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Focus on the Locus: Elucidating the roles of tau pathology and the locus coeruleus in Alzheimer's Disease

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Abstract

Focus on the Locus: Elucidating the roles of tau pathology and the locus coeruleus in Alzheimer's Disease

By Termpanit (Natty) Chalermpalanupap

Alzheimer's disease (AD) is an insidious, progressive neurodegenerative disorder with societal and financial burdens that are escalating every single day. Despite knowing about the disease for over a century now, we are still unable to slow, stop or prevent it. The lack of a clear understanding of the underlying neuropathological mechanisms that govern the production and interaction of the two main pathologies of the disease: extracellular beta-amyloid plaques and intracellular tau neurofibrillary tangles, greatly hampers our ability to develop effective diagnostics and therapeutics. It has been particularly difficult to catch the disease at its earliest stages prior to irreversible neurodegeneration and cognitive damage as the neuropathological changes occur decades prior to outward clinical onset. Recently, it was discovered that an early form of tau pathology, a hyperphosphorylated tau precursor to the neurofibrillary tangles that fill AD brains, can be detected in the subcortical region of the brain in relatively young, cognitively unimpaired individuals. This early tau pathology is detected in the locus coeruleus (LC), the main source of norepinephrine to the brain, prior to anywhere else and before any other AD-like pathology such as beta-amyloid deposits. These findings fomented more research into the role of the LC in AD as an area with the potential to act as a "ground zero" for AD tau pathology. We have demonstrated here that this early presence of hyperphosphorylated tau in LC neurons is detrimental to their structure, significantly reducing their neurite lengths, as well as sensitizing them to secondary insults. Degeneration of the LC is also an established hallmark of AD and has been shown to exacerbate beta-amyloid pathologies and related cognitive impairments, and we are the first to show that it interacts in a similar manner with tau. Together, these studies have characterized the intertwining roles of tau and the LC at both the early stages where pretangle tau deposition can negatively impact the LC as well as later stages where LC degeneration in turn exacerbates tau pathology. In light of this evidence, it is clear that LC and the noradrenergic system are important targets within the pathogenesis of AD and could provide a wealth of information to improve the development of novel diagnostics and therapeutics to treat and prevent AD.

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ABBREVIATIONS

Aβ	beta-amyloid
AD	Alzheimer's disease
APP	amyloid precursor protein
ANOVA	analysis of variance
CNS	central nervous system
DIV	days *in vitro*
DBH	dopamine beta hydroxylase
FTDP-17	Frontotemporal lobe dementia and parkinsonism linked to chromosome 17
GFP	green fluorescent protein
IL-6	interleukin 16
IL-1A	interleukin 1a
i.p.	intraperitoneal
ITI	inter-trial interval
LPS	lipopolysaccharide
LC	locus coeruleus
MAPT	microtubule associated protein tau
Ms-Prp	mouse prion promoter
DSP-4	n-(2-chloroethyl)-n-ethyl-2-bromobenzylamine hydrochloride
NFT	neurofibrillary tangle
NE	norepinephrine
NET	norepinephrine transporter
PFA	paraformaldehyde
PBS	phosphate buffered saline
PS1	presenilin-1
SEM	standard error of mean
SNP	single nucleotide polymorphism
TBS	tris buffered saline
TBS-Tw	tris buffered saline with tween
TH	tyrosine hydroxylase
WT	wildtype

CHAPTER 1: BACKGROUND AND LITERATURE REVIEW

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1.1 ALZHEIMER'S DISEASE

1.1.1 BRIEF HISTORY OF ALZHEIMER'S DISEASE

Discovery

In 1901, German psychiatrist Alois Alzheimer examined a 51-year-old woman after she was admitted to his hospital in Frankfurt. The patient, Auguste Deter, exhibited a remarkable collection of cognitive and psychosocial impairments including memory loss, disorientation, aphasia, paranoia and auditory hallucinations (Alzheimer, Stelzmann et al. 1995). When asked to write her name during her physical examination, she began with "Mrs" and subsequently forgot the rest of her name, citing "I have lost myself" (Maurer, Volk et al. 1997). Alzheimer continued to monitor Auguste as her condition steadily deteriorated over the next five years until her death in 1906. He performed a histopathological evaluation of her brain post-mortem and observed a cortex dotted with "numerous small military foci" and neurons filled with thick fibrils of "peculiar impregnability" (Maurer, Volk et al. 1997). At a conference in Tübingen later that year, Alzheimer went on to describe Auguste's case and the new form of early onset, presenile dementia he suspected was a result of the accumulating hallmark amyloid plaques and neurofibrillary tangles in her brain. Following the discovery of additional similar cases, another German psychiatrist, Dr. Emil Kraepelin, began using the eponym "Alzheimer's disease" (AD) for the first time in 1911 (Maurer, Volk et al. 1997).

Epidemiology

While the presenile dementia that Alzheimer described was originally thought to be distinct from the more well-known, later-onset senile dementia, it is now accepted that they are the same disease (Hardy and Selkoe 2002, Selkoe and Hardy 2016). Since its discovery over a century ago, AD has become recognized as the leading cause of dementia in the elderly, affecting approximately 11% of those over 65 in the United Sates (Tomlinson, Blessed et al. 1970, Hebert, Weuve et al. 2013). Age remains the most significant risk factor for developing AD, and the

incidence rate for the disease doubles every five years after 65 (Hendrie 1998, Kawas and Corrada 2006, Querfurth and LaFerla 2010). As modern medicine increases life span, the burden of this disease grows steadily along with it. Particularly daunting for the United States is the aging of the large population of "baby boomers" born shortly after World War II (1946-1964). As the last of the baby boomers reach 65 years of age in 2030, the proportion of Americans aged 65 or older will encompass over 20% of the population (a sharp increase from the 14% in 2012) (Ortman JM 2014). This surge in older individuals is predicted to result in a 35% increase in new cases of AD (Hebert, Beckett et al. 2001).

The burden of AD extends beyond its debilitating effects on the patient; it also has far-reaching implications for their caregivers and the overall healthcare system. The cost of caring for a family member with AD can be daunting even aside from the economic losses of unpaid care (totaling over 18.1 billion hours valued at $221.3 billion in 2015) (Alzheimer'sAssociation 2016). AD caregivers experience higher incidences of emotional stress and depression as well as physical health problems, especially as patients deteriorate and require more intensive care (Alzheimer'sAssociation 2016). The healthcare cost of AD for the United States is estimated at $236 billion for 2016, comprising primarily of costs from government systems such as Medicare (50%) and Medicaid (18%), but also a significant amount directly from out-of-pocket expenses (19%) (Alzheimer'sAssociation 2016). These burdens will continue to balloon as more patients requiring care emerge, pushing the projected cost of the disease to $1 trillion by 2050, with a quintupling in spending across all sectors.

AD is not only an expensive disease to care for, but it is also one that is deadly. Despite likely underreporting due to the constellation of AD-associated comorbidities (Wachterman, Kiely et al. 2008), the disease is one of the top ten leading causes of death in the United States (Xu JQ 2016). Strikingly, AD is the only one on this list that has experienced an increase in the last decade (up 71%) compared to other diseases such as heart disease, certain cancers, HIV or stroke that have been on a decline (Xu JQ 2016). It is clear that if these trends continue, the

disease will greatly jeopardize our ability to live long, healthy lives. Together, this information highlights the pressing need to elucidate, combat and treat Alzheimer's disease.

1.1.2 CLINICAL PRESENTATION OF THE DISEASE

While there are many causes for *dementia*, which is an umbrella term describing decline in mental ability serious enough to interfere with daily life (Alzheimer'sAssociation 2016), AD is the most common and encompasses between 60 -70% of neurodegenerative dementia cases (Tomlinson, Blessed et al. 1970, Barker, Luis et al. 2002). The disease typically presents in later adulthood, with the majority of patients aged over 65. The exceptions to this are rare, early (30-60 years), aggressive forms of AD that are inherited via autosomal-dominated mutations in genes that encode proteins involved in the disease (see below) (Bateman, Aisen et al. 2011). These monogenetic forms represent only a small fraction of the overall global burden of AD (1-5%), with sporadic, late-onset AD encompassing the majority of all cases (Blennow, de Leon et al. 2006, Goedert and Spillantini 2006, Turner 2006). Despite the differences in the timing and progression, both variants of AD share similar cognitive features and neuropathology.

Cognition

AD is a progressive neurodegenerative disorder that is characterized by an insidious and gradual onset of dementia over many months or years rather than a sudden loss of cognitive abilities (McKhann, Knopman et al. 2011). Its most recognizable symptom is amnestic presentation: patients struggle with their memory and are unable to learn and/or recall recently learned information. Other cognitive dysfunctions such as issues with language (reading or writing), as well as visuospatial (difficulty recognizing objects or faces) and executive processing (decision making, reasoning, judgement, problem solving) are also often present (McKhann, Knopman et al. 2011).

Affect

Despite being better known as a "memory" disease, AD has many other, often overlooked, non-cognitive symptoms. Over 50% of AD patients experience a range of behavioral symptoms of either non-psychotic or psychotic features (Lanari, Amenta et al. 2006). Non-psychotic symptoms (apathy, depression, agitation, a) can be observed early, often prior to the onset of cognitive dysfunction, while psychosis (hallucinations, delusions) is more prevalent in the later stages of disease progression.

A disruption to the delicate balance between the cholinergic and monoaminergic systems in the brain due to degeneration is thought to be at the root of these behavioral irregularities as there is severe degeneration of these systems in AD. Loss of the locus coeruleus (LC) and norepinephrine (NE) in its projection areas are associated with depression and aggression (Herrmann, Lanctot et al. 2004). Deficiencies in the cholinergic system and loss of the nucleus basalis of Meynert can affect corticolimbic connections important in regulating mood disturbances, resulting in apathy and indifference (Cummings and Kaufer 1996).

Clinical Diagnosis

Currently, AD cannot be definitively confirmed until post-mortem analysis of brain tissue, but a diagnosis of "possible or probable AD" can be given through clinical examination (Turner 2006). The standard method for diagnosis of AD begins with neurological and physical assessments, a comprehensive review of patient and family medical histories, and mental health and laboratory tests to rule out other potential causes of dementia such as medications, hypothyroidism, vitamin deficiency, systemic illness, vascular dementia, hydrocephalus, tumors and depression (Alzheimer'sAssociation 2016).

A diagnostic toolbox with tests like the Mini-Mental State Examination (MMSE) and guidelines such as the Diagnostic and Statistical Manual, 5th edition (DSM-V) or the National Institute of Neurologic, Communicative Disorders and Stroke-AD and Related Disorders

Association (NINCDS-ADRDA) criteria are also used to aid in the differential diagnosis of AD (Alzheimer'sAssociation 2016). Secondary methods such as neuroimaging and biomarkers are being introduced for use in conjunction with these assessments to substantiate the AD diagnosis (see below). Improvements in such diagnostics to facilitate early and specific detection of AD will be crucial for developing and implementing effective treatments.

1.1.2 NEUROPATHOLOGY

AD is characterized by two main neuropathologies, extracellular beta-amyloid (Aβ) plaques and intracellular tau neurofibrillary tangles (NFTs). There is still controversy in the field about if and how the two pathologies may interact to cause the disease (Holtzman, Goate et al. 2011, Holtzman, Morris et al. 2011), when and where each type of pathology first appears in the brain, and the physiological and pathological relevance of their presence.

Beta-amyloid

Aβ peptides are the main component of the senile or neuritic extracellular plaques seen in the brains of AD patients. While initially thought to be an abnormal protein, it is now known that Aβ is produced as a part of normal cell metabolism (Haass, Schlossmacher et al. 1992) through a sequential, proteolytic cleavage of the transmembrane amyloid precursor protein (APP).

APP is expressed throughout the body and in a variety of cell types including neurons, astrocytes and microglia (Ling, Morgan et al. 2003). The protein can be cleaved by a group of secretase enzymes via two main pathways (see Figure 1.1). The non-amyloidogenic pathway involves sequential cleavage by α- and γ-secretases to produce soluble sAPPα and the non-pathogenic p3 peptide. The alternative pathway involves cleavage by beta (β-) and gamma (γ-) secretase enzymes and produces Aβ fragments of varying lengths (Suh and Checler 2002, Olsson, Schmidt et al. 2014). The most prominent species in AD are $A\beta_{40}$ and $A\beta_{42}$, with $A\beta_{40}$ being more

abundant but $A\beta_{42}$ being more toxic and fibrillogenic (Butterfield 2002, Irie, Murakami et al. 2007).

Mutations in genes associated with Aβ production lead to familial AD. Some of the first mutations were discovered in the APP gene, favoring its proteolytic cleavage by β- and γ-secretases to form Aβ over the non-amyloidogenic pathway. Conversely, a recently discovered APP mutation (A673T) that lowers Aβ production by 40% has been associated with a lower risk of AD and of cognitive decline in the non-demented elderly (Jonsson, Atwal et al. 2012). Further examination of familial AD pedigrees uncovered more AD-linked mutations affecting the presenilin-1 (PSEN1) and presenilin-2 (PSEN2) catalytic protein subunits of γ-secretase that promote the production of $A\beta_{42}$ (Scheuner, Eckman et al. 1996). Patients with Down syndrome, who have an extra copy of the APP gene due to trisomy of chromosome 21 where it is located, also develop plaques and have a higher risk for early onset AD (Glenner and Wong 1984, Wiseman, Al-Janabi et al. 2015). In addition, the most significant genetic risk factor for sporadic late-onset AD is a variant of the gene encoding Apolipoprotein E (ApoE4) that impairs Aβ clearance (Castellano, Kim et al. 2011, Cerf, Gustot et al. 2011).

The discoveries of these genetic links resulted in a paradigm shift from the viewpoint that amyloid deposits were a result of AD to the idea that Aβ directly causes pathogenesis and etiology (Yankner 1989, Joachim and Selkoe 1992). This became the foundation for the "amyloid cascade hypothesis", first coined in 1992 by John Hardy and Gerald Higgins, which postulates that dysfunction in the production and clearance of Aβ is the initiator of a cascade of neuronal dysfunction (including oxidative stress, inflammation, neurodegeneration) as well as pathology and the underlying cause of the dementia seen in the disease (Hardy and Selkoe 2002, Selkoe and Hardy 2016).

Monomeric Aβ is soluble, but oligomerizes in pathological conditions before forming fibrils and aggregating into plaques. In the AD brain, plaques appear first as diffuse non-neuritic

structures in the temporal neocortex, progressing down to the entorhinal layers and hippocampus before filling most of the neocortex and gradually maturing into neuritic plaques (Thal, Rub et al. 2000). While these insoluble plaques are the main pathological hallmark of the disease, they are poorly correlated with other neuropathologies like neurofibrillary tangles, as well as cognitive impairments in AD (Arriagada, Growdon et al. 1992, Giannakopoulos, Herrmann et al. 2003), and many now believe that the final fibrillar form of Aβ may not be the main culprit. Aβ oligomers have begun to emerge as the potential toxic agent of Aβ, and their effects on many crucial neuronal processes such as synaptic and mitochondrial function, cell metabolism, neurotoxicity, and neurodegeneration are being investigated (reviewed in (Benilova, Karran et al. 2012, Larson and Lesne 2012, Kayed and Lasagna-Reeves 2013).

Despite the convincing genetic evidence and its popularity, the amyloid cascade hypothesis has encountered multiple challenges in the years since its conception. In addition to the lack of a strong correlation between plaques and cognitive decline in AD patients, imaging studies have identified numerous cognitively healthy individuals with brains riddled with amyloid plaques (Villemagne, Pike et al. 2011). The presence of such a population seems to suggest that amyloid deposits or even prolonged exposure to oligomeric amyloid (which such individuals presumably endured for many years prior to the arrival and accumulation of fibrillary deposits) alone may not be sufficient to cause AD. Moreover, while transgenic mice overexpressing human APP with the canonical genetic mutations mentioned above exhibit intense amyloid pathology in their brains as well as s cognitive impairments, they cannot faithfully replicate the entire AD phenotype, especially NFT development and neurodegeneration (Howlett and Richardson 2009, Lithner, Hedberg et al. 2011, Webster, Bachstetter et al. 2014). While promising results from clinical trials aimed at removing amyloid to ameliorate AD symptoms continue to emerge(Sevigny, Chiao et al. 2016) , the majority have also been disappointing, with all drugs to date failing to yield cognitive benefits even when reducing plaque load (Doody, Thomas et al. 2014, Salloway, Sperling et al. 2014).

These issues, coupled with a lack of an amyloid plaque-only form of dementia, suggest that Aβ may not be the sole source for AD-related dementia, and the etiology of the disease may involve other components such as tau playing an equally fundamental role.

Tau

Concurrent with the extracellular plaques that fill the brains of AD patients are intracellular neurofibrillary tangles comprised of paired helical filaments (PHF). The term PHFs was coined by Michael Kidd in 1963 after he observed NFTs from AD brains under an electron microscope and saw filaments that "wound helically around one another" (Kidd 1963). It took over two more decades before the main component of PHFs was revealed to be the microtubule-associated protein tau (MAPT) (Delacourte and Defossez 1986, Kosik, Joachim et al. 1986, Goedert, Wischik et al. 1988).

Tau normally exists in a highly soluble form that is abundant in most neuronal cells. The human MAPT gene is located on chromosome 17q21 and contains 16 exons. Exons 2, 3 and 10 of MAPT undergo alternative splicing, resulting in six different tau isoforms characterized by either 0, 1 or 2 N-terminal inserts and three or four repeat regions at the C-terminal (0N3R, 0N4R 1N3R, 1N4R, 2N3R, 2N4R). Unlike amyloid deposition, which is specific to only a few diseases (AD, Down's syndrome, dementia pugilistica), tau and tangle pathology comprise the foundation of numerous diseases - so named *tauopathies*. There are over 20 clinical tauopathies (reviewed in Williams, 2006 #163}, which can be grouped based primarily on the tau isoforms found in their aggregates: 4R tauopathies (progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), argyrophilic grain disease (AGD)), 3R tauopathies (Pick's disease (PiD)) and mixed tauopathies with both 4R and 3R (AD). Filamentous tau is also abundant in all tauopathies, but with great diversity in their structure and distribution pattern. While primarily confined to the intracellular compartments of neurons in AD and PiD, tau pathology proliferates in both neuronal and glial cells in PSP, CBD, and AGD.

Function

The structure of tau reflects its primary function: the C-terminal repeat regions act as positively charged domains that facilitate its binding to negatively charged microtubules in axons to stabilize them for axonal transport and cytoskeletal growth (Weingarten, Lockwood et al. 1975). Despite this integral role, multiple lines of tau knockout mice ($Tau^{-/-}$) have been generated that are viable and do not show any overt phenotypes initially (Harada, Oguchi et al. 1994, Dawson, Ferreira et al. 2001, Fujio, Sato et al. 2007). This is likely due to compensatory mechanisms from similar proteins in the microtubule-associated protein (MAP) family such as microtubule-associated protein 1A (MAP1A) or MAP1B. Young $Tau^{-/-}$ mice have increased expression of MAP1A (Harada, Oguchi et al. 1994, Fujio, Sato et al. 2007), and cross-breeding $Tau^{-/-}$ to $MAP1B^{-/-}$ mice exacerbates neuronal cytoskeletal dysfunction (Takei, Teng et al. 2000). With age, the ability of MAP1A diminishes and by 12 months, $Tau^{-/-}$ mice develop muscle weakness, motor deficits (Lei, Ayton et al. 2012, Lopes, Lopes et al. 2016), hyperactivity, cognitive impairments in spatial learning (Ikegami, Harada et al. 2000, Ma, Zuo et al. 2014, Regan, Piers et al. 2015), and neurodegeneration (Lei, Ayton et al. 2012). $Tau^{-/-}$ mice also have abnormal circadian rhythm, with longer periods of wakefulness, less non-rapid eye movement (NREM) sleep and shorter sleep bouts (Cantero, Hita-Yanez et al. 2010).

Post-translational modification/disease state

Although tau can be modified post-translationally in many ways (ubiquitination, oxidation, glycosylation, cleavage, truncation, or sumoylation, to name a few), it is exquisitely primed for phosphorylation, with 80 serine (Ser)/threonine (Thr) and 5 tyrosine (Tyr) phosphorylation sites spread along its structure (Martin, Latypova et al. 2011, Wang, Xia et al. 2013). When phosphorylated by kinases, tau loses its ability to bind to microtubules, thus permitting cytoskeletal fluidity that is integral to many neuronal processes (Drewes, Trinczek et al. 1995,

Sengupta, Kabat et al. 1998, Rodriguez-Martin, Cuchillo-Ibanez et al. 2013). As such, tau phosphorylation is highly regulated during development, beginning with high phosphorylation early in mammalian development in order to allow for maximal synaptic plasticity, before decreasing with age to stabilize and maintain the neuronal structure (Goedert, Spillantini et al. 1989, Kosik, Orecchio et al. 1989). By existing in equilibrium between its phosphorylated and native states, microtubule binding proteins like tau enable the dynamic nature of neuronal morphology and axonal transport (Drechsel, Hyman et al. 1992, Benitez-King, Ortiz-Lopez et al. 2006).

In disease states, however, this equilibrium is toppled (see Figure 1.2). Despite normally existing as a highly soluble protein, aberrant hyperphosphorylation of certain amino acid residues within tau's structure induces irreversible aggregation; first, into soluble, hyperphosphorylated pretangle material, which then contorts into less soluble PHFs before eventually maturing into the more insoluble NFTs (Goedert, Klug et al. 2006, Hanger, Anderton et al. 2009). It is postulated that dysfunction or dysregulation of the phosphatases and kinases that interact with tau is responsible for disease pathogenesis, and many researchers are interested in targeting them for pharmacological therapies (Dolan and Johnson 2010, Braithwaite, Stock et al. 2012, Martin, Latypova et al. 2013).

At the cellular level, hyperphosphorylated tau pre-tangle material mislocates from axonal areas to fill the soma and dendritic processes of cells where it incubates and aggregates into NFTs. Regional staging of tau pathology in the AD brain is believed to begin with early hyperphosphorylated tau in the LC and sub-LC regions, which spreads up to the entorhinal cortex, hippocampus, amygdala and on to the neocortex over time (Braak and Del Tredici 2011, Braak, Thal et al. 2011). This spatial and temporal pattern of spread mirrors the clinical symptoms, beginning with early changes in a variety of non-cognitive areas like sleep and affect, followed by subtle memory impairments and a steady decline across higher function cognitive domains leading to a vegetative state and eventually death (Bird 1998, Raudino 2013, Ringman,

Liang et al. 2015). NFT burden also correlates very well with the severity of disease and dementia in patients (Tomlinson, Blessed et al. 1970), suggesting that tau may be the critical player in the developments of symptoms in AD.

There is still debate on whether tau aggregates exert a gain of toxic function or a loss of normal function in disease states. Hyperphosphorylation of tau leads to its dissociation from microtubules and causes disassembly, impairing the axonal transport that is essential to neuronal survival and function (Iqbal, Alonso Adel et al. 2005, Spires-Jones, Stoothoff et al. 2009). On the other hand, hyperphosphorylated tau itself can induce neurodegeneration (Alonso, Di Clerico et al. 2010) and, as with Aβ, tau oligomers are emerging as key toxic agents in AD pathology (Ward, Himmelstein et al. 2012, Sahara and Avila 2014). For example, tau oligomers can impair long term potentiation (LTP), thought to be the underlying molecular mechanism of learning and memory, in hippocampal slices when applied in the bath *in vitro,* as well as memory formation when infused directly into the hippocampus *in vivo* (Fá, Puzzo et al. 2016).

