.05$) **E)** ppTau (Ser235) ($p<0.05$). Representative blots for **F)** pTau (Thr205) and **G)** pTau (Ser235). (ppTau – hyperphosphorylated Tau; DU – densitometry units).

in older mice ($p>0.05$). However, old mice who received either LPS ($p<0.05$) or saline ($p<0.0001$) showed significantly higher levels of $A\beta_{1-42}$ over their young and middle aged counterparts who received the same treatment.

To see if the lack of change in levels of $A\beta_{1-42}$ is mirrored by Tau, we injected aged mice (24-26 months) with either LPS or saline. Densitometry analysis showed no significant difference in Tau between saline and LPS treated groups (Figure 7A,F,G; $p>0.05$). Samples were then probed for pTau (Ser235) and pTau (Thr205).

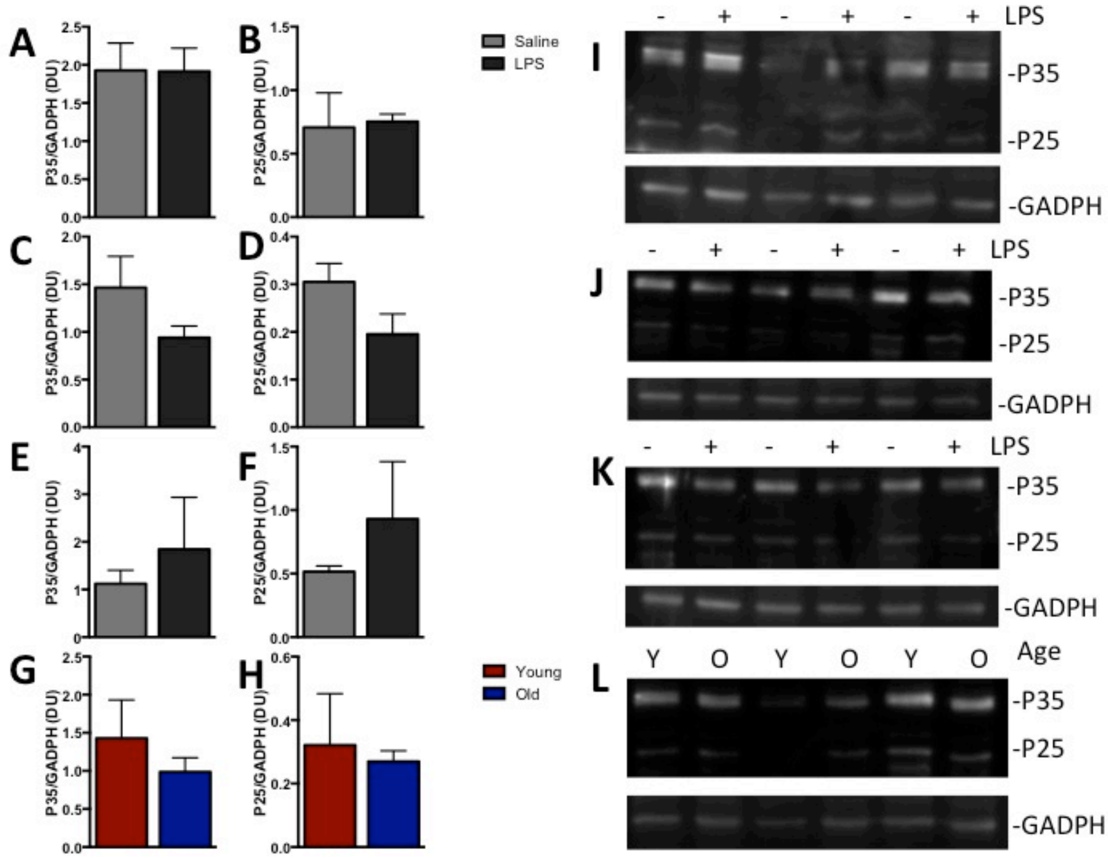

Figure 9 – Cdk5 subunit concentrations remains unchanged
2-6 month old C57BL/6J mice received 7 once-daily injections of either LPS (n=3) or saline (n=3). Western blot analysis of hippocampal lysates revealed **A**) no significant increase in P35 expression (p>0.05) or **B**) in P25 levels (p>0.05). After receiving 7 days of once-daily injections of either LPS (n=3) or saline (n=3), 2-6 month old C57BL/6J mice were allowed to recover in their cages for 15 days **C)** no difference was seen in P35 (p>0.05) or **D)** in P25 (p>0.05). Next 24-26 month old C57BL/6J mice received 7 once-daily injections of either LPS (n=3) or saline (n=3) **E)** no increase was seen in P35 (p>0.05) nor **F)** P25 (p>0.05). Finally Cdk5 subunits were compared between young (2-6 month) and old (24-26 month) C57BL/6J mice. **G)** No difference was seen in P35 (p>0.05) or **H)** P25 (p>0.05). Representative blots of **I)** young animals that received 7 LPS or saline injections, **J)** young animals that recovered for 15 days in their cages after receiving 7 LPS or saline, **K)** old animals that received 7 LPS or saline injections, and **L)** a comparison between young and old animals (DU – densitometry units).

No difference was seen in pTau or ppTau (Thr205) (Figure 7B,C,F; p>0.05). Also no difference was seen in pTau or ppTau (Ser235) (Figure 7D,E,G; p>0.05)

To determine if there are age related differences in the phosphorylation of Tau, like those that were seen in $A\beta_{1-42}$ levels, we compared pTau concentrations in old mice (24-26 months) to young mice (2-4 months). Densitometry analysis revealed that younger animals have a higher concentration of Tau (Figure 8A,F,G; $p<0.01$). However, older mice had a higher concentration of pTau (Ser235) and of ppTau (Ser235) (Figure 8D,E,G; $p<0.05$). Finally there was no difference in pTau or ppTau (Thr205) in young or old animals (Figure 8B,C,F; $p>0.05$).