Furthermore, tau appears to be necessary for Aβ excitotoxicity both *in vitro* and *in vivo*. Cultured neurons from $Tau^{-/-}$ mice are resistant to Aβ-induced degeneration (Rapoport, Dawson et al. 2002), defects in axonal transport (Vossel, Zhang et al. 2010), reductions in membrane potential (Pallo and Johnson 2015), and impaired LTP (Shipton, Leitz et al. 2011). Crossing mice expressing mutant human APP (hAPPJ20) mice to $Tau^{-/-}$ mice rescues the premature mortality and cognitive impairments that characterize the APP strain, despite having no impact on neuritic dystrophy (Roberson, Scearce-Levie et al. 2007). These protective effects (improved survival and cognition) were reproduced when crossing $Tau^{-/-}$ mice to another APP mouse model (APP23) (Ittner, Ke et al. 2010), and are attributed to a resistance to excitotoxins and abnormal neuronal over-excitation (Roberson, Scearce-Levie et al. 2007). Work showing that tau has an influence on postsynaptic NMDA receptor downstream signaling cascades also supports a protective role of tau against excitotoxicity (Ittner, Ke et al. 2010)

Most interestingly, the protective effect of tau deletion appears to be specific to AD. Crossing $Tau^{-/-}$ mice with mouse models of other neurodegenerative disorders such as ALS (Morris, Koyama et al. 2011) and Parkinson's disease (Roberson, Halabisky et al. 2011) does not improve their phenotypes. Tau deficiency also does not protect against prion disease pathogenesis (Lawson, Klemm et al. 2011). This specificity to AD suggests that tau may have a fundamental role in the disease and that its interaction with Aβ is highly important.

Nevertheless, there are also many pieces of evidence that suggest that, while tau may be necessary, it is not sufficient on its own it to induce AD. Unlike beta-amyloid, there are no known tau mutations that result in familial AD (Iqbal, Alonso Adel et al. 2005, Goedert and Spillantini 2006). Transgenic mice expressing tau mutations also never develop beta-amyloid plaques (Dujardin, Colin et al. 2015). Thus, to understand more about the disease, it is likely that we will need to target both pathologies to effectively combat it.

Advancements in Diagnostics Based on Neuropathology

Genetic Testing

Genetic screens for the mutations associated with familial early-onset AD (PSEN1, PSEN2 and APP) exist, however, due to the rarity of these mutations, they are only implemented in specific, high-risk cases. Although assessment of APOE variants continues to increase in predictive value with improvements in sequencing technologies, APOE genotyping remains a controversial way to diagnose sporadic AD.

Imaging

In addition to ruling out physical changes in the brain that can result in dementia but are unrelated to AD, imaging techniques are also used to probe for AD-specific features to corroborate the AD diagnosis. These techniques fall under three main categories: structural imaging to visualize

neurodegeneration, functional imaging to probe for reductions in brain metabolism and molecular imaging for the presence of Aβ or tau pathologies.

Structural change in the brain, especially the hippocampus, is a marker of AD progression (Braak and Braak 1995). Computerized tomography (CT) scans and magnetic resonance imaging (MRI) can be used to detect atrophy in the medial temporal lobe, where the hippocampus is located (Small 2006, Yuan, Gu et al. 2009). This can improve diagnostic accuracy over clinical guidelines alone, but has limited specificity for AD because hippocampal changes are common to many other non-AD dementias (Small 2006). Such a general diagnostic is also limited in its ability to catch early pathological changes, as it necessitates significant structural degeneration for detection.

Reduction in cellular activity in brain areas involved in learning and memory are well documented in AD patients. Diminished cerebral glucose metabolism can be seen via fluorodeoxyglucose (FDG) positron emission tomography (PET). Hypometabolism in certain brain areas are characteristic of AD (parietotemporal association area, posterior cingulate and precuneus), and FDG-PET can be used to predict progression from cognitively normal to MCI to AD (reviewed in (Kato, Inui et al. 2016). Single photon emission computed tomography (SPECT) can provide information about blood flow and brain perfusion and is more widely available and cost-effective, though it suffers from lower resolution and limited quantitative outputs compared to PET (Sanchez-Catasus, Stormezand et al. 2016). Like the neurodegeneration seen with structural imaging techniques, imaging for reduced brain activity at current resolutions still lacks specificity for AD and remains an adjunct to other diagnostic tests.

There has been significant progress in the last decade to improve imaging for AD by focusing on molecular tracers to visualize the disease’s pathological protein accumulations (amyloid and tau). There are many radioactive tracers available to detect amyloid in the brain through PET, including Pittsburg compound B (^{11}C PiB), ^{18}F florbetapir, ^{18}F flutametamol, and ^{18}F florbetaben. The use of amyloid imaging allows for potentially earlier detection as the

insidious nature of AD requires years or even decades of pathological accumulation prior to outward clinical manifestations.

The use of amyloid scans in clinical practice does have certain limitations. Despite being a hallmark pathology of AD, amyloid can also be found in the brains of healthy, cognitively unimpaired individuals as a result of non-pathological brain changes with age (Rowe and Villemagne 2011). Other neurological disorders such as Lewy body dementia and cerebral amyloid angiopathy can also result in an abnormal amyloid PET scan (Catafau and Bullich 2015). In addition, while the tracers used in these scans have received approval from the Food and Drug Administration (FDA), their use as a diagnostic in routine clinical practice has not been approved by Medicare and Medicaid services, meaning that patients are generally responsible for shouldering the (generally exorbitant) costs themselves.

PET tracers for tau remain in the early stages of development. Several tau PET tracers are being investigated, however many lack the specificity for tau neurofibrillary tangles (reviewed in (James, Doraiswamy et al. 2015). The most promising one so far, ^{18}F AV-1451, has been reported to allow for tau visualization with sufficient resolution to estimate the clinical staging of tau, though these claims remain unconfirmed by postmortem neuropathology (Scholl, Lockhart et al. 2016, Schwarz, Yu et al. 2016). With continuing improvements in sensitivity and specificity as longer lasting and more specific compounds are developed, a combination of imaging techniques will likely have improved accuracy for AD diagnosis.

Biomarkers

Many researchers believe that one of the most promising avenues to effective diagnosis for AD will be early detection via biochemical biomarkers. Cerebrospinal fluid (CSF) from lumbar punctures can be tested for levels the core elements of MCI and AD: beta-amyloid fragments ($A\beta_{40}$, $A\beta_{42}$) (Strozyk, Blennow et al. 2003, Fagan, Mintun et al. 2006), phosphorylated tau (Buerger, Ewers et al. 2006), and total tau (Blennow and Hampel 2003) (reviewed in (Blennow

2004, Scarano, Lisi et al. 2016). Other CSF biomarkers include different APP isoforms (Olsson, Hoglund et al. 2003, Zetterberg, Andreasson et al. 2008), beta-secretase-1 (BACE1) (Holsinger, Lee et al. 2006, Verheijen, Huisman et al. 2006, Zhong, Ewers et al. 2007), Aβ oligomers (Georganopoulou, Chang et al. 2005), and total Aβ turnover, although these have not fully evaluated for their accuracy and/or are still in early development.

Because lumbar puncture for CSF collection remains a daunting task for many patients despite the relative painlessness and low risk of side effects (Menéndez-González 2014), there is still a push for the development of peripheral blood- or urine-based biomarkers. However, early investigations of peripheral samples suggest they do not track changes in the brain as well as CSF; plasma $A\beta_{40}$ and $A\beta_{42}$ levels, for example, do not reliably track beta-amyloid load or predict AD (Vanderstichele, Van Kerschaver et al. 2000).

Other non-invasive methods for detection are in the early stages of development. Some interest in the correlation between early Aβ deposits in the eye and AD (Hart, Koronyo et al. 2016) has led to characterization of retinal screening as a potential non-invasive diagnostic (Heaton, Davis et al. 2015, Reed, Behar-Cohen et al. 2016). Further research into the early changes that characterize AD will greatly enhance our abilities to develop better diagnostic tests that can catch patients before they progress too far to be effectively treated.

1.2 LOCUS COERULEUS AND NOREPHINEPHRINE SYSTEM

1.2.1 BASIC CHARACTERISTICS

Anatomical locus

The LC is the major subcortical site for the synthesis of NE (Freedman, Foote et al. 1975). It was first identified in 1809 by Reil and named in 1812 by Wenzel and Wenzel (Maeda 2000). Its etymology, which comes from the Latin words for “blue spot”, was inspired by the nuclei’s dark blue appearance in unstained tissues due to the presence of neuromelanin inside of

its neurons (though this feature is missing in some lower mammals) (Maeda 2000). The LC is situated in the rostral pons as part of the brainstem, laterally flanking the fourth ventricle.

On average, the adult human LC has between 22,000 to 51,000 neurons bilaterally, compared to the billions of cortical neurons the nucleus innervates; thus, a single LC neuron must cover multiple neurons in its projection areas (Mouton, Pakkenberg et al. 1994, Maeda 2000). To do this, it has exceptionally far-reaching and highly ramified axonal projections that allow it to innervate nearly all areas of the brain as well as the spinal cord (Maeda 2000). Most of the neurons in the nucleus are grouped topographically by their terminal fields and functional targets (Berridge and Waterhouse 2003). As such, the nucleus is also very roughly compartmentalized rostrocaudally and dorsoventrally, with most of the neurons in the central and dorsal portions projecting to the forebrain, and the more ventral portions projecting to the spinal cord and cerebellum (Foote, Bloom et al. 1983).

Synthesis and metabolism of norepinephrine

Norepinephrine (NE) is the main small molecule neurotransmitter produced and released by the LC. It is synthesized from tyrosine, which is converted to dihydroxyphenyalanine (L-DOPA) by tyrosine hydroxylase, then readily converted into dopamine by aromatic amino-acid decarboxylase and finally into NE by dopamine β-hydroxylase. NE is packaged into synaptic vesicles by the vesicular monoamine transporter 2 (VMAT2), where it is stored until its release into the synaptic cleft via exocytosis induced by neuronal activation (Eiden, Schafer et al. 2004, Rang 2014). From the synapse, NE is taken back up into the presynaptic neuron by the NE transporter (NET). Any remaining NE in the synapse can also be enzymatically degraded either via monoamine oxidase A (MAO-A) or catechol-O-methyltransferase (COMT) into various metabolites, the primary end points being vanillylmandelic acid or 3-methoxy-4hydroxypehnylglycol (MHPG), both of which are relatively inactive biologically and easily excreted from the body (Eisenhofer, Kopin et al. 2004, Rang 2014).

Receptors and Signaling Pathways

Noradrenergic LC neurons preferentially project to the thalamus, hippocampus, the frontal and entorhinal cortices, but they also innervate most other brain regions to some extent (Foote, Bloom et al. 1983). Because NE signals via metabotropic G-protein coupled receptors (GPCRs) and their second messenger systems rather than directly gating ion channels, it has a slower, more gradual neuromodulatory effect. There are three main families of noradrenergic receptors: alpha-1 (α_1), alpha-2 (α_2) and beta (β) (reviewed in (Gilsbach and Hein 2008, Schmidt and Weinshenker 2014). Each family signals via different pathways: α_1 receptors couple to G_q proteins, α_2 receptors couple to $G_{i/o}$ proteins and β receptors couple primarily to G_s proteins.

α_1 receptors (including subtypes α_{1A}, α_{1B}, α_{1D}) are primarily excitatory, signaling via the G_q protein which activates phospholipase C-β (PLC-β), resulting in inositol trisphosphate (IP_3) and diacylglycerol (DAG) signaling to increase calcium release and the subsequent activation of protein kinase C (Marzo, Bai et al. 2009). These function as heteroreceptors and are expressed across the brain, especially in the hippocampus, cortex and brainstem, and are associated with many processes including motor coordination, pain perception and memory (Gilsbach and Hein 2008).

α_2 receptors (with subtypes α_{2A}, α_{2B}, α_{2C}), in contrast, are primarily inhibitory, signaling via the $G_{i/o}$ protein which inhibits the production of cyclic-adenosine monophosphate (cAMP) by adenylate cyclase, which in turn reduces the activities of ion channels as well as protein kinase A (PKA). Unlike α_1, α_2 receptors are found as both heteroreceptors and autoreceptors. The pre-synaptic α_2 autoreceptors on the LC are integral for modulating its own activity via negative feedback.

Most β-receptors (β_1, β_2, β_3) are again predominantly post-synaptic and have stimulatory effects via the G_s receptor, which has the opposite impact on the same pathway as $G_{i/o}$ coupled receptors: increasing cAMP and PKA signaling. The activation of β-receptors and the resulting

cascade of signaling in the hippocampus are crucial for the protein synthesis that underlies synaptic plasticity and long term potentiation essential to learning and memory (Benarroch 2009). Recent studies have determined that β_2 receptors also couple to $G_{i/o}$ and can play a role in the impairment of memory retrieval in the hippocampus (Schutsky, Ouyang et al. 2011, Schutsky, Ouyang et al. 2011).

Function

Due to its extensive innervation of multiple forebrain regions and the widespread distribution of its diverse receptors, the noradrenergic system is involved in many behavioral and physiologic processes. The role of the LC noradrenergic system in cognitive processes, stress responses, arousal, and wakefulness are covered in several extensive reviews (Berridge and Waterhouse 2003, Ramos and Arnsten 2007, Robbins and Arnsten 2009, Sara 2009, Berridge, Schmeichel et al. 2012). In addition to declining with normal aging, altered NE transmission has been reported in major brain disorders in psychiatry (depression, attention deficit disorder, Tourette's, psychosis, post-traumatic stress disorder, drug addiction), neurology (epilepsy, Parkinson's, AD) and sleep (Feinstein, Heneka et al. 2002, Szot 2012).

1.2.2 DEGENERATION AND DYSFUNCTION OF THE LOCUS COERULEUS IN ALZHEIMER'S DISEASE

Locus coeruleus loss in Alzheimer's

Extensive LC degeneration is nearly universal in AD (Bondareff, Mountjoy et al. 1987, Chan-Palay and Asan 1989, Lyness, Zarow et al. 2003, Zarow, Lyness et al. 2003, Haglund, Sjobeck et al. 2006) and is among the earliest pathologies (Forno 1966, Mann, Lincoln et al. 1980, Haglund, Sjobeck et al. 2006), with LC neuropathology detectable years before neurocognitive signs (Grudzien, Shaw et al. 2007, Braak and Del Tredici 2011, Braak and Del Tredici 2011). Alterations in NE are linked to cognitive, mood and neuropsychiatric symptoms (Amaral and

Foss 1975, Amaral and Sinnamon 1977, Forstl, Burns et al. 1994, Forstl, Levy et al. 1994, Ressler and Nemeroff 1999, Weinshenker 2008, Sara 2009). Importantly, several studies have also demonstrated significant correlations between LC cell death (or decreased cortical NE levels) with severity and duration of dementia in AD (Yates, Simpson et al. 1983, German, Manaye et al. 1992).

Preclinical studies of locus coeruleus and norepinephrine in Alzheimer's pathogenesis

The strong correlation between LC degeneration, NE depletion and AD severity has prompted multiple studies of the contribution of LC dysfunction to AD progression using animal models. The primary tool for studying the effects of LC degeneration and NE depletion *in vivo* is the neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4), which reliably lesions the LC while leaving other aminergic systems (including other central and peripheral NE neurons) intact (Ross and Stenfors 2015). Transgenic mice that overexpress human APP harboring disease-causing mutations recapitulate many aspects of AD neuropathology and cognitive deficits and have been used extensively to study AD. However, most of these mouse lines do not show the frank LC degeneration that occurs in human AD. Thus, to determine the functional consequences of LC loss in AD, several laboratories have used DSP-4 to lesion LC neurons in APP transgenic mice.

In general, DSP-4 lesions of the LC exacerbate AD-like neuropathology and cognitive deficits, suggesting that LC degeneration plays a causal role in AD progression. For example, the first study to use this approach showed that DSP-4 lesions of the LC resulted in increased Aβ deposition, neurodegeneration, neuronal loss, cognitive deficits and microglial activation, and reduced cerebral glucose metabolism, in APP23 mice (Heneka, Ramanathan et al. 2006). Importantly, the effects of DSP-4 were confined to forebrain areas that received projections directly from the LC, while brain regions that receive noradrenergic innervation from non-LC cell groups were unaffected. APP/PS1 mice that overexpress both mutant human APP and PS1 treated

with DSP-4 displayed severe loss of NET in LC and cortex, along with a loss of NET innervation (Jardanhazi-Kurutz, Kummer et al. 2010). Lesioning of the LC accelerated amyloid deposition and neuron death with age and conferred more severe deficits in spatial memory compared to vehicle-treated animals (Jardanhazi-Kurutz, Kummer et al. 2010). The mechanism underlying the increased amyloid deposition appears to be reduced clearance, as occurs in sporadic AD (Mawuenyega, Sigurdson et al. 2010), due to the inhibition of $A\beta_{1-42}$ (Aβ42) phagocytosis by microglia rather than an influence on APP production or processing (Heneka, Nadrigny et al. 2010). NE is a potent regulator of microglial function, and in general suppresses the production of pro-inflammatory cytokines and promotes the production of anti-inflammatory molecules. Thus, it is not surprising that DSP-4 treatment also exacerbates the neuroinflammatory response in multiple brain regions of APP/PS1 mice (Heneka, Nadrigny et al. 2010, Jardanhazi-Kurutz, Kummer et al. 2011). Interestingly, a recent study reported that in addition to increased Aβ deposition, DSP-4 lesions of the LC in APP/PS1 mice also resulted in olfactory deficits, another common and early pathology seen in AD patients (Rey, Jardanhazi-Kurutz et al. 2012).

Among the questions raised by these findings, an important issue with therapeutic implications is whether the effects of LC lesions in AD mouse models are due solely to the loss of NE itself, the loss of co-transmitters in LC neurons, collateral damage from the neurodegenerative process itself, or some combination thereof. To resolve these issues, our lab has previously crossed APP/PS1 mice with dopamine β-hydroxylase knockout (DBH-/-) mice that lack the ability to synthesize NE but have intact LC neurons and co-transmitters (Hammerschmidt, Kummer et al. 2012). While APP/PS1 and DBH(-/-) single mutant mice each displayed moderate hippocampal LTP and spatial memory impairments, the two mutations had an additive effect, resulting in double mutants with severely compromised LTP and maze performance. Somewhat surprisingly, the genetic loss of NE had no apparent effect on AD-like neuropathology in APP/PS1 mice, but DSP-4 still worsened neuropathology in the double mutant mice. Finally, non-degenerative loss of LC neurons produced by Ear2 knockout, which prevents

the development of most LC neurons, also exacerbated LTP and memory deficits but had no effect on plaque deposition in APP/PS1 mice. Combined, these results suggest that LC neuronal loss contributes to distinct aspects of AD; depletion of NE itself impairs synaptic plasticity and cognitive performance, while the physical process of LC neuron degeneration exacerbates AD-like neuropathology.

Neuroinflammation links LC degeneration to AD pathogenesis

There is growing evidence suggesting that the inflammatory response induced and/or augmented by LC degeneration is a key mechanism contributing to the initiation and progression of AD pathogenesis. Microglia, astrocytes, and endothelia are among the major targets of central NE neurons, and under normal conditions, these cells control the delicate balance of the inflammatory response. In general, NE is an anti-inflammatory molecule; acting via β-adrenergic receptors, NE suppresses the expression of multiple pro-inflammatory genes, including major histocompatibility complex class II, tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase (iNOS) and IL-1β, while simultaneously promoting the expression of anti-inflammatory molecules such as nuclear factor kappa B (NF-κB), inhibitory IkappaB (IκB), heat shock protein (HSP70), and chemokine monocyte chemotactic protein-1 (MCP-1) in astrocytes and microglia (Feinstein, Heneka et al. 2002, Marien, Colpaert et al. 2004). Thus, it is not surprising that NE deficiency results in undesirable proinflammatory effects.

One of first pieces of evidence connecting LC degeneration and neuroinflammation in an AD model was reported by Heneka et al (2002). Injections of Aβ42 in the cortex of rats induced severe cortical inflammation and increased the expression several pro-inflammatory genes, including iNOS/NOS2, interleukin-1β (IL-1β), and IL-6, within hours. This neuroinflammation was profoundly exacerbated when LC neurons were lesioned with DSP-4 prior to the cortical injection of Aβ42. In addition, DSP-4 pretreatment induced iNOS expression solely in neurons

rather than in microglial cells, more accurately replicating the expression pattern seen in AD patients (Heneka, Galea et al. 2002). Augmented forebrain microglial and astroglial activation and pro-inflammatory gene expression that coincided with the development of other AD-like neuropathologies such as Aβ plaques were also obtained using DSP-4 and the APPV171 and APP/PS1 transgenic mouse models of AD (Heneka, Nadrigny et al. 2010). LC lesions profoundly increased Aβ plaque load, brain inflammation, and spatial memory deficits concurrently in APP23 transgenic mice. In addition, DSP-4 treatment was associated with a switch in microglial cytokine expression from a neuroprotective anti-inflammatory profile to a pro-inflammatory and neurotoxic one (Heneka, Ramanathan et al. 2006, Kalinin, Gavrilyuk et al. 2007, Heneka, Nadrigny et al. 2010).

Because NE promotes microglia-mediated degradation and phagocytosis of Aβ in cell culture (Kong, Ruan et al. 2010), another deleterious effect of LC degeneration on the neuroinflammatory response is the dysfunction of cellular machinery involved in Aβ metabolism and clearance. For example, in V717F APP transgenic mice, DSP-4 lesions of the LC produced a 5-fold increase in Aβ plaques that was accompanied by microglial and astroglial activation and decreased expression of the Aβ plaque degrading enzyme, metallopeptidase neprilysin (Kalinin, Gavrilyuk et al. 2007). Another study showed that NE suppressed Aβ-induced cytokine and chemokine production and increased microglial migration and phagocytosis in cell culture, while DSP-4 lesions prevented the recruitment of microglia to Aβ plaques and impaired Aβ phagocytosis in APP/PS1 transgenic mice (Heneka et al., 2010).

Epidemiological studies investigating NE deficiency and neuroinflammation in AD also exist, but the findings remain to be fully validated in larger population samples. Single nucleotide polymorphisms (SNPs) have been discovered in the NE biosynthetic gene DBH that control serum DBH enzymatic activity, and a small epidemiological study in Spain examined the contribution of the low activity allele in the DBH gene and variants in the IL-1A or IL-6 genes to

the relative risk for developing AD. Though a single polymorphism in either the low-activity promoter variant of DBH, IL-1A or IL-6 alone did not correlate with risk for AD, the presence of both a polymorphism in DBH and one of the IL genes had a synergistic effect that significantly increased the risk of developing AD (Mateo, Infante et al. 2006). This interaction was further investigated in an independent study with a larger sample population and wider patient demographics, which found an association between the low-activity variant of DBH and a risk of AD (though it was mostly limited to males over the age of 75), but could only partially replicate the interactions between the DBH polymorphism and the IL-1A or IL-6 polymorphisms (Combarros, Warden et al. 2010).

SNPs that alter adrenergic signaling have also been linked to a risk for developing AD. People with homozygous C alleles for the ADRB1 (β1-adrenergic receptor) in addition to the GNB3 (G protein B3 subunit gene) T allele, both associated with increased cAMP levels and MAPK activation, have an increased risk of developing AD (Bullido, Ramos et al. 2004). A case-control study in the Han Chinese population found a polymorphism in the β2-adrenergic receptor that enhances responsiveness and is also associated with the risk of sporadic late onset AD (Yu, Tan et al. 2008). These studies seemingly refute the idea that the LC/NE system has anti-inflammatory properties, but caution is necessary when interpreting the signaling effects of receptor polymorphisms because they are typically only tested *in vitro* rather than *in vivo*, and in cultured cells (e.g. HEK cells, HeLa cells) that are not indicative of a neuronal or microglial phenotype. Thus, the role of adrenergic receptors in inflammation and microglia function may also be more complex than initially thought.

Pro-noradrenergic drugs limit Aβ toxicity *in vitro*.