The role of CDK5 in peripheral inflammation-induced Tau phosphorylation has yielded conflicting results in previous studies (Kitazawa et al., 2005; Sarlus et al., 2012). Due to the lack of phosphorylation on Thr205, it is unlikely that CDK5 activity was increased. As expected, in all tests, we did not see a significant change in CDK5s normal activating subunit, p35 ($p>0.05$), or the highly stable p25 form (Figure 9; $p>0.05$).

4. Discussion

The purpose of this study was to determine if our model of LPS-induced peripheral inflammation leads to an increase in pTau species. Previous studies have shown a correlation between inflammation and Tau phosphorylation using either allergy induced inflammation or an LPS-based model that does not lead to endotoxin tolerance (Kitazawa et al., 2005; Sarlus et al., 2012). Continuous exposure of cells to LPS leads to a reduction in the immune response known as endotoxin tolerance (Biswas & Lopez-Collazo, 2009). Although not completely understood, endotoxin tolerance is thought to be a self-preservation mechanism; restricting the immune response (and it's tissue damaging components) in order to protect the surrounding tissue during prolonged exposure to endotoxin (Biswas & Lopez-Collazo, 2009). Using our model of 7 consecutive days of once-daily ip injections of LPS we found a change in total Tau and in pTau species phosphorylated at Ser235 in the hippocampus. Oddly, this change was a decrease in the ratio of pTau/Tau in LPS treated animals over saline treated animals. As our model leads to endotoxin tolerance (Kahn et al., 2012) and other model systems do not (Kitazawa et al., 2005), we thought to eliminate this variable by taking tissue samples before endotoxin tolerance. At these earlier time-points Tau and pTau species remained unchanged between saline and LPS treated mice, with the exception of ppTau (Thr205) after one injection. Previously our lab has shown that $A\beta_{1\text{-}42}$ is not significantly elevated until after the 7^{th} injection. Furthermore, our lab has shown that our model of inflammation causes endotoxin tolerance after 3 injections (Kahn et al., 2012). As we do not see a change in Tau or pTau species until after the 7^{th} injection (the same time in which we have shown a noticeable increase in $A\beta_{1-42}$) it is less likely that proinflammatory cytokines are responsible for changes in Tau, and thus possible that the presence of $A\beta_{1-42}$ may be needed in order to induce changes Tau or pTau.

As previously stated, oxidative stress can lead to increased phosphorylation of Tau in neurons. Since the presence of $A\beta_{1-42}$ causes oxidative stress (Alvarez et al., 1999; Patrick et al., 1999), we investigated if prolonged exposure to $A\beta_{1-42}$ could lead to an increase in pTau. Previous work in our lab showed that $A\beta_{1-42}$ levels remain elevated for up to 15 days after the 7^{th} injection of LPS (Kahn et al., 2012). Unfortunately this prolonged exposure to $A\beta_{1-42}$ did not produce an increase in Tau or pTau species in our experiment.