In vitro challenge of human acute monocytic leukemia cells (THP-1) with Aβ42 induced cytotoxicity and provoked a neuroinflammatory response that was dose-dependently attenuated

by NE (Yang, Lee et al. 2012). Treatment with dibutyrl cyclic AMP (cAMP) or forskolin, a PKA activator, had similar effects, suggesting that NE's protective effect was regulated, at least in part, via stimulation of β-adrenergic receptors and the corresponding activation of the cAMP/PKA signaling pathway (Yang, Lee et al. 2012). Another *in vitro* study in hNT neuronal and primary hippocampal cultures revealed a neuroprotective effect of NE against both Aβ42- and $A\beta_{25\text{-}35}$-induced increases in oxidative stress, mitochondrial dysfunction and cell death (Counts and Mufson 2010). The neuroprotective effects were mediated by activation of β-adrenoceptor/cAMP signaling and also required the brain-derived neurotrophic factor (BDNF)/tropomyosin-related kinase B (TrkB) pathway, although some β-receptor-independent effects of NE persisted (Counts and Mufson 2010). Our lab followed up on this study and found that NE dose-dependently protected primary cortical and LC neurons from Aβ toxicity. The neuroprotective effects of NE were fully prevented by the Trk receptor antagonist K252a but only partially attenuated by adrenergic receptor antagonists and not mimicked by adrenergic agonists. These results indicate that NE can activate TrkB and protect against Aβ toxicity, at least in part, via adrenergic receptor-independent mechanisms.

Treatments that increase NE ameliorate AD-like pathology and cognitive decline in APP transgenic mice.

Animal studies have also provided the most compelling evidence that increasing NE could have beneficial effects on both AD neuropathology and cognitive symptoms. The earliest *in vivo* animal studies using noradrenergic pharmacotherapies focused on the α2-adrenergic autoreceptor. α2 antagonists that enhance NE release, such as piperoxane, reversed memory deficits in aged mice as assessed by performance in a step-down inhibitory avoidance response task (Zornetzer 1985). Another α2 antagonist, fluparoxan, prevented age-related decline in the spontaneous alternation task (a test of spatial working memory) in APP/PS1 mice, although it had

no effect in other memory tasks such as object recognition or the Morris water maze, and occurred in the absence of obvious concomitant change in pathology (Scullion, Kendall et al. 2011). Drugs targeting other NE receptors and transporters have also been tested in animal models of AD. Desipramine, a tricyclic antidepressant that inhibits NE reuptake, induced the production of the anti-inflammatory cytokine MCP-1 (Madrigal, Garcia-Bueno et al. 2010). CL316243, a selective β3-adrenergic receptor agonist, rescued performance in a learning paradigm by chicks given intracranial injections of Aβ42(Gibbs, Maksel et al. 2010).

Compelling evidence in favor of noradrenergic treatments for AD has also been observed using the NE precursor, L-threo-3,4-dihydroxyphenylserine (L-DOPS). For example, L-DOPS restored the balance of the brain inflammatory system, facilitated microglial migration and Aβ phagocytosis, and reversed learning deficits in DSP-4 lesioned APP transgenic mice (Heneka, Nadrigny et al. 2010) and partially rescued spatial memory deficits in the DBH(-/-), APP/PS1 double mutant mice (Hammerschmidt, Kummer et al. 2012). Treatment of 5xFAD mice, which have robust and early development of AD-like neuropathology, with a combination of L-DOPS and the NET inhibitor, atomoxetine, elevated brain NE levels, increased expression of Aβ clearance enzymes and BDNF, reduced inflammatory changes and Aβ burden, and improved spatial memory (Kalinin, Polak et al. 2012).

To generate further proof-of-principle for the efficacy of NET inhibitors in AD, our lab has used the NET knockout (NET KO) mice that lack the NET completely, and have elevated basal extracellular NE levels, similar to what might be observed with chronic NET inhibitor treatment (Xu, Gainetdinov et al. 2000).We crossed the NET KO mice to APP/PS1 mice, and examined AD-like neuropathology by Western blot at 6 months of age and by immunocytochemistry at 1 year of age. We found that APP/PS1 mice that carry wild-type copies of NET (NET WT, APP/PS1) contained heavy plaque load in the hippocampus and cortex, as detected by immunohistochemistry using antiserum 2964 against fibrillar Aβ42 (Wahle, Thal et

al. 2006). Remarkably, plaques were almost completely abolished in littermate APP/PS1 mice that lacked the NET (NET KO, APP/PS1). Similar results were obtained with western blots of brain homogenates. Interestingly, full-length APP and the C-terminal fragment of APP were also reduced. The reasons for this are not clear, but raise the possibility that a change in APP production or turnover contributes to the decrease in Aβ levels. In support of this idea, selective lesions of the ascending noradrenergic bundle with 6-OHDA in rats decreased cortical NE and increased cortical APP (Wallace, Ahlers et al. 1993). Combined with the results that atomoxetine + L-DOPS reduces AD-like neuropathology and cognitive deficits in 5xFAD mice (Kalinin et al., 2012), these results suggest that attenuating NET activity can reduce Aβ levels, perhaps by increasing phagocytosis or another NE-mediated mechanism. As such, atomoxetine is one drug currently in clinical trials at Emory University that is showing promise for the treatment of MCI (ClinicalTrials.gov ID #: NCT01522404).

While studies using NE pharmacotherapy in AD models show promise for disease treatment, these studies must be interpreted with caution because the effects of noradrenergic drugs are complicated by multiple adrenergic receptor subtypes with different distributions and signaling capabilities. For one, there appear to be compensatory changes in the degenerating noradrenergic system in AD; despite decreases in tissue forebrain NE in AD, surviving LC neurons show increased abundance of mRNA abundance for tyrosine hydroxylase, the rate-limiting NE biosynthetic enzyme, sprouting of dendrites and axonal projections (Szot, White et al. 2006), and increased CSF levels of NE are observed in AD patients (Raskind, Peskind et al. 1984, Raskind and Peskind 1994, Peskind, Wingerson et al. 1995, Elrod, Peskind et al. 1997). There are also several studies suggesting that noradrenergic transmission actually *increases* certain proinflammatory markers, and some adrenergic receptor blockade can in fact be therapeutic. Pharmacological activation of β-adrenergic receptors (especially β2) is reported to increase mRNA and protein levels for IL-1B and IL-6 in macrophages, microglia and brain

parenchyma (Tomozawa, Yabuuchi et al. 1995, Maruta, Yabuuchi et al. 1997, Tan, Nackley et al. 2007). Administration of adrenergic receptor antagonists *in vivo* can protect against the inflammatory response induced by footshock (Johnson, Campisi et al. 2005), peripheral bacterial challenge (Johnson, Cortez et al. 2008) or ischemia (Savitz, Erhardt et al. 2000, Heeba, Mahmoud et al. 2012). Nevibolol, a β1-blocker, can also reduce amyloid production in Tg2576 mice that have established amyloid and cognitive impairment, however it does not appear to be able to improve cognition(Wang, Wright et al. 2013). Finally, the α1-adrenergic receptor antagonist, prazosin, had no effect on plaque load but induced astrocytic proliferation and the release of ApoE and anti-inflammatory cytokines, and rescued memory deficits in APP23 mice.

These findings, together with the strong links between LC/NE loss in AD and disease progression in Aβ-based AD animal models, clearly demonstrate the exciting disease-modifying potential of drugs that facilitate NE transmission. However, what is largely missing from this discussion is what the impact of LC degeneration and the loss of the NE system has on the other canonical AD protein, tau.

1.3 TAU AND THE LOCUS COERULEUS IN ALZHEIMER'S DISEASE

1.3.1 POTENTIAL ROLES IN ALZHEIMER'S DISEASE PATHOGENESIS

As detailed above, the role of the LC in AD has been described primarily based on the neuroinflammatory response to its degeneration and the loss of the NE system, and the impact on Aβ pathology. New evidence has come to light suggesting that the LC may interact with tau as well. Understanding more about LC-tau interactions to complement what is already known about Aβ will make the noradrenergic system it a much more attractive therapeutic and diagnostic target, and could provide more insight into the underlying mechanisms of the disease.

Early deposition of subcortical tau pathology

The role of the LC in AD pathogenesis has been recently rejuvenated by the discovery of early tau pathology in the brains of young, cognitively normal individuals. A large-scale epidemiological study by Heiko Braak and his group examined over 2000 brains from individuals aged 1 to 100 years old and probed for immunohistological markers relevant to AD (Braak and Del Tredici 2011, Braak, Thal et al. 2011). In the youngest brains, they discovered deposition of hyperphosphorylated tau in the LC area that preceded AT8 pathology in all other brain regions and any Aβ immunoreactivity (via the 4G8 antibody). This, coupled with the constant presence of such LC tau pathology in all other brains that showed AT8 or 4G8 immunoreactivity, prompted the group to hypothesize that this subcortical tau pathology could be the earliest detectable sign and a potential initiation point of AD pathogenesis. The evidence also resulted in the creation of a new category of tau pathology in Braak's staging that precedes all of his previous stages.

These pathological changes in the LC that occur in prodromal stages of AD (i.e., MCI) have been replicated in a separate study (Elobeid, Soininen et al. 2012), however, whether the LC represents the initial site of pathology or reflects a non-specific response to brain insults is still under debate (Attems, Thal et al. 2012).

Still, this distribution of aberrant tau in the human population is especially interesting in light of previous research showing tau pathology can spread trans-synaptically *in vivo* (Clavaguera, Bolmont et al. 2009). Together, these findings suggest that AD may start as tau pathology in the LC that progresses from lower brainstem regions in discrete stages up to sub-cortical regions before finally reaching the cortex, matching how the neuropathology spreads in the brains of AD patients over time (Braak, Thal et al. 2011).

Capacity of locus coeruleus degeneration to aggravate tau pathology in projection areas

More recent evidence from another post-mortem study analyzing the LC from brains at various Braak stages suggests that while tau pathology in the LC is evident early (Braak stage a), LC cell

bodies do not begin to degenerate until later stages (Braak stages 4 -5). By these later stages, there is extensive AT8 pathology throughout most of the brain, including many of the LC's projection areas, and cognitive impairments become evident (Theofilas, Ehrenberg et al. 2016). This pattern begs the question of whether the loss of the LC contributes to the continuing aggravation of forebrain tau pathology and cognitive deficits. The evidence from studies of LC degeneration in the context of Aβ certainly points to its pervasive role in modulating that pathology and the consequential cognitive impairments, but a reciprocal role with tau remains to be seen.

1.3.2 KNOWLEDGE GAPS

The pathological effects of tau phosphorylation in the LC have not been studied, and its role in the cascade that leads to neuronal dysfunction and degeneration seen in AD is still unclear. The knowledge gaps present in these areas highlight the need for additional investigations into the mechanism by which LC loss contributes to AD. The proposed research is expected to fill these knowledge gaps and describe the consequences of (1) early aberrant hyperphosphorylated tau on LC neuron survival/function, and (2) LC degeneration on forebrain tau pathology and cognitive impairment. Most of the evidence supporting an interaction between LC degeneration and tau pathology comes from qualitative, cross-sectional epidemiological work. To investigate a potential cause-and-effect relationship, it was necessary to find suitable models of both aberrant tau as well as LC neuronal death.

1.3.3 MODELING TAU AND LOCUS COERULEUS DYSFUNCTION

Model of aberrant tau

Unlike Aβ, there are no known mutations in tau that result in AD. However, there are numerous tau mutations that are known to cause other neurodegenerative diseases resulting from tau

accumulations, collectively known as tauopathies. The first mutations in MAPT were discovered from frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17), a neurodegenerative disease characterized by extensive frontotemporal degeneration and gliosis. This disease has been the predominant source of tau mutations used to model tauopathies, with over 40 different mutations identified thus far (Chaunu, Deramecourt et al. 2013, Dujardin, Colin et al. 2015). While there are many other mouse models of tauopathies that do not rely on these mutations and instead induce tau pathology via deletion of genes involved in tau phosphorylation or the insertion of wild-type human tau, these generally do not faithfully develop the aggregated tau pathology that is reminiscent of AD and desired for most experiments (reviewed in (Dujardin, Colin et al. 2015)). The FTDP-17 mutations allow for an aggressive tau accumulation that begins with hyperphosphorylation of tau, followed by its somato-dendritic localization, the development of insoluble aggregates and eventually neurodegeneration, akin to the progression of tau pathology in AD (Dujardin, Colin et al. 2015).

The P301S Mouse

For our studies, we chose to use the P301S mutation. P301S is found within exon 10 of MAPT, in its microtubule-binding region, and impacts the ability of tau to interact with and stabilize microtubules and promotes fibrillary assembly (Bugiani, Murrell et al. 1999, Sperfeld, Collatz et al. 1999). Since its discovery in a Dutch family (Sperfeld, Collatz et al. 1999) in 1999, the mutation has been reported in many other populations across the globe, including Italian (Bugiani, Murrell et al. 1999), Japanese (Yasuda, Yokoyama et al. 2000) and Algerian-Jew (Lossos, Reches et al. 2003). The clinical presentation reported from affected individuals within these families were highly variable but included various movement disorders, behavioral changes, seizures, progressive cognitive deterioration and dementia (Bugiani, Murrell et al. 1999, Sperfeld, Collatz et al. 1999, Yasuda, Yokoyama et al. 2000, Lossos, Reches et al. 2003). A unifying factor among all patients included very early onset of symptoms, rapid progression and deterioration

and early death (between 36-46 years) (Bugiani, Murrell et al. 1999, Sperfeld, Collatz et al. 1999, Yasuda, Yokoyama et al. 2000, Lossos, Reches et al. 2003). Neuropathological findings showed extensive hyperphosphorylated tau pathology in both neurons and glia, coupled with neuronal loss and vacuolation, and gliosis and demyelination of white matter (Bugiani, Murrell et al. 1999).

The P301S mutation has been used to create 2 independent lines of transgenic mice. One was created by Michel Goedert's group in 2002 and expresses the P301S mutation in the 0N4R isoform of human tau under the neuron-specific murine Thy-1 promoter (Allen, Ingram et al. 2002). The other was created by Virginia Lee's group in 2007, and expresses the P301S mutation in the 1N4R isoform of human tau under another neuron-specific promoter, murine prion protein (Prnp) (Yoshiyama, Higuchi et al. 2007). Both models develop age-dependent hyper-phosphorylation of tau and neurofibrillary tangle-like pathologies, neurodegeneration, neuroinflammation and cognitive impairments (Allen, Ingram et al. 2002, Yoshiyama, Higuchi et al. 2007, Hampton, Webber et al. 2010, Takeuchi, Iba et al. 2011).

We selected the Lee group's mouse for our experiments because the pathological progression is less aggressive and more akin to the slow, insidious onset of pathology and cognitive deficits in AD (neuronal loss begins only at around 9 -12 months, as opposed to 3 months in the Goedert mouse, NFT deposition occurs at 6 months, as opposed to 4 months, and cognitive impairments in the Morris water maze appear only at 6 months compared to 2.5 months) (Allen, Ingram et al. 2002, Hampton, Webber et al. 2010, Takeuchi, Iba et al. 2011, Xu, Rosler et al. 2014). This slower progression was especially important for us because we were interested in looking at the early pathological changes that may be missed or reach a ceiling in the more rapidly progressing model.

N-(2-chloroethyl)-N-ethyl-bromo-benzylamine (DSP4)

To induce LC degeneration in our studies, we chose the neurotoxin DSP-4 which, as described above, has been used in many studies to selectively lesion the noradrenergic neurons of the LC. It readily passes the blood brain barrier, making it an attractive method for lesioning the LC because it can be easily administered systemically via an intraperitoneal (i.p.) injection, yet results in a selective lesion that can be hard to achieve otherwise (for example via focal intracranial injections of toxins or electro-lesions due to the small size and location of the nucleus).

DSP-4's mode of toxicity in the brain begins with its uptake by the NET into noradrenergic nerve terminals, which it can also irreversibly inhibit (reviewed in (Ross and Stenfors 2015)). Once inside, it cyclizes via a nucleophilic substitution reaction to its reactive aziridinium derivative and slowly accumulates in the terminal. It is not entirely clear how the reactive derivative induces toxicity, but xylamine, a close relative of DSP-4, inhibits mitochondria function, thereby reducing the formation of adenosine triphosphate (ATP), the main source of energy within the cell. It is likely that DSP-4 acts in a similar manner (Dudley, Howard et al. 1990).

In vivo, DSP-4 causes a pronounced depletion of the NE in the brain. NE is significantly reduced in the rat LC projection areas within the cerebral cortex and spinal cord in a dose-dependent manner beginning at 6 mg/kg i.p. up to the most commonly used dose of 50 mg/kg, which results in a 80-90% reduction in NE concentration one week after a single injection (Archer, Jonsson et al. 1984, Wolfman, Abo et al. 1994). This depletion can be recovered very gradually in a time-dependent manner, with NE concentrations back to 50% by 3 months, 75% by 10 months and fully recovered within one year, suggesting that noradrenergic terminals are capable of regrowth or resprouting (Wolfman, Abo et al. 1994).

Interestingly, the time course of NE loss and decrease in DBH immunoreactivity is staggered: NE loss occurs within hours of DSP-4 administration, while DBH immunoreactivity is not impacted until four days later as thick, swollen noradrenergic axons can be seen before

completely disappearing within 2 weeks as the terminals die back (Fritschy and Grzanna 1990). At the level of the LC itself, it is generally agreed that DSP-4 also induces some neurodegeneration, but following a much longer delay. A single DSP-4 injection results in marked degeneration and neuronal cell loss a few months later (with a 34% reduction reported by 3 months and 57% reduction by one year) (Fritschy and Grzanna 1991, Zhang, Zuo et al. 1995). This suggests that DSP-4 first causes an acute loss of NE in projection areas, which is later followed by a neurodegenerative phase where the neurons first lose their axons as they die back, and finally death of the cell bodies.

In rodents, DSP-4's neurotoxicity is more selective for the noradrenergic neurons of the LC compared to other noradrenergic neurons in the brain. Immunohistochemistry for DBH in rats injected with DSP-4 showed intense elimination of the LC's projection terminals, while those of the A5 (superior olivary complex of the pontine tegmentum) and A7 (pontine reticular formation) groups remained intact (Fritschy and Grzanna 1989, Fritschy and Grzanna 1990). More work is required to fully understand why this selectivity exists, but it is hypothesized that the cerulean and noncerulean noradrenergic cells make up distinct subsystems with potentially different pharmacological properties, especially at their terminals (Ross and Stenfors 2015).

In vitro studies of DSP-4 are more limited but appear to echo its effects *in vivo*. DSP-4 irreversibly inhibited human NET expressed in embryonic kidney (HEK293) cells (Wenge and Bonisch 2009). DSP-4 also induced cytotoxicity, as measured by decreases in ATP content and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test, in a dose dependent manner (Wenge and Bonisch 2009). DSP-4 also reduced DBH mRNA, protein and NET levels in a dose dependent manner in SH-SY5Y neuroblastoma cells expressing NET and DBH (Wang, Musich et al. 2014).

1.3.4 THESIS AIMS

Using P301S tau transgenic mice and DSP-4, our aims are to 1) characterize the impact of early hyperphosphorylated tau in LC cells and 2) ascertain the role that LC degeneration may have on the development of tau pathology in the forebrain and its resulting cognitive deficits.

To answer the first question, we cultured LC neurons from P301S mice and assessed whether the presence of aberrant, hyperphosphorylation-prone tau impacts structural integrity or survival of the cells by measuring neurite length and performing cell counts. We also determined whether the presence of the aberrant tau sensitizes the LC cells to other insults by treating them with different toxins. Our results provide a quantitative look at the role of the early deposition of hyperphosphorylated tau in the LC seen in the young, cognitively normal individuals from Braak's studies.

To answer the second question, we lesioned the LC with i.p. injections of DSP-4 in P301S mice and assessed the development of neuropathology and cognitive deficits. We probed for exacerbations of the tau accumulations, neurodegeneration and neuroinflammation that characterize the P301S mice and determined if there are also functional changes in cognitive performance through various tasks such as the Morris water maze, fear conditioning, social interaction and circadian rhythm. The results shed light onto how the ubiquitous degeneration of the LC seen in AD impacts tau pathogenesis and its interplay with cognition.

We believe the LC plays an important part in the pathogenesis of AD, both in the early stages of the disease when the pathogenic process is emerging, as well as in the later stages in which the pathology is advancing and inducing neuropathological and cognitive changes. Understanding more about the role of the LC at both of these stages will help steer the development of better diagnostics and therapeutics that could target this region and the noradrenergic system and improve our ability to combat this disease.

FIGURES

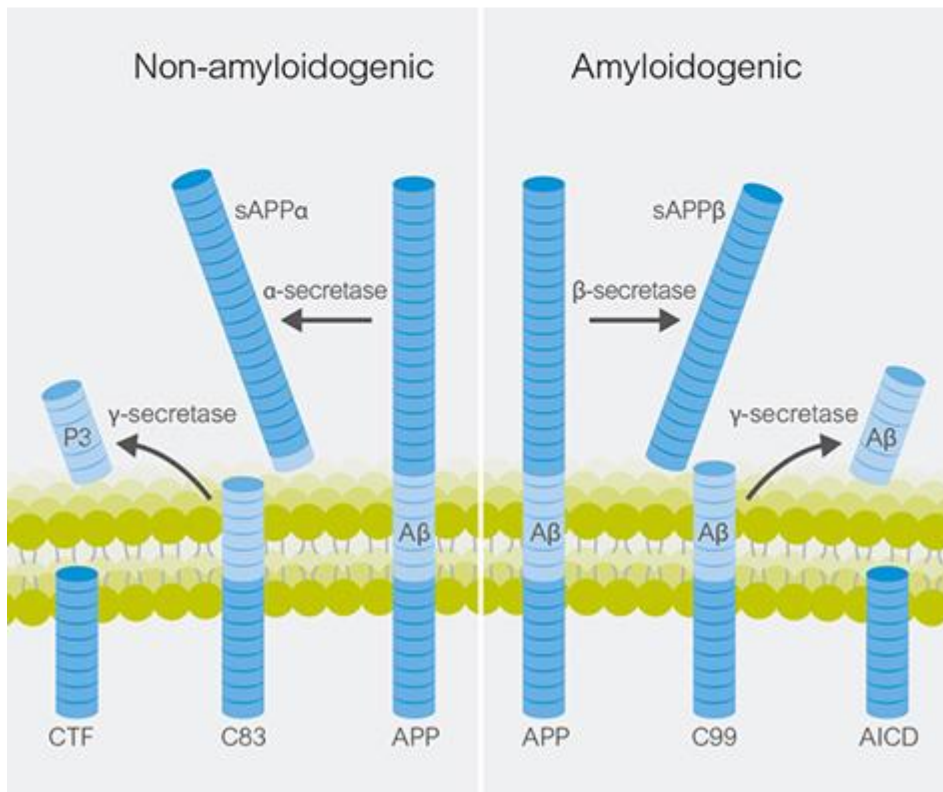


Figure 1.1: *The non-amyloidogenic and amyloidogenic processing pathways of the amyloid precursor protein.* The amyloid precursor protein (APP) can be processed in two ways. The non-amyloidogenic pathway (left) involves cleavage by α-secretase followed by γ-secretase to release a non-toxic sAPPα and P3 fragments. The amyloidogenic pathway (right) involves cleavage by β-secretase followed by γ-secretase to release sAPPβ and the toxic beta-amyloid (Aβ) peptide. Source of image: Abcam (http://www.abcam.com/neuroscience/beta-amyloid-in-alzheimers-disease-app-processing)

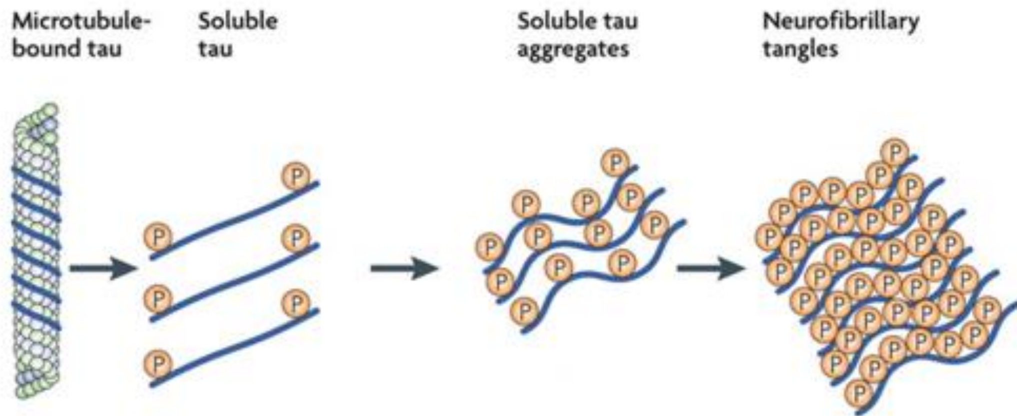


Figure 1.2: *The pathological processing of microtubule associated tau.* Tau normally exist freely in the cytosol or bound to microtubules to promote their stability. Its hyperphosphorylation leads to unbinding from microtubules. Oligomeric tau becomes more and more aggregated with further phosphorylation forming soluble pre-tangle tau aggregates. Eventually this takes on a misfolded conformation and eventually forming insoluble neurofibrillary tangles. Source of image: modified from (Citron 2010)

CHAPTER 2: ABERRANT TAU IN THE LOCUS COERULEUS IMPACTS NEURITE LENGTH AND NEURONAL SENSITIVITY TO TOXIN *IN VITRO*

2.1 ABSTRACT

Elucidating the mechanisms underlying the initiation of Alzheimer's disease (AD) at its earliest stages is the key to developing effective therapeutics and diagnostics. The locus coeruleus (LC) is the major brainstem noradrenergic nucleus that supplies norepinephrine (NE) to the forebrain and eventually degenerates in nearly all AD patients. Recent studies suggest that aberrant hyperphosphorylated tau can be found in the LC of young, cognitively normal individuals before the appearance of any other AD-like pathologies in the rest of the brain and prior to widespread depositions and neurodegeneration. It is unclear what effect this early presence of aberrant tau has on the function and survival of LC neurons, especially in conjunction with other genetic and environmental risk factors. By crossing mice expressing green fluorescent protein (GFP) driven by the tyrosine hydroxylase promoter with mice expressing a form of mutant tau (P301S) that is prone to hyperphosphorylation and aggregation and closely recapitulates AD-like tau pathology, we have successfully isolated and cultured primary LC neurons expressing aberrant human tau to study their morphology and survival, as well as their susceptibility to toxic challenges associated with AD. We find that the presence of hyperphosphorylated tau reduces average LC neurite length, but is not sufficient to induce rapid LC neuronal death. However, the aberrant tau can interact in a synergistic manner with a noradrenergic toxin, DSP-4, to induce LC neuronal death at concentrations that are otherwise non-lethal in the absence of tau. This synergy appears to be toxin-specific, as we did not observe the same hypersensitivity to immune stress challenge with lipopolysaccharide (LPS). These findings support the idea that early hyperphosphorylated tau in the LC impairs neurite morphology and may put the nuclei at risk for dysfunction and degeneration because of other additional, external factors.

2.2 INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia and poses immense burdens on society, yet current treatments are limited in effectiveness because the disease's underlying molecular mechanisms, particularly what initiates its onset, are not fully understood. AD is thought to have a particularly insidious prodromal stage, where neuropathology incubates for decades before clinical symptoms appear, leaving patients with irreversible neurodegeneration and dysfunction by the time they are diagnosed and undergo clinical interventions (Ritchie, Carriere et al. 2016). One important question that remains to be answered is whether the two main neuropathologies of AD (extracellular beta-amyloid plaques and intracellular tau neurofibrillary tangles (NFTs)) are interacting to induce disease.

Not surprisingly, currently available therapies for AD remain palliative and only result in modest and transient improvements in certain symptoms without significantly delaying disease pathogenesis (Casey, Antimisiaris et al. 2010). Promising disease-modifying therapies in recent years have continued to disappoint in clinical trials, presumably due to how far the neuropathology and neurodegeneration have already progressed before patients begin displaying outward cognitive symptoms (Mangialasche, Solomon et al.). The "amyloid cascade" hypothesis, which posits that beta-amyloid deposition is the main driver of AD, has directed most clinical investigations towards amyloid modifying therapies, however, this strategy has yet to be successful despite many different candidates (Hardy and Selkoe 2002). The most recent failure of solanezumab, an amyloid antibody therapy from Eli Lilly, has prompted many to suggest this strategy may not be effective or appropriate (Abbott and Dolgin 2016). Those still resolute in their support of amyloid suggest the hypothesis requires earlier implementation to be effective, while others critical of it suggest a different mechanism may be needed (Abbott and Dolgin 2016). Given the growing impact of the disease, there is an urgent need to ascertain the early changes that initiate AD and identify more effective and early biomarkers and interventions. One alternative area to target are early changes in tau pathology.

In 2011, large scale case studies of brains from young and cognitively normal individuals by Dr. Heiko Braak's group suggested that the formation of soluble hyperphosphorylated tau aggregates (precursors to mature tau NFTs) (Bancher, Brunner et al. 1989) in the locus coeruleus (LC) may be the first AD-like pathology to appear in the brains of individuals who are still cognitively intact (Braak and Del Tredici 2011, Braak, Thal et al. 2011, Elobeid, Soininen et al. 2012). This pre-tangle tau pathology always accompanied brains filled with severe and widespread Aβ plaques (the other distinctive hallmark of the disease), but was also found on its own in younger brains in the absence of all other AD-related pathologies (Braak, Thal et al. 2011). With these findings, Braak amended his staging of tau in AD to include the predominately subcortical LC pathology as the first stage and proposed that AD tau pathology may be occurring earlier and deeper in the brain than the field has previously shown (Braak, Thal et al. 2011). The detrimental role of hyperphosphorylated tau (both due to loss of normal function as well as gain of toxicity) has been heavily investigated, but mainly with regards to cortical neuronal populations (reviewed in(Khan and Bloom 2016, Medina, Hernandez et al. 2016)), and it is unknown whether this kind of pre-tangle pathology impacts the subcortical LC neurons as well.

Interestingly, the LC has maintained a quiet, unassuming role in the AD field for many decades since the discovery of its ubiquitous degeneration in patients (Tomlinson, Irving et al. 1981, Mann 1983, Haglund, Sjobeck et al. 2006, Weinshenker 2008). Acting as the major source of noradrenergic innervations to the forebrain, its degeneration correlates well with other aspects of AD neuropathology such as beta-amyloid plaques and tau neurofibrillary tangles as well as cognitive impairment (Bondareff, Mountjoy et al. 1987, German, Manaye et al. 1992). The surviving LC neurons in AD patients are morphologically ragged, with "swollen and misshapen" somas, reduced arborization and thick, shortened neurites (Chan-Palay and Asan 1989). While it is not clear what may be contributing to this degeneration pattern, the cross-sectional studies from Braak et al. allude to the potential role of aberrant tau.

No study has directly looked at the consequences of early aberrant tau on LC neurons and whether it contributes to the degeneration seen in AD. The aim of this study is to fill this knowledge gap and determine if aberrant hyperphosphorylated tau is detrimental to LC neurons by developing a method to culture LC neurons expressing hyperphosphorylation-prone tau and determining (1) whether the presence of aberrant tau impairs morphology or survival, and (2) whether they are more sensitive to toxic challenges that AD brains are susceptible to. This *in vitro* approach provides a quantitative cellular complement to the more qualitative cross-sectional clinical reports available thus far characterizing the involvement of LC tau pathology in AD. It also allows for more control over toxin exposure and lets us further examine the effect of aberrant tau on AD-related toxicity through numerous toxins. We selected two toxins to begin our investigation: a noradrenergic-specific toxin (DSP-4) as well as an immunostress agent (LPS). We selected DSP-4 for its robust and selective effect on noradrenergic neurons and because of its structural similarities to chemicals found in tobacco smoke, diesel exhaust and plant toxins that humans are commonly exposed to (Pamphlett 2014). We selected LPS because of the mounting evidence in recent years supporting a major contribution of neuroinflammation to AD (Heneka, Carson et al. , Heneka, Carson et al. 2015, Heppner, Ransohoff et al. 2015), as well as the documented impact of LPS on the LC itself (Bardou, Kaercher et al. 2014). If LC neurons with aberrant tau are impaired and that results in dysfunction or a greater sensitivity to insults, it may provide a potential mechanism for how LC degeneration occurs in AD.

2.3 MATERIALS AND METHODS

Transgenic mice

The tau transgenic mice used express 4R1N human tau with a pathogenic mutation (P301S) under the control of the mouse prion promoter (Ms-Prp) (Yoshiyama, Higuchi et al. 2007). Transgenes driven by the PrP promoter are targeted exclusively to neurons, with high expression throughout the brain that typically begins starting on embryonic day 11.5 (E11.5) and continues for life

(Maskri, Zhu et al. 2004, Tremblay, Bouzamondo-Bernstein et al. 2007). The P301S missense mutation in tau is responsible for an autosomal dominant form of frontotemporal lobe dementia and parkinsonism linked to chromosome 17 (FTDP-17), a disease characterized by cognitive decline, personality changes and motor abnormalities (Bugiani 2000, Goedert and Spillantini 2000, Lossos, Reches et al. 2003). The P301S mice have a fivefold higher expression of mutant human tau compared to endogenous mouse tau and show tauopathy, brain atrophy and neuroinflammation by 6-8 months in the hippocampus (Yoshiyama, Higuchi et al. 2007). As no tau mutation has been associated with AD, the FTDP-17 mutations have been accepted as close substitutes. The P301S mutation impacts the ability of tau to interact with microtubules and promotes its hyperphosphorylation and formation of filamentous structures (Goedert and Spillantini 2000), making it an effective model of hyperphosphorylated tau, though there are distinctions between the tau pathology of AD compared to FTDP-17 .

To visualize the LC, the P301S mice were crossed to another line of transgenic mice expressing green fluorescent protein (GFP) under the tyrosine hydroxylase promoter (TH) (Matsushita, Okada et al. 2002). All experiments were conducted at Emory University in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and approved by the Emory IACUC.

Primary Neuron Culture

LC neuronal cultures were derived from post-natal day 1 TH-GFP/P301S and TH-GFP-only mouse pups. Following brief anesthesia on ice, pups were cleaned with ethanol and decapitated. A tail snip was taken from each pup and PCR genotyping for TH-GFP and P301S was run after neuronal cell count and neurite length were calculated to keep experimenters blind to genotype. Brains were removed and the LC was dissected out under an inverted fluorescent microscope (MZ FLIII, Leica, Deerfield, IL, USA) in ice-cold dissection buffer made of Hank's balanced salt

solution (HBSS, H-4891, Sigma-Aldrich) with penicillin/streptomycin (1%, 17-602E, Lonza) and HEPES (25-060-CI, Cellgro).

Briefly, a coronal cut was made at the level of the superior and inferior colliculi to separate the brainstem from the forebrain. The LC neurons were visualized fluorescently using the GFP channel on the microscope and the fourth ventricle as a landmark. Bilateral LC were micro-dissected out with fine tip tweezers from each brain. Tissue was incubated for 30 min at 37°C in dissociation media (DM), which consisted of papain (1mg/ml, P4762, Sigma-Aldrich), Dispase I (0.5U/ml, 07923, StemCell Technologies), DNase I (20µg/ml, DN25, Sigma-Aldrich) in Plating Media (PM). PM was made with MEM with L-Glutamine and phenol red (11095-080, ThermoFisher Scientific) supplemented with glucose (20%, 15023-021, Gibco), heat-inactivated horse serum (26050088, ThermoFisher Scientific), 1% penicillin-streptomycin and used to wash tissue several times following incubation in DM. Cells were dissociated by gentle trituration with a fire-polished Pasteur pipette. The suspension was centrifuged at 1200 rpm for 4 min and the pellet was resuspended in PM. Cells were counted prior to plating onto an individual 12mm glass coverslip pre-coated with poly-d-lysine and laminin (08-774-384, Fisher Scientific) in a well of a 12-well culture plate (CLS3615, Sigma Aldrich). Three coverslips were plated per mouse brain, with approximately 50,000 cells suspended in 150µl PM per coverslip. Plates were incubated for 30 min in a humidified 5% CO2 incubator at 37°C before each well was flooded with 2ml of warm PM. Two h later, half of the PM was replaced with warm Neurobasal media (NM) (21103-049, Gibco) supplemented with 1% penicillin/streptomycin, B-27 (17504-044, Gibco) and 2mM L-glutamine (25-005-CI, Cellgro). After 1 day *in vitro* (DIV), half of the media was replaced with NM containing aphidicolin (final concentration 0.5µg/ml, A-1026, AG Scientific) to control glial proliferation for neuronal enriched cultures. Thereafter, media changes in this manner occurred every 3 days. Cells underwent the toxic challenge on DIV 11. Glial proliferation was required for the immuno-stress challenge experiments, and aphidicolin was not added during media changes for those cultures to support a mixed glial/neuronal environment.

Toxin challenge

On DIV 11, half of the media in each well was replaced by warm NM with double the desired final concentration of toxin. N-(2-Chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4, 40616-75-9, Sigma Aldrich) was administered at 0, 100μM or 1mM final concentrations in neuronal enriched cultures. Lipopolysaccharide (LPS, from Escherichia coli 0111:B4, L4391, Sigma Aldrich) was administered at 0, 10, or 100 ng/ml final concentration in mixed glial/neuronal cultures. No aphidicolin was added to the NM for either challenge. Cultures were returned to the incubator and challenged for 24 h before fixation and immunocytochemistry.

Immunocytochemistry

Following 24 h of toxin challenge, half of the media was replaced with warm 4% paraformaldehyde (PFA) and incubated at room temperature for 5 min for an initial fix. All media in each well was aspirated and 2ml of 4% paraformaldehyde was added for another 20 min for full fixation. Wells were washed 3 times with phosphate buffered saline (PBS). Nonspecific binding sites were blocked with PBS containing 10% normal goat serum (31872, ThermoFisher Scientific), 1% bovine serum albumin (BP1600, Fisher) and 0.1% Triton-X (X100, Sigma Aldrich) for 1 h at room temperature, before incubation with primary antibodies overnight at 4°C (rabbit anti-tyrosine hydroxylase (TH), 1:1000 (P40101, Pel-Freez), mouse anti-human tau (HT7), 1:500 (MN1000, Abcam)). This was followed by 3 washes with PBS and incubation with fluorescently tagged anti-rabbit and anti-mouse IgG secondary antibodies at a dilution of 1:600 for 1 h. Wells are washed again 4 times with PBS, with a penultimate wash containing Hoechst dye (1%, 34580, SigmaAldrich) to visualize nuclei. Coverslips were mounted onto SuperfrostPlus slides with Fluoromount™ Aqueous Mounting Medium (F4680, Sigma Aldrich) and visualized under a fluorescent microscope (DM6000B, Leica).

Neuronal Cell Count & Neurite Length

TH-positive LC cells on each coverslip were hand counted by an experimenter blind to the genotypes. Each coverslip was counted 3 times and the average was taken. Three random images of LC cells were taken at 10x magnification and average neurite length was calculated using the NeuriteTracer plugin for ImageJ (Pool, Thiemann et al. 2008). Cell count data is shown as a percent of control (TH-GFP with no treatment) and average neurite length is shown in pixels.

Western blot

Microdissected LC tissue from TH-GFP and TH-GFP/P301S mice was homogenized in RIPA buffer (ThermoFisher Scientific, #89900) containing 1x phosphate/protease inhibitor cocktail (ThermoFisher Scientific, #78440) and centrifuged at 13,000rpm at 4°C for 15 min. To remove non-specific mouse Igs from homogenates and reduce non-specificity from tau antibodies, supernatants were boiled at 95°C for 10 min and centrifuged at 13,000rpm at 4°C for 15 min. Protein concentration from this supernatant was determined using the Pierce™ BCA Protein Assay Kit (ThermoFisher Scientific, #23225). 15 ug of protein was loaded into each well of a NuPAGE® 4-12% Bis-Tris Novex® gel, together with a ladder (Precision Plus Protein™ All Blue Prestained Protein Standards, 1610373, Bio-Rad) and run at 165mV for 50 min in NuPAGE® MOPS SDS Running Buffer (NP0001, ThermoFisher Scientific), before transferring at 10mV for 120 min in Transfer buffer to a 0.2 µm pore size nitrocellulose blot (LC2009, ThermoFisher Scientific). Blots were washed briefly with Tris-buffered saline (TBS) before blocking non-specific binding sites with 5% non-fat milk in TBS for 1 h at room temperature. Blots were incubated overnight at 4°C with primary antibodies diluted in 1% non-fat milk in TBS. Primary antibodies included rabbit anti-human tau (1:2000, Craig Heilman, Emory University, USA) and mouse anti-β-actin (1:2000, ab6276, Abcam). Blots were washed with TBS with 0.5% Tween-20 (TBS-Tw) before incubating with fluorescent secondary antibodies in TBS-Tw before visualizing

with the LI-COR Odyssey® imaging system. Band densitometry was analyzed using the LI-COR Image Studio™ Version 4.0 software.

Statistical Analysis

Neuronal cell count and neurite length were analyzed by 2-way ANOVA with genotype and treatment as the factors. Tukey's or Sidak's post-hoc tests were used to for multiple comparisons. Western blot data were analyzed by an unpaired t-test. Significance was set at $p<0.05$, with 2 tailed variants of tests implemented. Multiplicity adjusted p-value was reported for each comparison. Data are presented as means ± standard error of mean (SEM). Calculations were performed and figures created using GraphPad Prism version 6 for Windows (GraphPad Software, Inc., San Diego, CA, USA).

2.4 RESULTS

Using TH-GFP mice to visualize LC during dissection.

The LC in an adult mouse is only about 1mm long, with a highly non-uniform shape, making it very difficult to effectively micro-dissect, especially in young post-natal day 1 pups. The use of the fourth ventricle as the only landmark for dissection is also imprecise and requires a large area of the brainstem to be removed to encapsulate the whole LC. Using TH-GFP mice allowed for easy location and cleaner dissection of the LC from mouse brains and resulted in a higher density of plated LC cells relative to non-LC cells compared to blind microdissections based on previous, unpublished observations (Figure 2.1a). By DIV 11, the LC neurons had long, healthy looking highly arborized neurites (Figure 2.1b), and multiple LC neuronal cells types could be seen, including large multipolar cells as well as medium and small sized fusiform cells (Figure 2.1c), similar to what has been previously described (Masuko, Nakajima et al. 1986).

LC neurons cultured from TH-GFP/P301S mice express human tau.

LC neuronal cultures from TH-GFP/P301S mice show clear immunoreactivity for human tau (HT7) co-localized to LC cells (TH) (Figure 2.2a, bottom), while HT7 immunoreactivity was completely absent in LC cultures from TH-GFP only mice (top). The presence of human tau in the TH-GFP/P301S mice was also quantified in a western blot utilizing another human tau antibody (hTau) (Figure 2.2b). There was a significant difference in human tau levels between TH-GFP/P301S mice (1.234×10^7 densitometry units ± 2.479×10^6, n=5) and TH-GFP only mice (1999 ± 1303, n=3); $t(6) = 3.734$, $p < 0.01$).

P301S tau reduces LC neurite length but does not impact survival.

All cultures were fixed and stained for TH to improve visualization of LC cells and neurites because the endogenous TH-GFP signal is diminished by paraformaldehyde fixation. LC neurons were hand counted under a fluorescent microscope. Three images of LC cells with neurites per coverslip were also randomly taken and neurite length was calculated using the NeuriteTracer Image J macro (Fournier Lab, McGill). To determine if the presence of aberrant human tau prone to hyperphosphorylation alters LC cellular structure and survival, we compared LC cultures from TH-GFP only and TH-GFP/P301S mice. LC neurons with P301S tau had a significantly lower average neurite length compared to control LC neurons. However, there was no difference in cell survival between the two groups in either neuronal enriched or mixed glial/neuronal cultures (Figure 2.3e&f), indicating that while the presence of P301S tau may influence LC neurite structure, it does not independently induce LC neurodegeneration under these conditions. This reduction in neurite length was seen in LC neurons from both the neuronal enriched culture (Figure 2.3g, mean difference = -8653 pixels ± 2392, $t(7)=2.728$, $p=0.0294$) as well as the mixed glial/neuronal culture (Figure 2.3h, mean difference = -5454 pixels ± 1999, $t(10)=3.617$, $p=0.0047$). Compared to P301S tau harboring LC cells, average neurite length in control LC cells are approximately 3 times longer (7916 vs 2462 pixels) in the neuronal enriched culture and 2 times longer in the mixed glial/neuronal culture (16488 vs 7835 pixels).

LC neurons expressing P301S tau are more susceptible to DSP-4 induced cell death

To determine whether P301S tau may work synergistically with a secondary insult to induce structural changes and/or cell death, we calculated neuronal survival and average neurite length in LC cultures that underwent a 24-hour challenge with 0, 100, and 1000µM DSP-4 before fixation and immunocytochemistry for TH (Figure 2.4). Multiple comparisons via Tukey's post hoc test showed that there was no significant difference in cell survival between TH-GFP versus TH-GFP/P301S LC cells in the absence of DSP-4 ($p>0.99$) or at the highest dose of 1000µM ($p>0.99$) where there was comparable cell loss in both groups compared to no DSP-4 ($p<0.0001$). However, at the moderate dose of 100µM, which does not induce a significant change in cell survival compared to no DSP-4 in the TH-GFP only LC cells ($p=0.98$), there was a significant reduction in LC cell survival in the TH-GFP/P301S LC cells ($p<0.05$). These data suggest that a dose of DSP-4 that is not toxic to normal LC neurons becomes lethal in the presence of P301S tau.

Sidak's post hoc test showed a significant difference between the average neurite lengths of LC neurons from control versus P301S tau mice at 0 ($p<0.05$) and 100µM DSP-4 ($p<0.01$) and1000µM, where control neurites show significant reductions making them comparable to those from P301S tau expressing cells ($p=0.99$).

LC neurons expressing P301S tau are not more susceptible to LPS toxicity.

To determine whether P301S tau may sensitize LC neurons to other types of toxins, we examined LC cultures that underwent a 24-hour challenge with 10n or100ng/ml LPS before fixation and immunocytochemistry for TH (Figure 2.5). Similar to what we observed in the neuron-enriched cultures (see above), LC neurite length was reduced by P301S tau in mixed neuron-glia cultures at baseline ($F(1,30) = 11.97$, $p<0.05$). However, LPS exposure did not further affect neurite

length in LC neurons of either genotype (F(2,30) = 0.4910, p=0.62). These data suggest that the cell death susceptibility for cell death conferred by P301S tau may be toxin-specific.

2.5 DISCUSSION

Early accumulation of hyperphosphorylated tau is seen in the LC of young and cognitively unimpaired individuals and may represent the initial steps in the cascade of events leading to AD (Braak, Thal et al. 2011). This pre-tangle pathology often colocalizes with active caspases in the LC of AD brains, pointing towards its potential role in the characteristic degeneration of the nuclei (Wai, Liang et al. 2009). However, these observations rely solely on cross-sectional data and it is difficult to draw conclusions about the direct mechanism through which this aberrant protein accumulation could induce dysfunction.

Here we show through *in vitro* experiments that the expression of hyperphosphorylation-prone tau significantly reduces LC neurite length. While this was not sufficient on its own to trigger cell death, the aberrant tau did sensitize the cells to a secondary insult. Together, these data suggest that early tau in the LC may compromise the morphology of its neurons and sensitize the LC to other insults that accumulate and promote dysfunction over time before resulting in the characteristic degeneration observed in AD.