Another factor that leads to an increase in oxidative stress is aging. As oxidative stress leads to increases in $A\beta_{1-42}$, we sought to investigate if there are age related differences in $A\beta_{1-42}$ levels in the hippocampus. Using our inflammation model in aged mice we showed that aging has a significant effect on the level of $A\beta_{1-42}$, whereas LPS-induced inflammation did not. Although LPS and saline treated old animals did not significantly differ from each other in $A\beta_{1-42}$ levels, both groups had significantly more $A\beta_{1-42}$ than their young or middle-aged counterparts. This suggests that age alone is sufficient to produce significant levels of hippocampal $A\beta_{1-42}$.

Tau and pTau followed this trend, in that we were unable to see a significant difference in the phosphorylation state of Tau between old LPS and saline treated animals. However, as expected, when comparing aged mice to young mice, we did find a significant difference in Tau, and Tau species phosphorylated at Ser235.

Together these data suggest that the process of aging plays a role in the pathogenesis of AD, predisposing the older population to the disease.

The process of aging leads to a multitude of cellular changes, including the silencing and disregulation of genes. The immune system is not immune from this, and as such, the immune response in aged mice differ from that of young mice. A micro array analysis conducted by Chelvarajan et al. showed 362 genes that are regulated differently in splenic macrophages between young and aged mice in response to LPS. Included in these are the pro and anti-inflammatory cytokines IL-1β, IL-6, IL-12 and IL-10. With the exclusion on IL-10, all of these factors are higher in young mice (Chelvarajan et al., 2006). These differences in how aged animals are able to upregulate inflammatory factors, could explain why we do not see a difference in $A\beta_{1\text{-}42}$, Tau or pTau in aged animals treated with LPS as opposed to saline.

An important factor in the phosphorylation of Tau is the activation of specific kinases that target Tau. As we did not see a change in the levels of CDK5 activating subunits, another kinase must be responsible. Several other kinases are known to phosphorylate Tau at Ser235, including AMPK, PKA, GSK-3, JNK, p42/p44 mitogen-activated protein, p38MAPK, PhK, stress activated protein kinase, SAPK2a, SAPK2b, SAPK3, SAPK4 and Alzheimer PHF-tau. However, a great number of these also phosphorylate Tau at Thr205, and we did not detect this change in any of our experiments. As such the most likely kinase is AMPK, as it phosphorylates Ser235 but not Thr205 (Hanger). Although PhK appears to be another potential candidate, active PKA is needed, which would have led to an increase in the phosphorylation of

Tau at Thr205 (Brushia & Walsh, 1999). Mairet-Coello et al. recently showed AMPK-dependent Tau phosphorylation in response to neuronal exposure to $A\beta_{1-42}$ (Mairet-Coello et al., 2013). As the change in Tau and pTau species in our model corresponds to an increase in $A\beta_{1-42}$, there could suggest a potential link to AMPK activity.

An interesting factor that could be confounding these data is the presence of heat-shock proteins (HSPs). Heat shock proteins are a major class of molecular chaperones that interact with other proteins in order to carry out a variety of functions within the cell. Some of these functions include regulating gene expression by binding to transcription factors, aiding in the proper folding of other proteins and tagging aberrant proteins for degradation (Dickey et al., 2007; Schlesinger, 1990). HSPs have been identified as being either beneficial or detrimental to a number of different diseases including cancer, diabetes and inflammatory bowel disease (Barbatis & Tsopanomichalou, 2009; J.-H. Lee et al., 2013; Luo, Rodina, & Chiosis, 2008). Interestingly, in the case of AD, the interaction of HSP40, HSP70 and HSP90 determines the fate of pTau. HSP70 and HSP40 are responsible of the initial binding of pTau. HSP90 is then able to take pTau from this complex through the aid of the HSP70/HSP90-organizing protein (HOP). HOP is a co-chaperone that facilitates the interaction between HSP70 and HSP90 (Scheufler et al., 2000). Failure of pTau to be transferred to HSP90 results in pTau being ubiquinated by CHIP (C terminus of HSC70 interacting protein)(Dickey et al., 2007). CHIP binds to the C-terminus of HSP70/HSC70 and has E3 ubiquitin ligase activity. This allows it to ubiquinate HSP70/HSC70 client proteins, effectively tagging them for degradation (Jiang et al., 2001). If pTau is successfully transferred to HSP90, then it is either refolded, or held in the cell and protected from degradation (a process that favors NFT formation) (Dickey et al., 2007).