The effects of hyperphosphorylated tau on LC neuron morphology and survival

In non-pathological conditions, tau is necessary for microtubule stability and plays an integral role in cytoskeletal structure and cell protein trafficking and signaling. Its aberrant hyperphosphorylation in disease conditions reduces its affinity for microtubules, which results in microtubule instability and dysfunction (Iqbal, Alonso Adel et al. 2005). On the cellular level, this has detrimental consequences for neurites, negatively impacting their outgrowth, stability, axonal transport and neurotransmission (Johnson and Stoothoff 2004, Alonso, Li et al. 2008,

Sydow, Van der Jeugd et al. 2011, Khan and Bloom 2016, Medina, Hernandez et al. 2016). This integral role of tau has been further confirmed with transgenic mice expressing hyperphosphorylation-prone mutant human tau (such as the P301S tau mice used in this study) that first develop tau pathology and altered synaptic function, followed by neurodegeneration in aged animals (reviewed in (Dujardin, Colin et al. 2015). Consistent with these data, we found that expression of P301S tau reduced LC neurite length in *in vitro* primary cell culture, both in neuronal enriched as well as mixed neuron/glia environments. Average neurite lengths were higher in the mixed cultures for both P301S tau expressing and control LC cells, likely a result of the more hospitable environment conferred by glial cells. While the NeuriteTracer plugin developed by the Fournier lab is a user-friendly and effective tool for streamlined assessment of total neurite lengths, it is not able to report on each separate neurite. Thus, our analysis cannot determine whether the reduction in neurite length is due to a reduction in average neurite lengths overall, a loss of individual neurite outgrowths, or a combination thereof. More in-depth analyses of whether the neurite length reduction seen is indicative of lower neurite branching complexity and/or changes in morphology of individual neurites could be elucidated with other programs such as Sholl's analysis (Ferreira, Blackman et al. 2014) or NeurphologyJ (Ho, Chao et al. 2011).

Despite the nearly three and two-fold differences in neurite length in the neuronal enriched and mixed cultures respectively, there was no significant difference in LC cell survival between cells expressing P301S tau and control in the short period of time the cells were incubated. These results are consistent with our finding that, in TgF344-AD rats that express mutant human APP and PS1 genes and develop plaques and NFTs (Cohen, Rezai-Zadeh et al. 2013), pre-tangle tau pathology in the LC is observed at 16 months together with LC dysfunction (reduced projection fibers and NE tissue content in the forebrain) but no LC degeneration (our unpublished data). These data also echo recent findings from stereological studies of the LC in postmortem human brains showing a significant gap of time between accumulation of tau pathology in the LC and its frank degeneration (Theofilas, Ehrenberg et al. 2016). While tau

pathology may be accumulating in the brainstem early on (Braak stages a-c), it is not until later stages, when the pathology has breached the temporal lobe and limbic regions (Braak stage III), that the LC begins to show signs of neuronal loss (Theofilas, Ehrenberg et al. 2016), suggesting that a secondary insult may be necessary to push the LC over the threshold into a degenerative state. It is not clear from this study or ours whether a secondary insult is *necessary* to induce neurodegeneration in aberrant tau harboring LC cells or simply accelerates it.

It is possible that with more time, this aberrant tau accumulation, that only induces structural dysfunction in the LC within our 12-day *in vitro* protocol, could result in degeneration. Longer term studies for both our *in vitro* and *in vivo* experiments could clarify whether tau pathology on its own can induce neurodegeneration in the LC, given enough time. Nevertheless, the current findings of this study have important implications for diagnostics, as the mere presence of hyperphosphorylated tau in the LC can cause rapid morphological changes in neurites that could impair noradrenergic transmission.

Interaction of hyperphosphorylated tau in the LC with other toxins

The delay in LC neuronal loss seen by Theofilas et al. and our data examining the effect of aberrant tau on the LC suggests that a secondary trigger may be necessary to push LC cells ridden with tau pathology into a degenerative state. There are many possibilities for what the secondary trigger could be. Risk factors ranging from toxins or nutritional factors, to underlying health conditions or medications have been discussed over the decades-long investigation of AD prevalence (Lindsay, Laurin et al. 2002, Povova, Ambroz et al. 2012). LC neurons themselves are known to be sensitive to environmental toxins and can easily accumulate heavy metals and toxicants from the environment, making DSP-4, which has similar chemical properties to these toxins, an appropriate toxin to start with (Pamphlett 2014).

The highest concentration of DSP-4 in our experiments induced significant LC cell death in both the tau expressing as well as control LC cells, confirming its toxicity. While the

mechanisms underlying DSP-4 induced toxicity are not entirely clear, it is believed to be taken up by NE transporters at the synaptic terminal, where it cyclizes to a reactive aziridinium derivative that interacts with cellular machinery such as the mitochondria to shut down and destroy the terminal (Ross and Stenfors 2015). *In vivo,* this manifests as a "dying back" of the LC projection neurites seen as decrease in immunoreactivity for noradrenergic components in projection areas (Ross and Stenfors 2015). *In vitro*, similar deleterious consequences have been reported with DSP-4 treatment of chromaffin cells at the concentrations used in our study (Boksa, Aitken et al. 1989). In that study, NE levels were immediately reduced within the first hour and the cells began showing retraction of their processes within 2 hours following incubation with 1000 µM DSP-4. This was followed by significant death or severe changes in morphology by 24 hours together with a complete ablation of NE. At 100µM, there was a moderate reduction in NE levels without any noted morphological or survival changes. Our data similarly demonstrated that at 1mM DSP-4, even control LC neurons had significant reductions in neurite length and neuronal survival. However, at the 100 µM concentration, which was reported to be non-lethal in the Boksa et al. study and did not impact neurite length or neuronal survival in our control TH-GFP only cells, the presence of P301S tau sensitized the LC neurons, leading to significant degeneration in mutant tau expressing cells.

We also investigated the impact of immunostress (induced via LPS) in conjunction with aberrant tau in the LC as there are many documented connections between the LC and neuroinflammation. For example, a polymorphism in the promoter of the gene for the noradrenergic enzyme dopamine beta-hydroxylase (DBH) that results in NE deficiency, can interact with genes for proinflammatory interleukin (IL-1A and IL-6) to increase risk for AD (Mateo, Infante et al. 2006, Combarros, Warden et al. 2010). In addition, both direct intracranial infusion of LPS into the LC and peripherally injected LPS induce hyperactivity in the LC that is modulated by IL-1 (Borsody and Weiss 2002, Borsody and Weiss 2004).

Interestingly, we did not observe a synergy between tau and LPS. While there was significant cell death at our high concentration of LPS, it was of similar magnitude in control and P301S LC neurons, and lower concentrations had no effect on cells of either genotype. This contradicts a recent study showing that tau-deficient mice are protected against the neurotoxic effects of LPS (Maphis, Xu et al. 2015). The interaction between tau and neuroinflammation may be complicated; there are contradictory studies reporting that LPS treatment attenuates or increases tau pathology, with some highlighting its potential to induce tau hyperphosphorylation (Gardner, White et al. 2016, Liu, Wang et al. 2016) and others showing its ability to as enhance neuronal autophagy and clearance of the same pathology (Qin, Liu et al. 2016).

It is possible that while aberrant P301S tau is postulated to induce loss of function phenotypes by sequestering normal tau away from microtubules, it may not necessarily equate to a system that is completely devoid of tau, which could explain the differences in our findings. Various concentrations or durations of treatment could also result in differentially protective or deleterious effects and perhaps the protocol used in our experiment was not sufficient to see synergy. LPS may also be inducing cell death and/or dysfunction within our cultures in a manner that we could not detect with our current study design. For example, testing conditioned media from treated cultures could elucidate whether there is an impact of the toxins on NE release. Employing other immunostress agents that better mimic AD's neuroinflammatory state and are more physiologically relevant like proinflammatory cytokines instead of LPS may also yield better results.

Further studies are still needed to fully elucidate the interaction between P301S tau in LC neurons and LPS, and whether immunostress could act synergistically with aberrant tau to induce dysfunction. Our studies represent only glimpses at the exhaustive potential insults LC neurons may face, and follow-up experiments investigating how aberrant tau interacts with beta-amyloid, oxidative stress and other potential insults, especially those that induce structural

deficits, are necessary to fully understand how this early pathology may be contributing to the degeneration of the LC and AD pathogenesis.

Our results also do not clarify the mechanism through which P301S may act synergistically with a secondary insult, but given the deleterious effects of both DSP-4 and P301S independently on neurite length, combined with the lack of synergy with LPS (which does not impact neurite length), the disruption of neurite stability and function is a likely candidate. Many microtubule stabilizing agents are being investigated as possible AD therapies, and their compensatory effects for the loss of normal tau function appears to be very promising (reviewed by (Ballatore, Brunden et al. 2012). Future experiments with LC cultures harboring the deleterious P301S tau could incorporate a “rescue” or “protection” against the conferred susceptibility to insults by pretreatment with a microtubule stabilizing agent. Abrogation of P301S-induced toxin susceptibility would support a contribution of dysfunctional neurites for LC neurodegeneration in disease states and further our understanding of the impact of early tau pathology in the LC.

A final, yet important, limitation for this study is our reliance on the P301S mutation to model hyper-phosphorylated tau. While the P301S mutation makes tau exquisitely susceptible to hyperphosphorylation, it is not a genetic mutation that results in AD, and thus, may lack face validity. Tauopathies show great heterogeneity in their histopathology. FTDP-17, the family of diseases which the P301S mutation is linked to, results in a significantly less hyperphosphorylated tau load and different ultrastructural appearances than early onset AD despite also forming NFTs (Shiarli, Jennings et al. 2006). However, the P301S mutation still induces hyperphosphorylation of tau at several AD-relevant epitopes (such as AT8) and we believe it is highly relevant to investigate early tau changes for AD. Performing this experiments using transgenic mice with other tau mutations such as V337M or R406W, which *do* produce filamentous tau pathology that appear identical to that of AD may be more physiologically

relevant and strengthen the findings from this study (Spillantini, Crowther et al. 1996, Reed, Grabowski et al. 1997, Hutton, Lendon et al. 1998).

Conclusion

Our experiments indicate that hyperphosphorylated tau reduces neurite length in LC neurons, but is not sufficient to cause cell death. However, we found that hyperphosphorylated tau can interact synergistically with the noradrenergic toxin, DSP-4, to induce LC cell death, supporting the idea that the presence of this aberrant tau species may sensitize LC neurons to secondary insults and make them more susceptible to degeneration. The lack of synergy with LPS on cell death could point towards a specific mechanism of action, whereby the aberrant tau only interacts with toxins or insults that also impact neurite health. These results should be interpreted with caution and further studies investigating other toxins with or without impacts on neurite length such as oxidative stress or beta-amyloid will improve our understanding of how tau pathology impacts LC degeneration. Further experiments elucidating the details of how aberrant tau reduces neurite length (e.g. whether it is a reduction in overall neurite length or a reduction in numbers of neurites) will also provide more information on the mechanism underlying these pathological changes. The present findings suggest that hyperphosphorylated tau in the LC may represent a preclinical stage of AD and further illustrates the need for earlier detection and implementation of therapeutics (Stratmann, Heinsen et al. 2016).

FIGURES

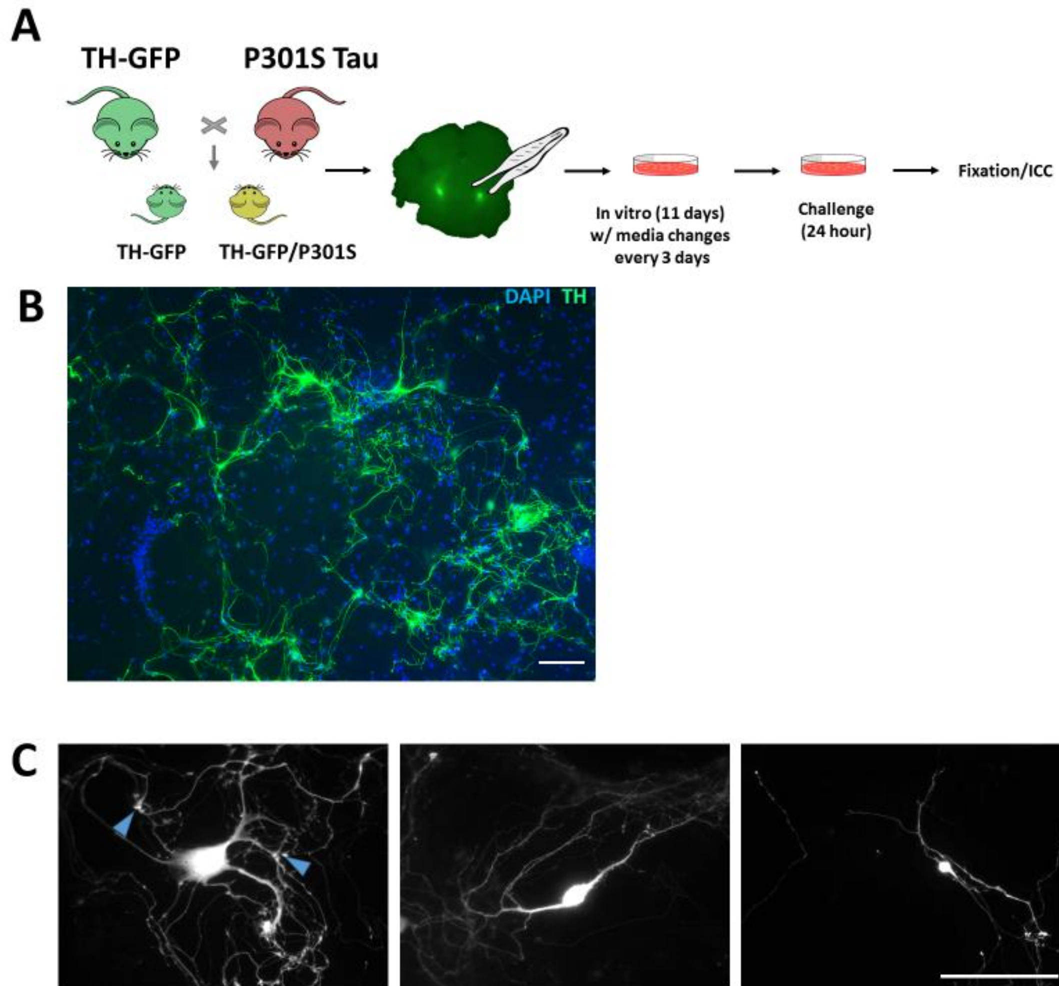


Figure 2.1 *Cultured primary LC neurons from TH-GFP mice*. **(a)** Schematic diagram of LC primary culture protocol in which LC from P1 pups of P301S mice crossed with TH-GFP mice were cultured and maintained *in vitro* for 11 days prior to fixation and immunocytochemistry for TH. **(b)** TH positive LC cells (green) with DAPI nuclear staining (blue), scale bar: 100µm **(c)** Close up view of LC cells show diverse morphologies including large multipolar cells (left) with varicosities (arrowheads), medium sized fusiform cells (center) and smaller cells (right), scale bar: 100 µm.

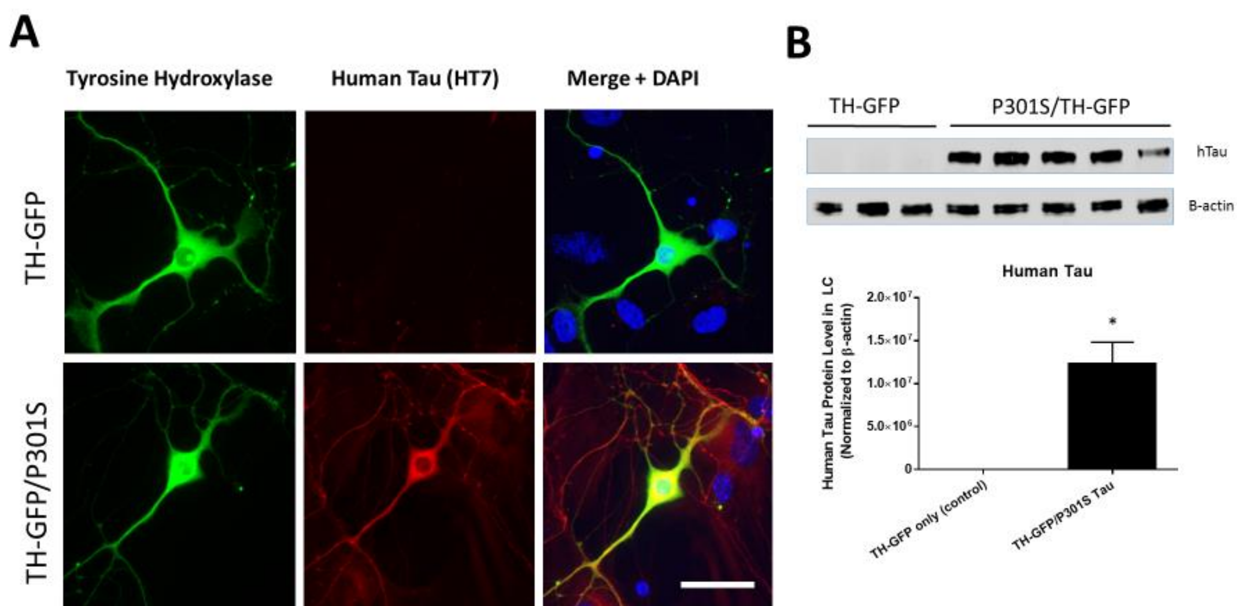


Figure 2.2 *Cultured primary LC neurons from TH-GFP/P301S mice express human tau while control TH-GFP cells do not* **(a)** Representative images from DIV 11 cultures of TH-GFP (top) and TH-GFP/P301S (bottom) mice, showing LC neurons (TH, green) with nuclei (DAPI, blue) and co-immunostaining for human tau (HT7, red). **(b)** Mean ± SEM human tau abundance as measured by western blot using another antibody against human tau (hTau), *$p<0.05$ compared to TH-GFP, n=3-5, scale bar = 50μm.

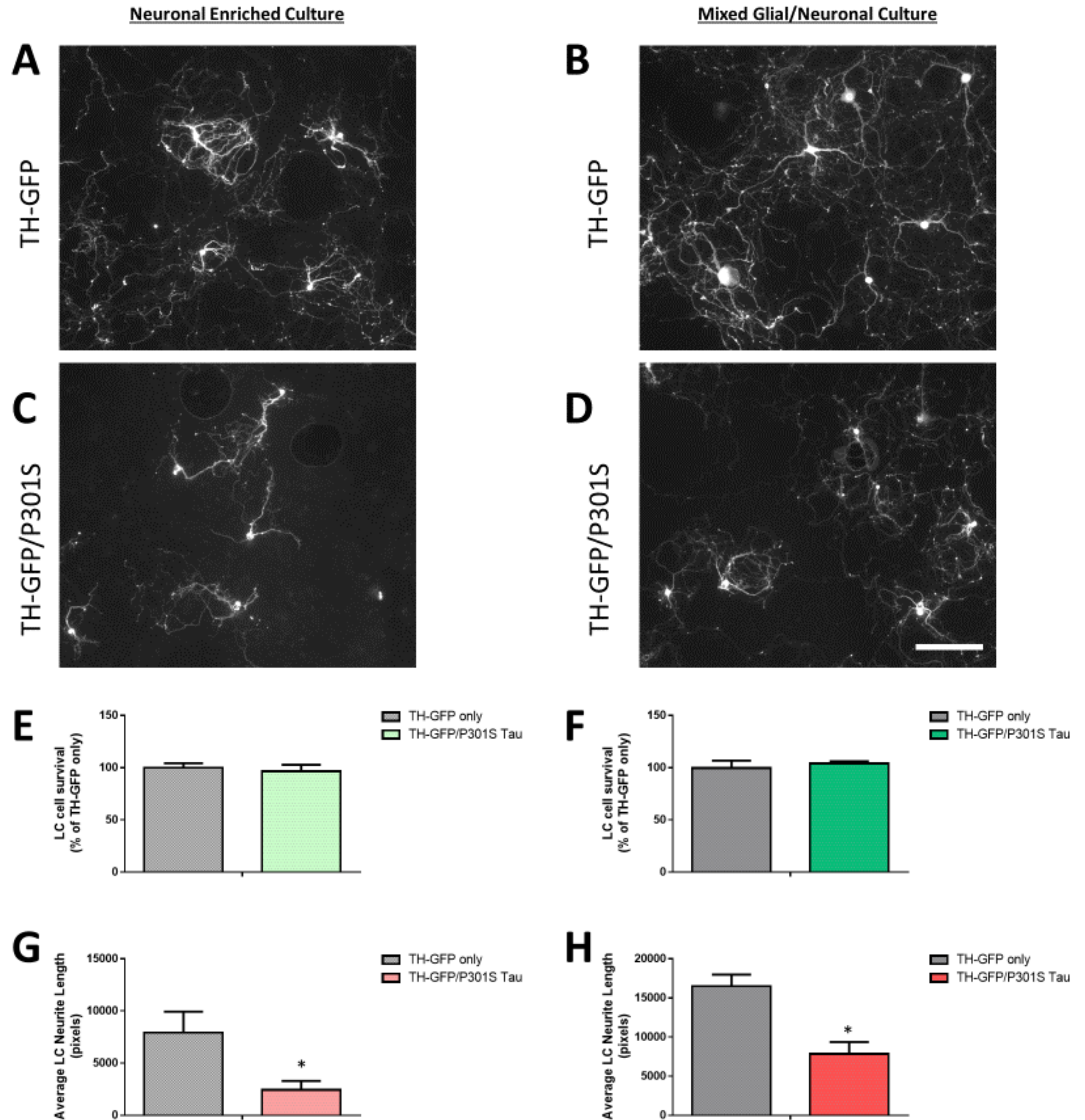


Figure 2.3 *P301S tau reduces neurite length but not neuronal survival in LC cells from both neuronal enriched and mixed glial/neuronal cultures.* Representative images of LC neurons with and without P301S tau from **(a&c)** neuronal enriched and **(b&d)** mixed glial/neuronal cultures maintained *in vitro* for 12 days prior to fixation and immunocytochemistry for TH. **(e&g)** LC neuronal survival was quantified as percent of TH-GFP only group. **(f&h)** Neurite length is shown as pixels. (Mean ± SEM, *$p<0.05$ compared to TH-GFP only, n=4-9 per group, scale bar: 200µm).

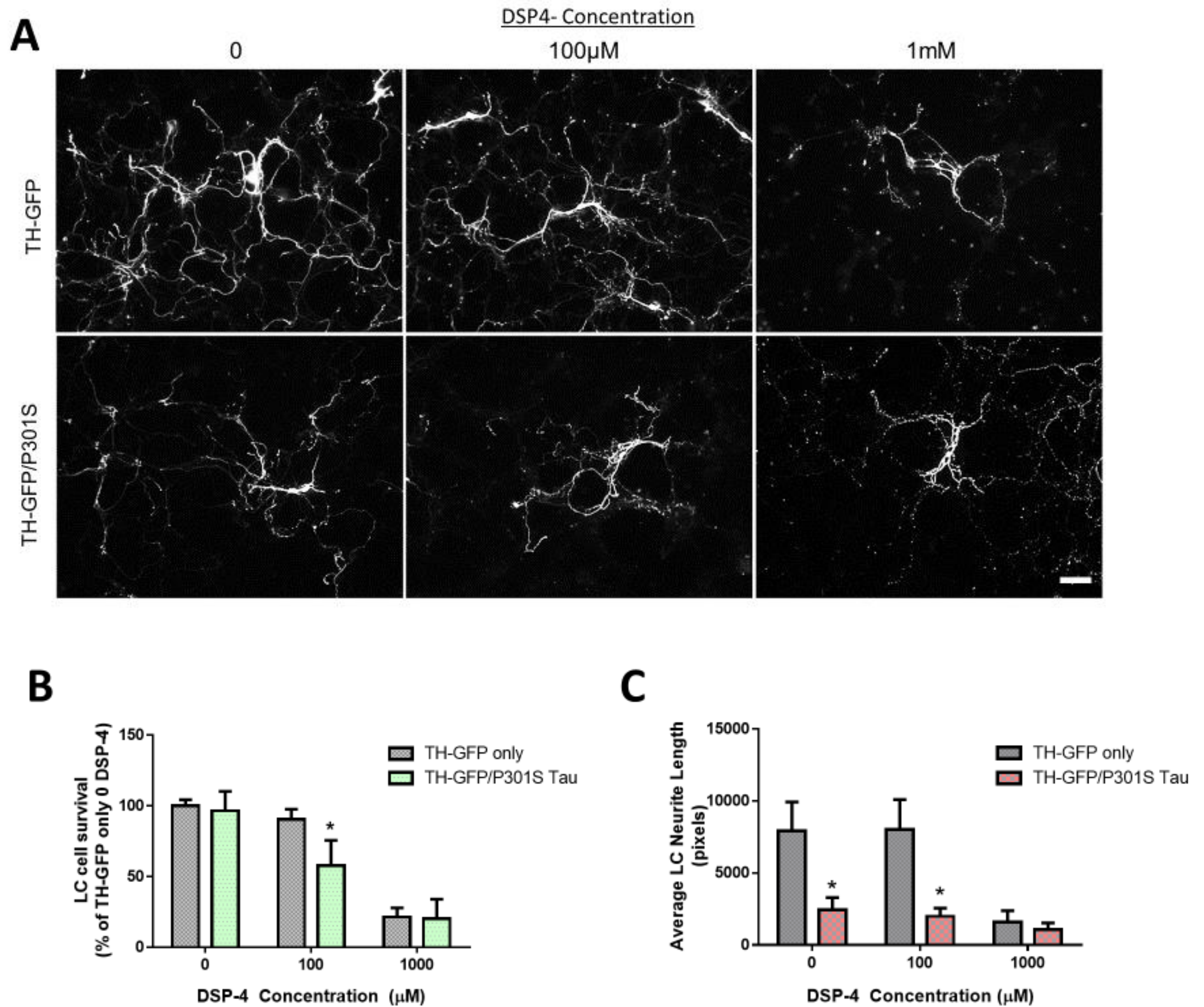


Figure 2.4 *P301S tau expression sensitizes LC neurons to DSP-4 toxic challenge*

(a) Representative images of LC primary cultures from TH-GFP (top) and TH-GFP/P301S mice maintained *in vitro* for 11 days before a 24 hour challenge with DSP-4 prior to fixation and immunocytochemistry for TH. **(b)** LC neuronal survival was quantified as percent of TH-GFP only + 0 DSP-4 group. **(c)** Neurite length is shown as pixels. (Mean ± SEM, (*p<0.05 compared to TH-GFP only, n=4-9, scale bar: 100µm).

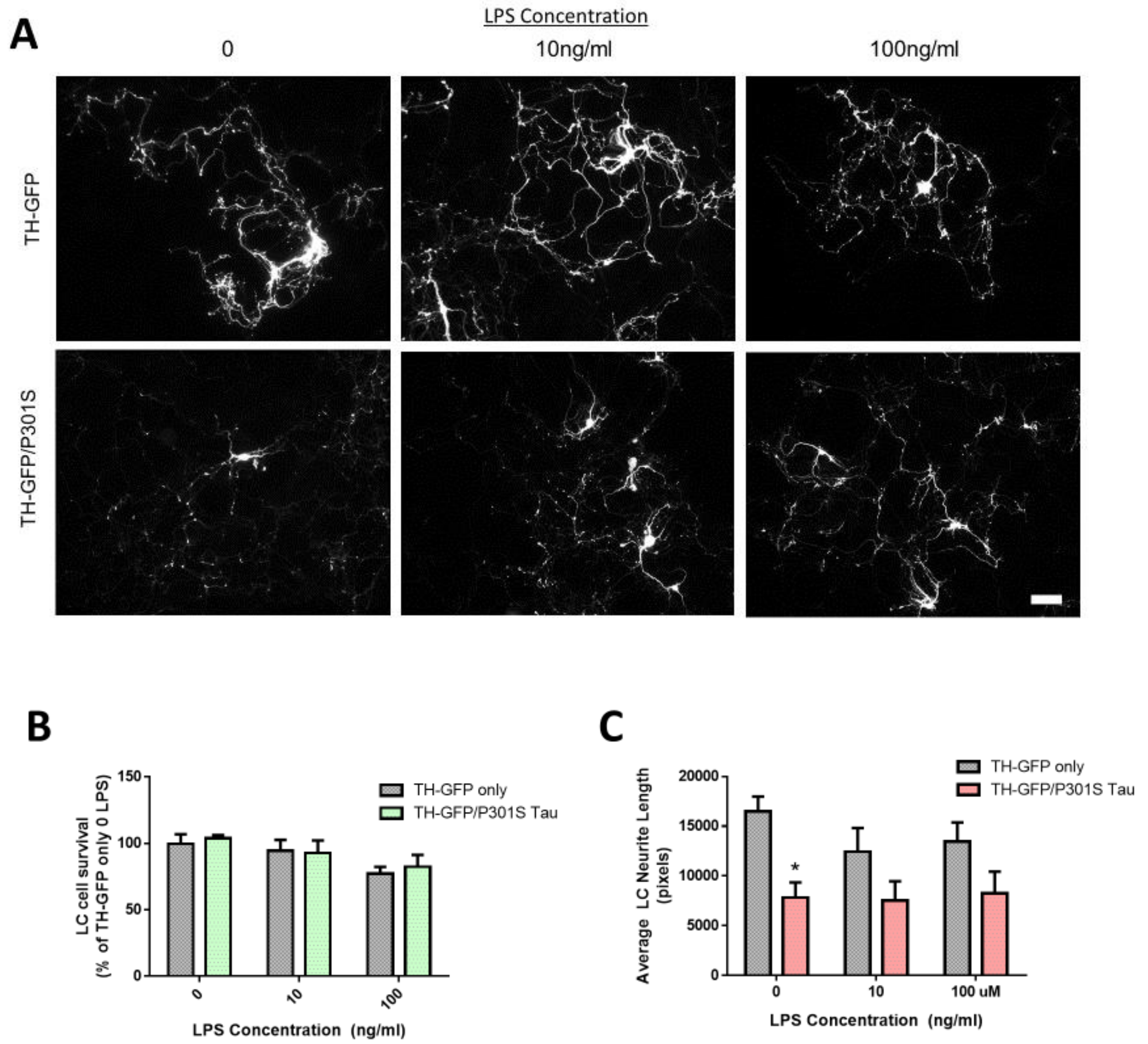


Figure 2.5 *P301S tau expression does not impact LC neuron sensitivity to LPS toxicity*

(a) Representative images of LC primary cultures from TH-GFP (top) and TH-GFP/P301S mice maintained *in vitro* for 11 days before a 24 hour challenge with LPS prior to fixation and immunocytochemistry for TH. **(b)** LC neuronal survival was quantified as percent of TH-GFP only + 0 LPS group. **(c)** Neurite length is shown as pixels. (Mean ± SEM, (*p<0.05 compared to TH-GFP only, n=4-9, scale bar: 100µm).

CHAPTER 3: DSP-4 LESIONS OF THE LOCUS COERULEUS EXACERBATE NEUROPATHOLOGY IN A MOUSE MODEL OF TAUOPATHY

3.1 ABSTRACT

Elucidating the mechanisms that initiate Alzheimer's disease (AD) at its earliest stage is the key to developing effective therapeutics and diagnostics. The locus coeruleus (LC) is a brainstem nucleus that supplies norepinephrine (NE) to the forebrain and degenerates early in nearly all AD patients. Loss of LC neurons is correlated with increased severity of other hallmarks of AD such as beta-amyloid plaques, tau neurofibrillary tangles and cognitive deficits, suggesting that it may have a critical role in the initiation and progression of the disease. Lesions of the LC in beta-amyloid mutant mouse models of AD significantly exacerbate amyloid pathology and cognitive deficits, but it is unknown how LC ablation affects tau pathology. Here we investigate the impact of LC degeneration in a mouse model of hyper-phosphorylated tau by lesioning the LC of P301S tau transgenic mice with systemic injections of the selective noradrenergic neurotoxin DSP-4 and assessing the development of tau pathology, neuroinflammation and neurodegeneration. DSP-4 resulted in clear lesions of the LC and its noradrenergic projections in both wildtype and P301S mice. We found that by 6 months, there is an exacerbation of tau pathology in the both the LC and hippocampus of lesioned P301S mice compared with unlesioned transgenics. This is followed by a significant upregulation of both astroglial and microglial activation and neurodegeneration in the hippocampus by 10 months. These experiments highlight the dual role LC degeneration has on exacerbating tau pathology, in addition to beta-amyloid, in AD.

3.2 INTRODUCTION

Degeneration of the brainstem locus coeruleus (LC) is an early and ubiquitous feature of Alzheimer's disease (AD) that correlates well with many of its other features such as beta-amyloid plaques, tau neurofibrillary tangles (NFT) and cognitive impairment (Iversen, Rossor et al. 1983, Mann 1983, Bondareff, Mountjoy et al. 1987, Chan-Palay and Asan 1989, Haglund, Sjobeck et al. 2006, Grudzien, Shaw et al. 2007). The LC projects to many of the brain areas that are prominently affected in AD such as the hippocampus and frontal cortex, and is the sole source of norepinephrine (NE) in these regions. NE has been implicated in many cognitive and behavioral processes including attention, learning and memory, stress and arousal, thus, loss of the LC and the resulting reduction of NE transmission in the brain possibly contributes to many of the affective symptoms and cognitive impairments seen with AD patients (Marien, Colpaert et al. 2004, Gannon, Che et al. 2015).

Despite the pervasive loss of the LC seen in AD, only a handful of animal models of AD report endogenous LC degeneration or dysfunction. 16-month-old mice expressing mutated amyloid precursor protein and presenelin-1 (APP/PS1) genes related to beta-amyloid production have 23% fewer noradrenergic cells in their LC compared to wild-type mice, but their remaining LC neurons are hypertrophic, resulting in a paradoxically larger LC volume (Liu, Luo et al. 2013). Strikingly, the LC degeneration seen in these mice begins as a breakdown of distal axons before progressing to cell bodies, which is reminiscent of the pattern seen in patient brains (Liu, Yoo et al. 2008). Another line of mice expressing aberrant beta-amyloid related mutations (Tg2576) also shows reduced LC cell numbers by 8 months, but does not result in the same increase in cell size seen with the APP/PS1 strain (Guerin, Sacquet et al. 2009). Transgenic models of Down's syndrome, which incorporates a triplication of the APP gene, have also reported LC degeneration (Salehi, Faizi et al. 2009). Other models, such as the PDAPP mouse that expresses APPV717F, show only a shrinkage of LC cells without any overt degeneration of the nuclei even at the old age of 23 months (German, Nelson et al. 2005). LC cell loss in AD

patients is reported to range from 40 - 80% (German, Manaye et al. 1992, Lyness, Zarow et al. 2003), which has not been faithfully recapitulated in the majority of animal models of AD on their own, thus, other methods have been developed to investigate the impact of LC cell loss and dysfunction of the NE system within the context of AD.

The NE system can be manipulated in AD transgenic animals by crossing them to other transgenic animals with NE deficits. APP/PS1 mice crossed with dopamine beta-hydroxylase knockout ($DBH^{-/-}$) mice unable to synthesize NE show a profound synergistic exacerbation of the only modest cognitive impairments seen in the single mutants (Hammerschmidt, Kummer et al. 2013). Similarly, crossing APP/PS1 mice with mice lacking Ear2, the nuclear receptor transcription factor important for the specification of LC neurons, also results in an exacerbation of cognitive deficits (Kummer, Hammerschmidt et al. 2014). In both studies, these impairments could be rescued with the administration of a NE pro-drug, L-threo-3,4-dihydroxyphenylserine (L-DOPS), further supporting the role of NE in mediating these cognitive deficits. Interestingly, while the disruption of the NE system in both studies resulted in exacerbated cognitive deficits, this was not accompanied with an exacerbation of beta-amyloid pathology. This suggests that while loss of NE itself may be critical for the cognitive deficits seen in AD, it may play less of a role in the development and progression of neuropathology. As the $DBH^{-/-}$and the Ear2 crosses do not result in an actual physical degeneration of the LC that is more reminiscent of the pathology seen in AD brains, other methods for modeling this feature were needed (Hammerschmidt, Kummer et al. 2013, Kummer, Hammerschmidt et al. 2014).

To investigate whether the physical degeneration of the LC nuclei may be involved in AD neuropathogenesis, other groups have experimentally lesioned the LC in APP transgenic mice with the site-specific noradrenergic neurotoxin, *N*-(2-chloroethyl)-*N*-ethyl-bromo-benzylamine (DSP-4). These lesions in APP23, F717F APP, APP/PS1 mice not only lead to a similar exacerbation of cognitive and behavioral deficit as seen with the genetic depletion of NE, but also a robust inflammatory response and increased Aβ plaque deposition (Heneka,

Ramanathan et al. 2006, Kalinin, Gavrilyuk et al. 2007, Rey, Jardanhazi-Kurutz et al. 2012). Together, these findings emphasize the importance of the LC/NE system in the gating of beta-amyloid pathology and downstream cognitive deficits in AD. However, there are currently no published studies investigating the role of LC degeneration in mouse models of tau, and it remains unclear whether there is a similar deleterious effect for the other main aspect of AD pathology as well.

Here we examined whether LC degeneration and NE depletion via a DSP-4 lesion impacts neuropathology in a mouse model of tauopathy. We find that DSP-4 exacerbates tau pathology, neuroinflammation and neurodegeneration in transgenic mice expressing mutant human tau (P301S) prone to hyper-phosphorylation. These results support a role for LC degeneration in AD pathogenesis through interactions with tau, in addition to beta-amyloid.

3.3 MATERIALS AND METHODS

Animals

Male and female P301S transgenic mice (B6N.Cg-Tg(Prnp-MAPT*P301S)PS19Vle/J, Jackson Laboratory, #024841), which express the 4N1R isoform of human microtubule-associated protein tau (MAPT) with the P301S mutation under the mouse prion promoter (Prnp), were used. P301S mice were purchased as hemizygotes on a (C57BL/6 x C3H)F1 background and backcrossed to C57BL/6. Non-transgenic littermates were used as wild-type controls. P301S mice are reported to develop extensive astro- and microgliosis beginning at 3 months, followed by a progressive accumulation of NFTs beginning at 6 months (Yoshiyama, Higuchi et al. 2007, Takeuchi, Iba et al. 2011). From 9-12 months, neuronal loss is observed in the hippocampus and entorhinal cortex (Yoshiyama, Higuchi et al. 2007).

DSP-4 lesions

There were four groups of animals within each age group (4 month, 6 month and 10 month): P301S transgenic mice (P301S) and non-transgenic littermate controls (WT) with either DSP-4 or vehicle (bacteriostatic saline) treatment. At around 2 months of age, mice began their treatment regime with two initial doses of DSP-4 (50 mg/kg, i.p.) or saline were administered on day 1 and day 7, after which animals received a monthly booster (50 mg/kg), with the final dose ending one month prior to behavioral testing which began at 4, 6 and 10 months. Behavioral testing spanned 4 weeks, thus mice were 5, 7 and 11 months at the time of sacrifice for histology.

Perfusion and Brain Dissection

Mice were anesthetized with a mixture of ketamine (100 mg/kg, i.p.) and xylazine (20 mg/kg, i.p.). To prevent tau hyper-phosphorylation due to anesthesia-induced hypothermia (Planel, Richter et al. 2007, Bretteville, Marcouiller et al. 2012, Carrettiero, Santiago et al. 2015), mice were kept on top of a heating pad until fully anesthetized. Mice were transcardially perfused with cold 0.1M potassium phosphate-buffered saline (KPBS) until their livers were cleared of blood. Brains were removed and carefully split sagitally into two hemispheres using an acrylic mouse brain mold (Ted Pella, #15050, Redding, CA). One hemisphere was drop-fixed overnight in 4% paraformaldehyde in KPBS for immunohistochemistry. The other hemisphere was micro-dissected on ice and the olfactory bulb, prefrontal cortex, hippocampus, brain stem and remaining cortex were individually frozen and kept at -80°C for biochemical processing.

Immunohistochemistry

Mouse brain hemispheres were dehydrated in ethanol, embedded into paraffin blocks, sliced at 8 μm and mounted onto slides. Slides were processed by deparaffinization in xylene and rehydration through an ethanol series. Slides to be processed with DAB-immunohistochemistry were incubated with 3% hydrogen peroxide in methanol for 20 min before rehydrating in distilled water. Antigen retrieval was performed by incubating slides in a 10mM sodium citrate solution at

pH 6.0 with 0.5% Tween-20 for 10 min at 90°C. Slides were allowed to cool to 50°C before washing in distilled water and transferring to PBS. Non-specific binding of proteins was blocked by incubating in a solution containing 3% bovine serum albumin and 0.1% Triton-X 100 in PBS for 30 min at room temperature before washing with PBS prior to antibody incubation in a humidified chamber overnight, also at room temperature. Primary antibodies included rabbit anti-tyrosine hydroxylase (1:1000, Pel-Freez, P40101), rabbit anti-Iba1 (1:1000, Wako, #019-19741), rat anti-GFAP (1:1000, Invitrogen, #130300), mouse anti-Ser202,Thr205 (AT8) (1:500, ThermoScientific, MN1020), mouse anti-Ser396,404 (PHF1) (1:500, courtesy of Peter Davies, Albert Einstein College of Medicine, New York), mouse anti-NeuN (1:100, Millipore, MAB377). Slides were washed multiple times with PBS and incubated with either biotinylated or fluorescently labeled secondary antibodies for 2 h at room temperature. Immunofluorescent slides were treated for 5 min with Hoechst dye (12 µg/ml, Sigma-Aldrich, B1155) prior to the final PBS wash and cover-slipping with Fluoromount™ Aqueous Mounting Medium (Sigma Aldrich, F4680). Biotinylated slides were washed in PBS, incubated for 2 h with ABC solution (Vectastain® Elite ABC HRP Kit, PK-6100) at room temperature, washed again in PBS and developed using SIGMA*FAST*™ 3,3′-Diaminobenzidine (DAB, SigmaAldrich, D4293) for 10 min. Slides were washed in PBS again before dehydrating with an ethanol and xylene gradient and coverslipping with Permount™ mounting medium (FisherSci, SP15-100). All slides were air dried before viewing with a microscope (DM6000B, Leica).

High Performance Liquid Chromatography

Frozen micro-dissected tissue was sonicated with 10 sec of 1 sec pulses in 10x (volume/weight) 25 mM Tris buffer pH 7.4 and split into equal volumes for use in multiple biochemical tests. A 0.2N perchloric acid solution was added to one aliquot of sonicated tissue, followed by gentle tapping to mix and centrifugation at 13,000rpm at 4°C for 15 minutes. Supernatant was collected and filtered through a 0.45µm filter on a table-top centrifuge at 13,000rpm at 4°C for 10 minutes.

Samples were processed with the kind assistance of Dr. Rong Fu, Dr. Ellen Hess and the HPLC core at Emory. They were run using an ESA 5600A CoulArray detection system, equipped with an ESA Model 582 pump and an ESA 542 refrigerated autosampler. Separations were performed at room temperature using an MD-150 × 3.2 mm C18 column. The mobile phase consisted of 1.7 mM 1-octanesulfonic acid sodium, 75 mM NaH2PO4, 0.025% triethylamine, and 9% acetonitrile at pH 3.01. 20 µl of sample was injected. The samples were eluted isocratically at 0.4 mL/min and detected using a 6210 electrochemical cell (ESA, Bedford, MA) equipped with 5020 guard cell. Guard cell potential was set at 600 mV, while analytical cell potentials were −175, 100, 350 mV and 425 mV. The analytes were identified by the matching criteria of retention time and sensor ratio measures to known standards (Sigma Chemical Co., St. Louis, MO). Compounds were quantified by comparing peak areas to those of standards on the dominant sensor.

Statistical Analysis

Data was analyzed via 2-way ANOVA with varying factors depending on the test. Tukey's or Sidak's post-hoc tests were used to for multiple comparisons. Significance was set at $p<0.05$, with 2 tailed variants of tests implemented. Multiplicity adjusted p-value was reported for each comparison. Data are presented as means ± standard error of mean (SEM). Calculations were performed and figures created using GraphPad Prism version 6 for Windows (GraphPad Software, Inc., San Diego, CA, USA).

3.4 RESULTS

DSP-4 induces degeneration of LC cell bodies.

We visualized the effect of DSP-4 using immunoreactivity (IR) for the NE transporter (NET), a reliable marker of noradrenergic neurons that also shows marked decrease in AD patients (Gulyas, Brockschnieder et al. 2010). Systemic administration of DSP-4 (50 mg/kg, i.p.; 2 initial doses then monthly booster doses) induced a substantial lesion of the LC, significantly reducing

NET IR in both WT and P301S mice at all age groups (Figure 3.1a). Data are shown as the raw % area of the LC (Figure 3.1b) as well as a relative % of the average LC NET IR of the 4-month saline treated WT mice (Figure 3.1c). A two-way ANOVA (genotype x treatment) at each age found a main effect of treatment (4 months: $F(1, 41) = 78.19$, $p < 0.0001$; 6 months: $F(1, 28) = 40.13$, $p < 0.0001$; $F(1, 37) = 27.22$, $p < 0.0001$), but not genotype. Sidak's multiple comparisons showed marked loss of % area of NET IR in DSP-4 lesioned animals compared to saline treated animals (4 months, WT: $t(41)=6.692$, $p < 0.0001$, P301S: $t(41)=5.790$, $p<0.0001$; 6 months, WT: $t(28)=4.554$, $p=0.0002$, P301S: $t(28)=4.404$, $p=0.0003$; 10 months, WT: $t(37)=4.422$, $p=0.0002$, P301S: $t(37)=3.013$, $p=0.0093$).

When comparing relative changes using 4-month unlesioned WT mice as a baseline, the lesions produced a ~50% reduction in NET IR starting at 4 months, which increased to ~60% by 6 months and ~70% by 10 months. A two-way ANOVA (age x group) revealed a main effect of age ($F(2, 108) = 29.84$, $p<0.0001$) and group ($F(3, 108) = 48.26$, $p<0.0001$). All 10-month groups have significantly reduced NET IR compared to their 4-month counterparts (WT + Saline: -39.81% ± 7.592, $p<0.0001$; WT + DSP-4: -23.44% ± 7.560, $p=0.0069$; P301S + Saline: -34.54% ± 7.401, $p<0.0001$; P301S + DSP-4: -19.33 ± 7.818, $p=0.0395$). No differences were seen between genotypes for either the lesioned or unlesioned animals at the same age. These results confirm that the LC lesions were successful, comparable to LC loss observed in AD, and were unaffected by the presence of human mutant tau.

DSP-4 induces degeneration of LC fibers and loss of NE in the hippocampus.

NET IR revealed that NE projection neurites from the LC to the hippocampus were significantly reduced with DSP-4 lesions (Figure 3.2a-d). Two-way ANOVA (genotype x treatment) revealed a main effect of treatment at each age (4 months: $F(1,43) = 61.96$, $p < 0.0001$; 6 months: $F(1,37) = 37.71$, $p < 0.0001$; 10 months: $F(1,41) = 34.31$, $p<0.0001$), but not genotype. DSP-4 treated animals had significantly lower hippocampal NET IR (calculated as % area covered of total area)

compared to vehicle for every age group and both genotypes: 4-month WT: $p<0.0001$; 4-month P301S: $p<0.0001$; 6-month wildtypes: $p=0.0002$; 6-month P301S: $p=0.002$; 10-month wildtype: $p<0.0001$; 10-month P301S: $p=0.0074$.