Previous work done in our lab shows that the stress of an injection leads to an increase in HSP90 in the hippocampus (unpublished data). We have also recently shown that after 7 days of LPS injections, HSP70 is significantly elevated in mice that received LPS over those who received saline. With such neuro-protective mechanisms in place, it may be hard to fully demonstrate the effects of peripheral inflammation and $A\beta_{1-42}$ accumulation on the phosphorylation of Tau. Elimination of these HSPs could reveal a greater change in pTau than we are currently able to observe. However, inhibition of HSPs in the CNS poses a unique issue, as few HSP inhibitors are capable of crossing the BBB. For example, while MKT-077 (an HSP70 inhibitor) actively affects Tau levels in vitro, its use as a therapeutic is limited as it is not capable of crossing the BBB. Miyata et al. recently developed a derivative of MKT-077 (YM-08) that is capable of interfering with HSP70 activity, that could be useful in our line of experiments (Miyata et al., 2013).

Another way to factor out the effect of HSPs on the level of phosphorylated Tau is to instead check for kinase activity. Whereas HSPs may be masking a rise in the presence of pTau, the activity of kinases responsible for the phosphorylation of Tau could give great insight into the possibility of an increase in Tau phosphorylation.

As stated earlier, AMPK is most likely the kinase responsible for the observed inflammation-induced phosphorylation of Tau. An important residue that AMPK phosphorylates is Ser262. As mentioned earlier, phosphorylation of Tau at this residue leads to its almost complete loss of affinity for microtubules. As such, using an antibody specific to Tau phosphorylated at Ser262, it would be interesting to see if our model is capable of phosphorylating this residue. This evidence would substantially contribute to the possibility of inflammation leading to not only increases in $A\beta_{1-42}$ but also the dissociation of Tau from microtubules.

These data presented here suggest that there is a relationship between the presence of $A\beta_{1-42}$ and pTau. An investigation into deciphering this relationship would prove interesting. A previous study in our lab showed that Imatinib selectively eliminates $A\beta_{1-42}$ in the hippocampus while not effecting inflammatory cytokine activity (Weintraub et al., 2013). In this paradigm, if pTau were also reduced, it would suggest that $A\beta_{1-42}$ is necessary for the phosphorylation of Tau. A potential flaw in this paradigm is that LPS could potentially lead to an increase in permeability of the blood, brain barrier (BBB), leading to an increase of Imatinib in the CNS. Imatinib is highly specific tyrosine kinase inhibitor (although it has a strong affinity for the tyrosine kinase, BCR-Abl). (Deininger & Druker, 2003) As such the preservation of the BBB would have to be verified in order to assure that Imatinib is not directly interfering with the phosphorylation of Tau by inhibiting kinases in the CNS.

Although we have not demonstrated the perpetuation of AD in our model, it does provide a possible explanation for the genesis of the disease. Our model not only leads to an increase in $A\beta_{1-42}$ in the hippocampus, but as demonstrated by these experiments, also leads to changes in the expression and phosphorylation of Tau. Furthermore, these data show that the process of aging could predispose a person to AD. Together, these data add further evidence to inflammation being a potential contributing factor in the onset of AD, as well as providing a novel inherent route for the formation of the disease.

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ABSTRACT

INFLAMMATION- AND AGE-RELATED ALTERATIONS IN TAU EXPRESSION AND PHOSPHORYLATION

by

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Alzheimer's disease (AD) is characterized by neurodegeneration associated with the formation of amyloid-beta (Aβ) plaques and neurofibrillary tangles in the brain. Our lab has previously shown that inflammation can lead to an increase in central Aβ, following 7 consecutive injections of lipopolysaccharide (LPS). Using this LPS-induced inflammation model, we demonstrated an increase in Tau expression and a decrease in the phosphorylated Tau (pTau)/Tau ratio at Ser235 and Thr205. Furthermore, we demonstrated that older mice have higher basal levels of Aβ and LPS does not induce a further increase of Aβ in this population. Interestingly, in this population, we found that increases in Aβ correspond to a higher proportion of pTau and a decrease in total Tau. Together these data demonstrate that inflammation is capable of altering Tau phosphorylation and expression, and the detrimental effects of aging exacerbate pathological markers of AD.