To confirm the functional loss of the NET-positive fibers, we used high performance liquid chromatography for electrochemical detection of NE tissue content (Figure 3.2e-g). Two-way ANOVA (genotype x treatment) revealed that NET IR reflects NE levels for 4 and 6-month WT and P301S mice, showing a main effect of treatment at those ages (4 months: $F(1,40) = 50.96$, $p < 0.0001$; 6 months: $F(1,38) = 105.6$, $p<0.0001$) but not genotype. At 10 months however, there was a main effect of treatment ($F(1,42) = 209.6$, $p<0.0001$), genotype ($F(1,42) = 5.839$, $p=0.0201$), and a treatment x genotype interaction ($F(1,42) = 4.879$, $p=0.0327$). Similar to the NET IR results, all DSP-4 groups had lower hippocampal NE levels compared to saline treated groups: 4-month WT: -0.1896 ng/mg ± 004415., $p=0.0006$; 4-month P301S: -0.2551 ng/mg ± 0.04397, $p<0.0001$; 6-month WT: -0.2446 ng/mg ± 0.03323, $p<0.0001$; 6-month P301S: -0.2633 ng/mg ± 0.03658, $p<0.0001$; 10-month WT -0.3175 ng/mg ± 0.02558 $p<0.0001$; 10-month P301S: -0.2334 ng/mg ± 0.02817, $p<0.0001$. At 10 months, saline treated P301S mice also had slightly less hippocampal NE tissue content compared to saline treated WT mice (-0.088 ng/mg ± 0.02558, $p=0.007$).

DSP-4 exacerbates tau burden in P301S mice

The P301S mutation makes tau exquisitely prone to hyper-phosphorylation, and the P301S mouse develops extensive pre-tangle tau pathology that can be visualized via a variety of phosphorylation-dependent antibodies. AT8 is an antibody commonly used as a marker of hyper-phosphorylated tau; it detects phosphorylated serine and threonine residues at the 202 and 205 codons of tau, respectively. We utilized conventional colorimetric immunohistochemistry to detect levels of phosphorylated tau in the LC (Figure 3.3) and hippocampus (Figure 3.4) of all our mice.

There was no AT8 immunoreactivity in any WT mouse (lesioned or unlesioned) at any age (data not shown). This is congruent with previous reports that WT mouse tau is resistant to hyper-phosphorylation (Clavaguera, Bolmont et al. 2009). At 4 months, we observed weak AT8 staining in the LC of both lesioned and unlesioned P301S mice, mainly as diffuse neuritic deposits that matured to fill a handful of cell bodies by 6 months in the lesioned animals and 10 months in unlesioned animals. A two-way ANOVA (age x treatment) showed a main effect of age ($F(2, 49) = 3.996$, $p=0.0247$) and treatment ($F(1, 49) = 4.046$, $p=0.0498$) for AT8 IR in the LC. Sidak's multiple comparison tests showed significantly increased LC AT8 IR in 6 and 10-month lesioned P301S mice compared to 4-month animals ($p=0.0440$, $p=0.0302$) but not for unlesioned mice ($p=0.9965$; $p=0.4256$).

In the hippocampus, weak AT8 IR was present beginning at 4 months as primarily neuritic staining, in the mossy fiber pathway in the CA3 region. By 6 and 10 months, we observed significantly more AT8 IR in the CA3 region as well as in the CA1 and dentate gyrus (DG) regions, including neuronal cell bodies in addition to neurites. An initial two-way ANOVA (age x treatment) of total AT8 IR for the entire hippocampus showed a main effect of age ($F(2, 54) = 25.46$, $p<0.0001$), but not treatment ($p=0.1043$). Upon closer inspection of the staining pattern, we decided to investigate CA3 and CA1/DG separately to try and tease out more region-specific differences in the tau burden.

Two-way ANOVA of the CA3 region (age x treatment) alone revealed a main effect of age ($F(2, 54\ 9.032$, $p=0.0004$) but not treatment. The CA1 region revealed a similar pattern with an effect of age ($F(2,54)= 15.77$, $p<0.0001$) but not treatment. However, in the DG region there was a significant effect of age ($F(2,54)=11.82$, $p<0.0001$) as well as an interaction between age and treatment ($F(2,54) = 3.845$, $p=0.0275$). Post-hoc tests found that lesioned P301S mice had significantly higher AT8 IR in the DG than unlesioned mice at 6 months ($t(54)=2.956$, $p=0.0138$). There were no significant differences at the two other ages.

We next evaluated the LC and hippocampus with another antibody that recognizes later stage phosphorylation of serine residues at the 396 and 404 sites (PHF1) indicative of conformational changes towards paired helical filaments. No PHF1 IR was observed in the LC of 4-month old mice (data not shown), but it was detectable at 6 months and evident at 10 months (Figure 3.5). In the hippocampus (Figure. 3.6), at 6 months, there was no significant difference in PHF1 IR in the CA1 or DG region, but in the CA3 region, a two-way ANOVA revealed there a main effect of treatment ($F(1,31)= 5.466$, $p =0.0260$), but not age. A multiplicity adjusted t-test showed significantly increased PHF1 IR in lesioned compared to unlesioned P301S mice ($t(14)=2.51362$, $p=0.0248$). There were no differences in PHF1 IR in the hippocampus at 10 months.

DSP-4 exacerbates neurodegeneration in P301S mice

To determine the impact of LC degeneration on neurodegeneration, we assessed cell loss via IR for NeuN, a neuronal marker (Figure 3.7). No significant differences in neuronal density in the hippocampus (CA1/DG or CA3) were observed between groups at 4 or 6 months. At 10 months, P301S mice treated with DSP-4 showed significant neuronal loss. In the CA1 region, a two-way ANOVA (genotype x treatment) revealed a main effect of genotype ($F(1,34) = 15.37$, $p=0.0004$) and a genotype x treatment interaction ($F(1, 34) = 8.377$, $p=0.0066$). Sidak's post hoc tests showed significant neuronal loss only in DSP-4 vs saline treated P301S mice ($t(34)=2.523$, $p=0.0327$). In the CA3 region, there was a main effect of genotype ($F(1, 34) = 20.54$, $p<0.0001$), treatment ($F(1, 34) = 6.067$, $p=0.0190$) and a genotype x treatment interaction ($F(1, 34) = 4.508$, $p=0.0411$). Again, Sidak's post hoc tests revealed significant neuronal loss in DSP-4 vs saline treated P301S mice ($t(34)=3.343$, $p=0.0041$).

DSP-4 increases neuroinflammation in P301S mice

The hippocampus was also stained for activated microglia (via Iba1 antibody) and astrocytes (via GFAP antibody) to assess neuroinflammation (Figure 3.8). For microglia, a two-way ANOVA (genotype x treatment) revealed only a main effect of genotype at 6 months ($F(1,38) = 10.50$, $p = 0.0025$). Tukey's multiple comparison post hoc tests showed that DSP-4 lesioned P301S mice had a significant increase in hippocampal Iba1 IR. In DSP-4 lesioned P301S mice compared to both saline and DSP-4 treated mice (+2.322% ± 0.7754, p=0.0239; +2.390% ± 0.7912, p=0.0223 respectively). At 10 months, a 2-way ANOVA showed a main effect of genotype ($F(1,43) = 76.38$, $p<0.0001$), treatment ($F(1,43) = 11.01$, $p=0.0019$) and a genotype x treatment interaction ($F(1,43) = 11.49$, $p=0.0015$). At this age, both saline and DSP-4 treated P301S mice had elevated levels of Iba1 compared to saline treated WT (+6.127% ± 0.1553, p=0.0016; +13.81% ± 1.711, p<0.0001), but microglial activation was even greater in lesioned animals (p=0.0002).

For astrocytes (Figure 3.9), no genotype or treatment differences in GFAP IR were observed until 10 months, when there was a main effect of genotype ($F(1, 39) = 96.29$, $p<0.0001$), treatment ($F(1, 39) = 7.805$, $p=0.0080$) and a genotype x treatment interaction ($F(1, 39) = 11.59$, $p=0.0015$). Post hoc tests revealed that while both saline (p=0.0002) and DSP-4 treated (p<0.0001) P301S mice had elevated levels of GFAP IR compared to saline treated WT mice; +14.24% ± 1.614,), neuroinflammation was more pronounced in the lesioned animals (p=0.0004).

3.5 DISCUSSION

AD neuropathology is progressive, and accumulates over a long period of time. The lack of clear mechanisms for how and why the two main protein aggregates (beta-amyloid plaques and tau NFTs) form and induce neuronal dysfunction is a major roadblock in combating this disease. Since degeneration of the LC is a ubiquitous event in AD that correlates well with the deposition of the beta-amyloid and tau deposition as well as cognitive impairment, it may contribute to AD pathogenesis (Bondareff, Mountjoy et al. 1987). We and others have shown previously that loss

of the LC and/or NE can exacerbate pathology and cognitive impairments in mouse models of beta-amyloid (Heneka, Ramanathan et al. 2006, Jardanhazi-Kurutz, Kummer et al. 2010, Hammerschmidt, Kummer et al. 2013, Kummer, Hammerschmidt et al. 2014). This study provides the first longitudinal analysis of how LC degeneration impacts tau-mediated pathology. We show that DSP-4 lesions of the LC exacerbate tau deposition, neuroinflammation, and neurodegeneration in the P301S mouse model of tauopathy.

Effects of DSP-4 on the LC noradrenergic system

Consistent with previous studies using a similar DSP-4 regimen, we observed LC cell body, neurite, and NE loss in a dose/time-dependent manner. In the 4 month old group (2 injections of DSP-4), there was already a dramatic reduction in NET IR in the LC for both WT and P301S mice. At the level of the hippocampus, one of the densest projection areas of the LC, there was also significant NET IR reduction in both lesioned P301S and wildtype mice. Our HPLC data matched the NET IR and also showed a significant reduction in the tissue NE content in the hippocampus of DSP-4 treated mice. The reductions in both NET IR and tissue NE are commensurable with previous literature demonstrating about a 70 – 80% reduction in NE content in the projection areas (Jardanhazi-Kurutz, Kummer et al. 2010, Ross and Stenfors 2015).

Exacerbation of tau pathology by LC lesions

P301S mice progressively accumulate tau pathology throughout the brain, beginning first with hyperphosphorylated pre-tangle tau that aggregates into more misfolded forms, eventually resulting in the characteristic insoluble and Gallyas positive neurofibrillary tangles (Yoshiyama, Higuchi et al. 2007). Because reductions in NE and degeneration of the LC are pathologically significant events in AD, and have been shown to exacerbate amyloid pathology in APP transgenic mice, we predicted there would be similar exacerbations within the P301S model of tau.

To test for the progression of tau pathology, we utilized two different antibodies. The AT8 antibody detects earlier epitopes of hyper-phosphorylation, while the PHF1 antibody reveals phosphorylation of epitopes more commonly associated with later stage conformational changes. At the level of the LC, we observed an age-dependent accumulation of both AT8 and PHF1 pathology. DSP-4 lesions exacerbated AT8 accumulation at 6 and 10 months compared to 4 months, while LC degeneration did not affect PHF1 accumulation.

We saw similar DSP-4 induced exacerbations of tau pathology in the hippocampus, but there was subregion specificity. The CA3 region appeared to be most vulnerable to developing tau pathology; AT8 IR could be seen as neuritic depositions in the mossy fiber pathway starting at 4 months, while the CA1 region remained mostly devoid of AT8 pathology at this age. By 6 months, both the CA3 and CA1 regions showed intense AT8 pathology that filled neurites as well as cell bodies. At this age, lesioned mice had significantly exacerbated AT8 pathology in the CA1 region. However, this difference disappeared by 10 months, when both lesioned and unlesioned P301S mice showed relatively similar, high levels of AT8 staining suggestive of a ceiling effect.

However, we also considered the possibility that that perhaps lesioned P301S mice at this age had progressed to more severe tau pathology. To test this idea, we assessed PHF1 IR, which detects a more advanced form of hyperphosphorylated tau. Interestingly, we found increased PHF1 pathology specifically in CA3 of lesioned mice at 6 months of age. This finding supports our hypothesis that the CA3 region is more vulnerable and progresses with tau pathology more quickly than the CA1 region, and it appears that LC lesions significantly augment the pathological accumulation of tau to appear much earlier than in unlesioned P301S mice (see Figure 3.10).

Neuroinflammation and neurodegeneration

In studies of LC lesions in beta-amyloid models, the impact of NE deficiency and LC dysfunction has been more thoroughly investigated and the exacerbation of pathology can be attributed (at

least in part) to the LC/NE system's neuroprotective and anti-inflammatory role in (Feinstein, Kalinin et al. 2016). The heightened inflammatory state induced by loss of LC neurons is thought to consequently increase the beta-amyloid load either by influencing the beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) activity to increase beta-amyloid production (Rossner, Sastre et al. 2006) and/or reduce beta-amyloid enzymatic degradation or microglial phagocytosis (Kalinin, Gavrilyuk et al. 2007). It is likely that similar mechanisms govern the relationship between LC dysfunction and tau pathologies, as we observed an increase in activated microglia and astrocytes in the hippocampus of lesioned P301S mice at 10 months, following the intensification of tau pathology.

Previous studies have also linked neuroinflammation to tau pathologies. The triggering receptor expressed on myeloid cells 2 (TREM2) is expressed primarily on microglia, and a rare variant of its gene is now recognized as a risk factor for AD, correlating well with tangle pathology as well as global cognitive decline in recent genome wide association studies (Cruchaga, Kauwe et al. 2013, Lill, Rengmark et al. 2015). Levels of TREM2 expression also correlate very well with the degree of tau phosphorylation in post-mortem analysis of brains from AD patients (Lue, Schmitz et al. 2015). As expected, TREM2 levels are upregulated in P301S mice, and silencing its expression exacerbates tau pathology, likely because of hyperactivation of tau kinases due to the neuroinflammatory response (Jiang, Tan et al. 2015). This protective effect can also be seen in the reverse, as overexpression of TREM2 in P301S mice ameliorates neuropathologies like tau pathology as well as neuronal loss (Jiang, Zhang et al. 2016).

Levels of tau oligomers have also been shown to correlate well with inflammatory markers for activated microglia and astrocytes. Microglial, as detected via immunohistochemistry for Iba1, colocalize with (and presumably engulf) tau oligomers in the retina of P301L mice, which expresses a mutant form of hyperphosphorylation-prone tau very similar to P301S (Nilson, English et al. 2016). The engulfment and degradation of tau pathology via microglia has also been demonstrated *in vitro* and *ex vivo* (Luo, Liu et al. 2015). Interestingly, despite similar levels

of upregulation and presence near tau oligomers *in vivo*, astrocytes (detected via GFAP) do not colocalize with them in the same way as microglia, suggesting that while they may interact with the tau oligomers, they may not directly engulf and degrade them (Nilson, English et al. 2016).

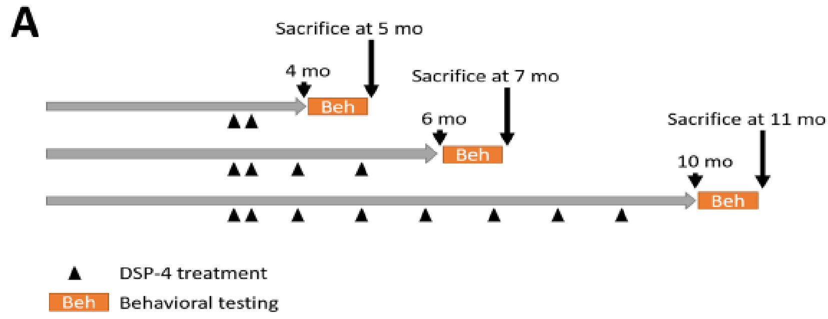
The delicate balance of pro and anti-inflammatory states makes it likely that neuroinflammation has a dual role in relation to tau: a protective anti-inflammatory response to the presence of tau pathology and a deleterious pro-inflammatory response because of the rampant pathology and neurodegeneration. It is likely that the neuroinflammation that may start out as a protective response spirals into a self-perpetuating loop where tau pathology induces neuroinflammation, which damages the cells, and may also induce more tau pathology which continues to increase neuroinflammation. Our findings of exacerbated cell loss in the hippocampus of lesioned P301S mice supports this hypothesis. Further analysis of the qualitative states of the microglia and astrocytes upregulated in our lesioned 10 month old P301S mice may provide more insight as to the mechanism through which LC dysfunction may be contributing to tau.

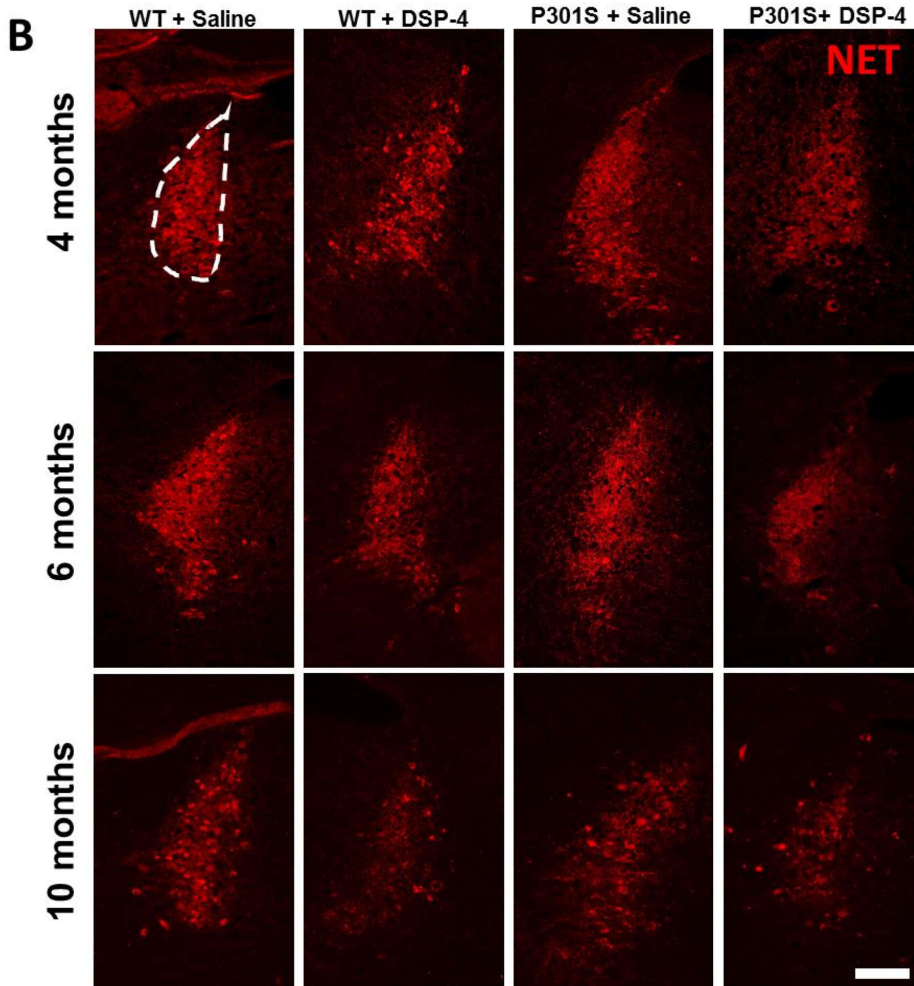
Conclusion

The ubiquitous degeneration of the LC and subsequent loss of noradrenergic tone appear to be important events in the pathogenesis of AD. Our experiments highlight a systematic shift in tau pathology and resulting neurodegeneration and neuroinflammation with the loss of the noradrenergic system in the P301S mice. We observed an exacerbation of both pre-tangle and paired helical filament tau pathology in the hippocampus of DSP-4 lesioned mice at 6 months. Although the levels and severity of tau pathology levels are more similar between lesioned and unlesioned mice at 10 months, hippocampal neuroinflammation and neurodegeneration is significantly increased by LC degeneration. This study is the first to thoroughly investigate the impact of an LC lesion and loss of NE in a mouse model harboring mutated tau prone to hyper-phosphorylation. Our studies provide an important complement to the extensive work that has

been done characterizing the effect of LC degeneration in beta-amyloid mouse models of AD, and suggest the parallel impact that LC dysfunction has on both major aspects of AD pathology. This further highlight the integral role that the LC and its dysfunction may have in the pathogenesis of AD, and merits more intensive examination of this nuclei as a potential therapeutic target, especially for early interventions.

FIGURES





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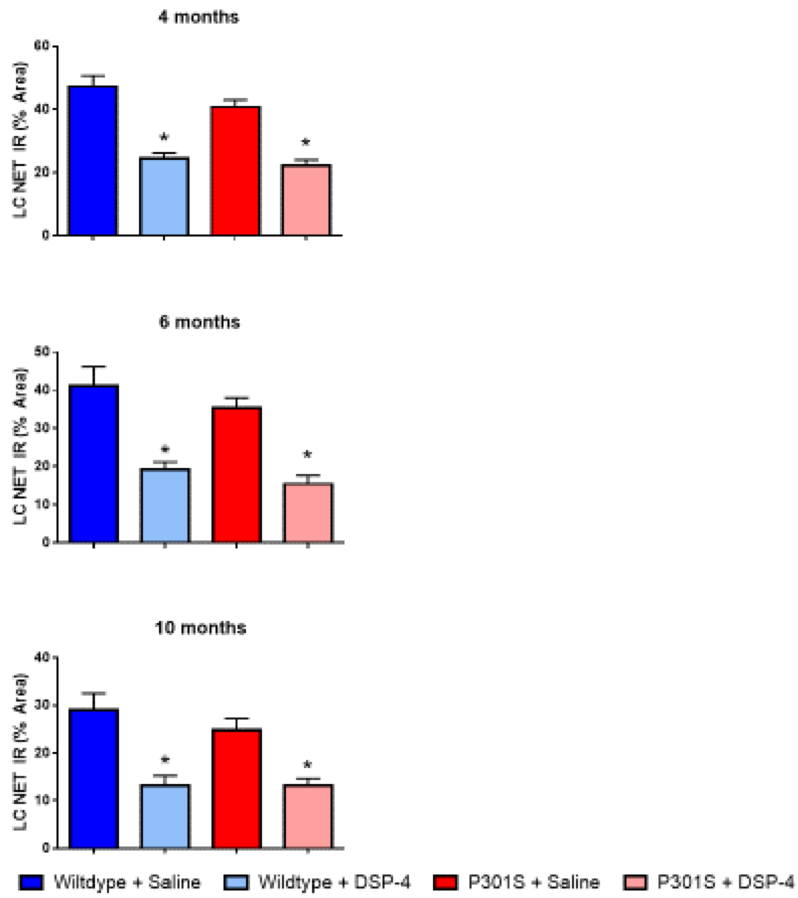

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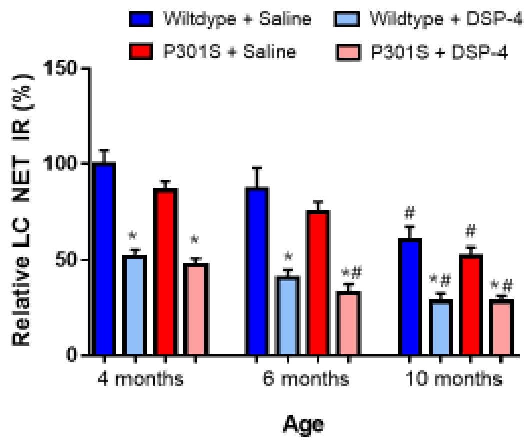
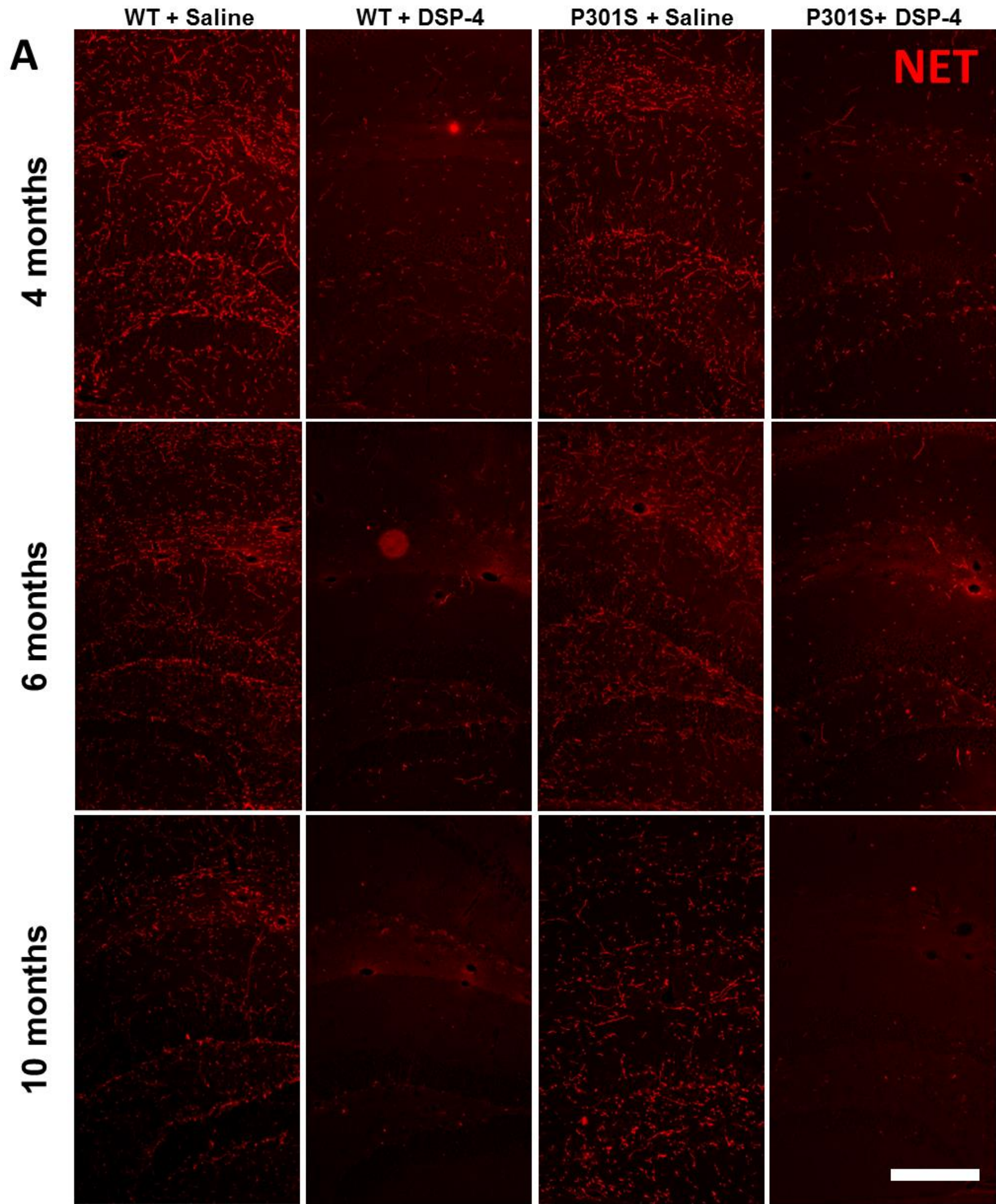


Figure 3.1 *DSP-4 lesions reduce NET immunoreactivity in the LC of both WT and P301S mice* **(a)** Experimental design for administration of lesions **(b)** Representative immunofluorescence images and quantification of the NET immunoreactivity in the LC **(c)** as % of total LC area and **(d)** as percent of baseline (4 month unlesioned wildtypes) showing significant reduction with DSP-4 treatment in both wildtype and P301S transgenics that increases with age.


A
WT + Saline
WT + DSP-4
P301S + Saline
P301S+ DSP-4
NET
4 months
6 months
10 months

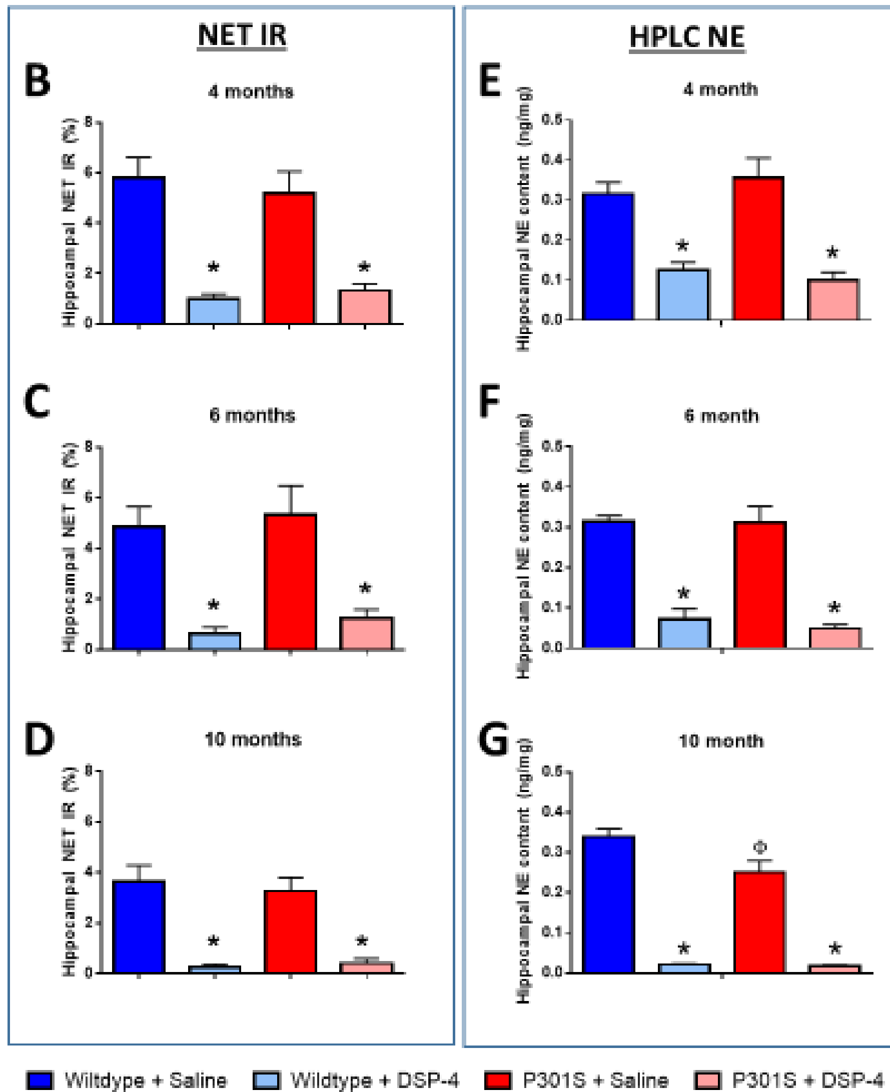
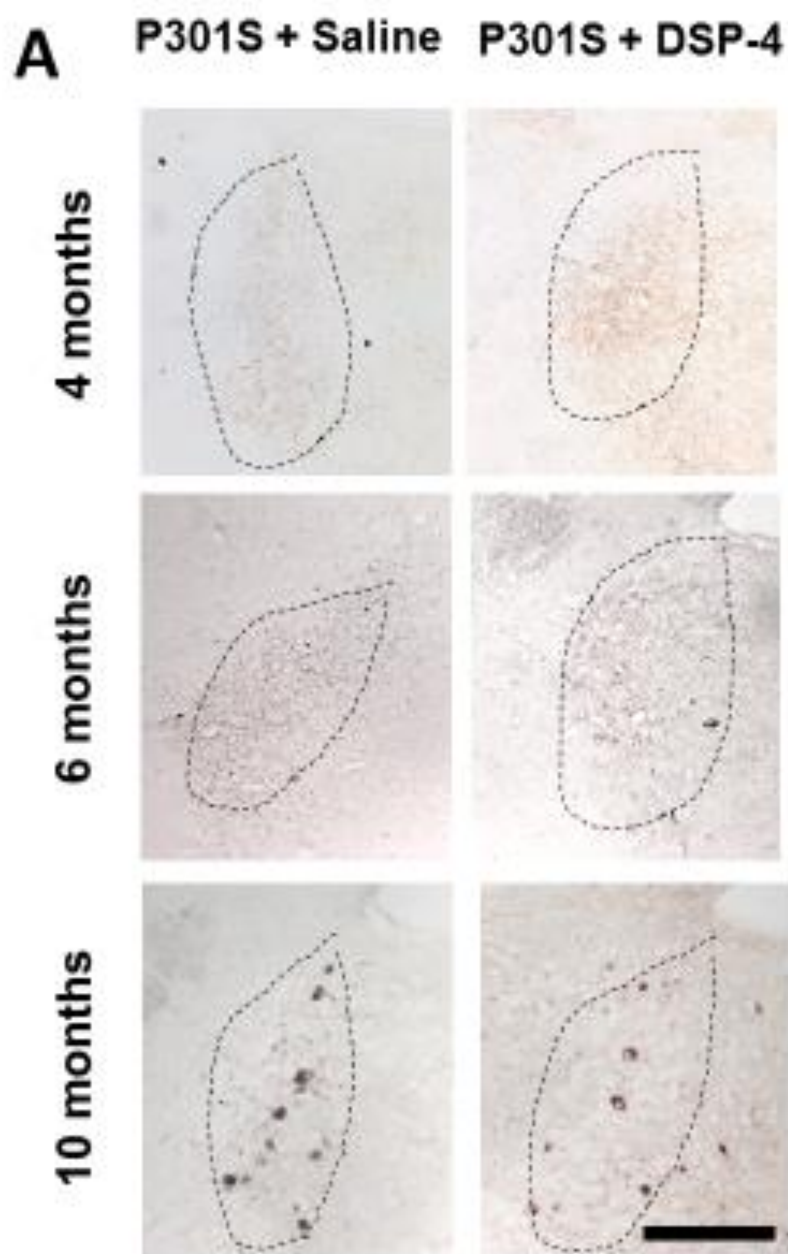


Figure 3.2 DSP-4 lesions *reduce NET immunoreactivity and tissue NE content in the hippocampus* **(a)** Representative immunofluorescence images and **(b)** quantification of the NET immunoreactivity in the hippocampus show significant reduction with DSP-4 treatment in both WT and P301S transgenics. **(c)** HPLC of NE concentrations in the hippocampus. DSP-4 lesions reduce NE in the hippocampus in both AT and transgenic mice at all ages (* donates $p<0.05$, comparing DSP-4 to saline groups). At 10 months, there is also a significant reduction in NE concentration in saline treated P301S mice compared to saline treated WT mice (Φ denotes $p<0.05$). Data shown as mean ± SEM, n = 8 – 12 per group, scale bar = 100 µm.


A
P301S + Saline
P301S + DSP-4
4 months
6 months
10 months

B

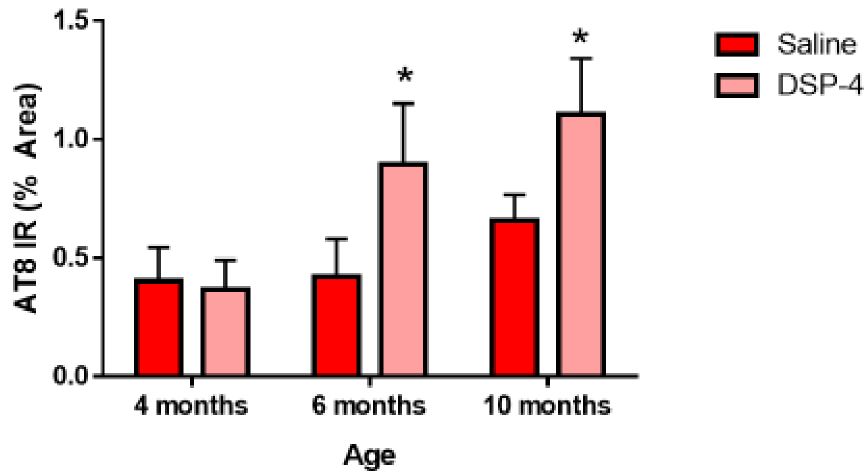


Figure 3.3 *DSP-4 lesions exacerbate pre-tangle tau pathology in the LC of P301S mice* **(a)** Representative images and **(b)** quantification of AT8 immunoreactivity in the LC show light deposition of hyperphosphorylated tau beginning at 4 months as mainly neuritic staining that progresses to fill cell bodies with age. (* indicates $p<0.05$, compared to 4-month group) Data shown as mean ± SEM, n = 5 – 12 per group, scale bar = 100 µm, LC outlined in dashed lines.

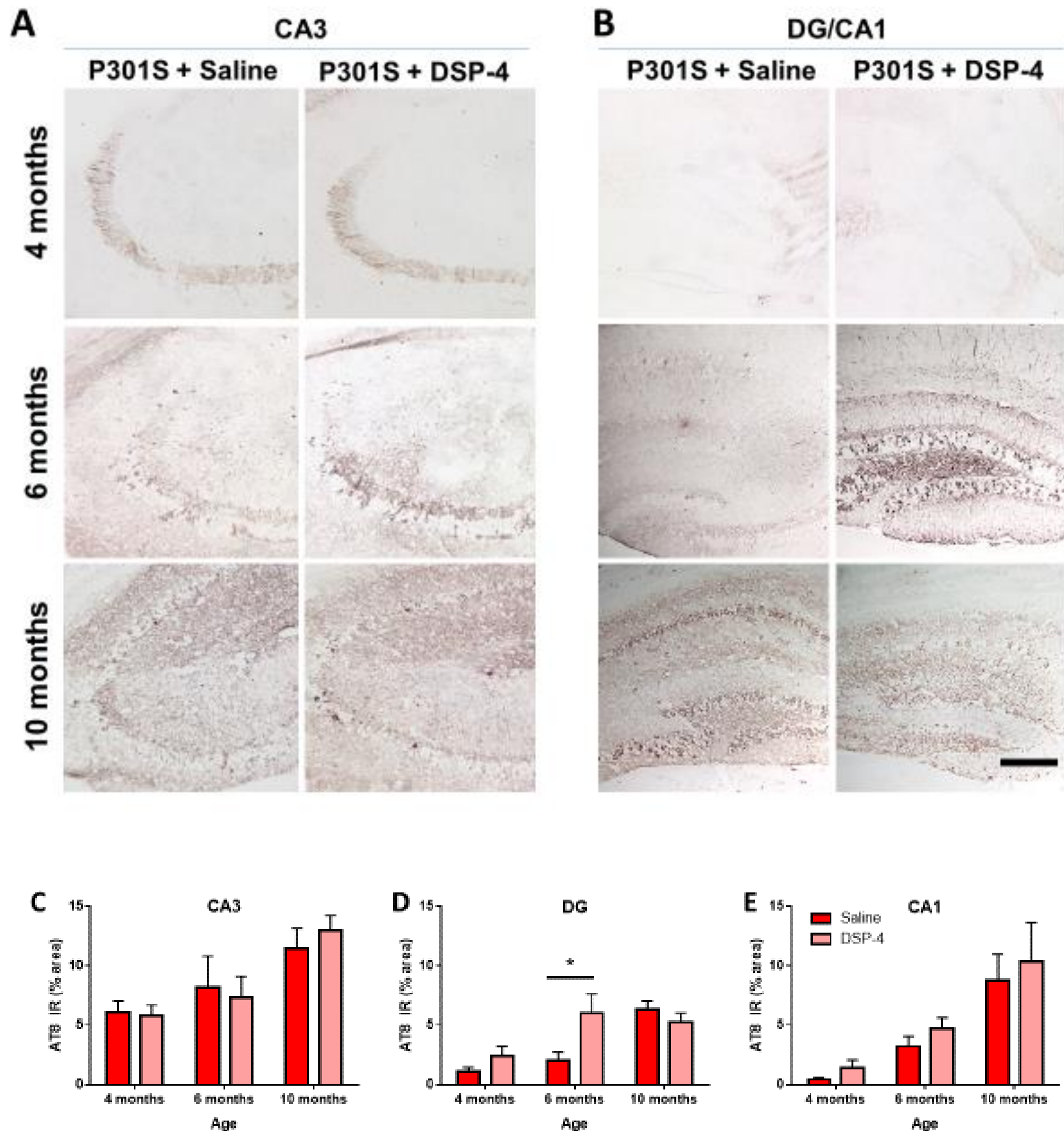


Figure 3.4 *DSP-4 lesions exacerbate pre-tangle tau pathology in hippocampus of P301S mice* **(a&b)** Representative images and **(c-e)** quantification of AT8 immunoreactivity in the hippocampus show significant exacerbation of hyperphosphorylated tau immunoreactivity in the CA1 region with DSP-4 treatment in P301S transgenics at 6 months. (* indicates $p<0.05$) Data shown as mean ± SEM, n = 8 – 12 per group, scale bar = 100 μm.

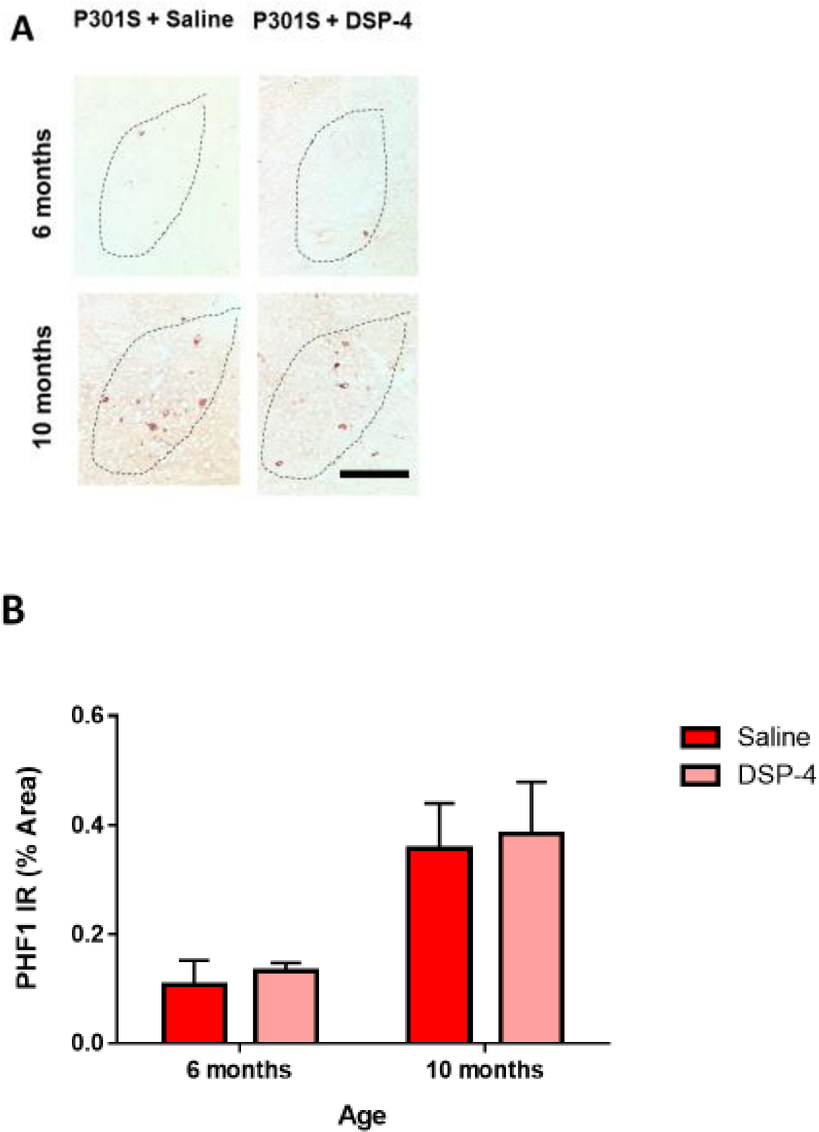


Figure 3.5 *DSP-4 lesions have no effect on paired helical tau pathology in the LC* **(a)** Representative images and **(b)** quantification of PHF1 immunoreactivity in the LC show mainly neuronal tau pathology that increases with age. Data shown as mean ± SEM, n = 8 – 12 per group, scale bar = 100 μm, LC outlined in dashed lines.

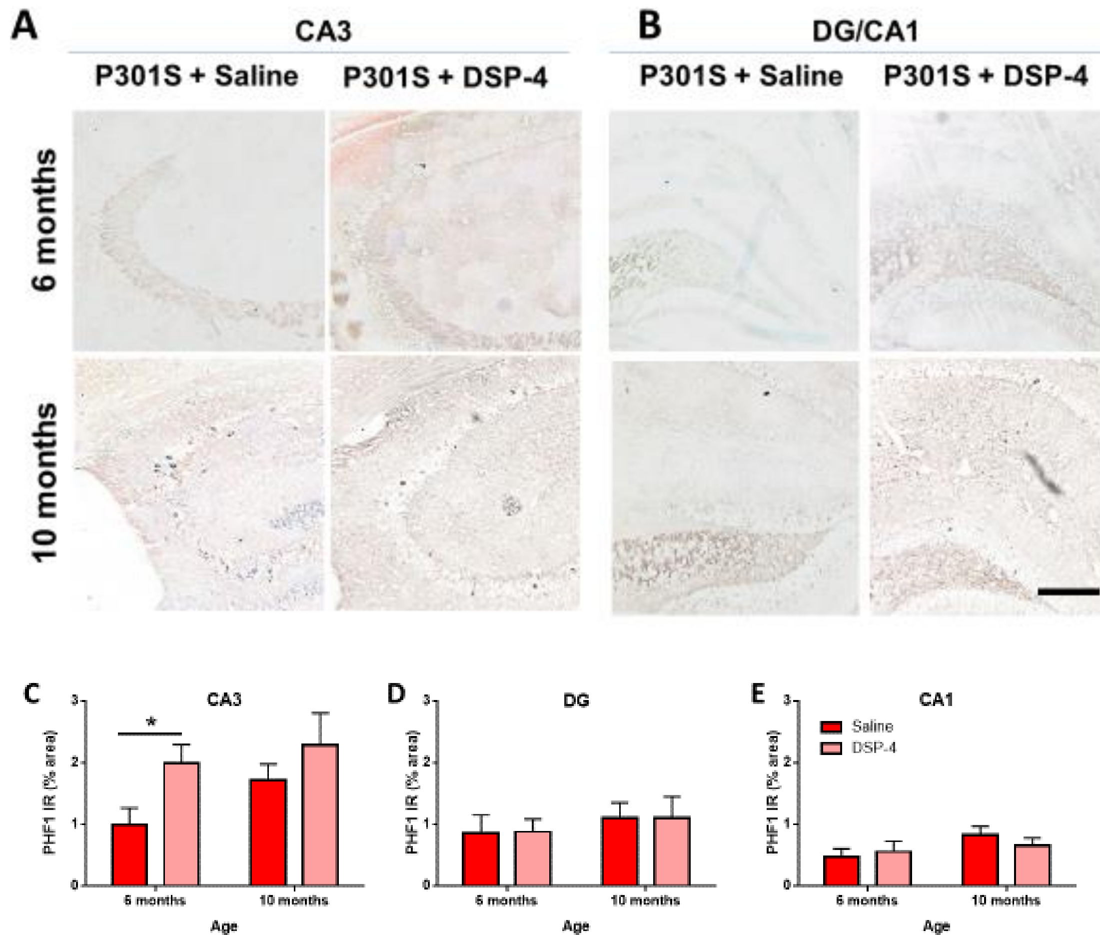
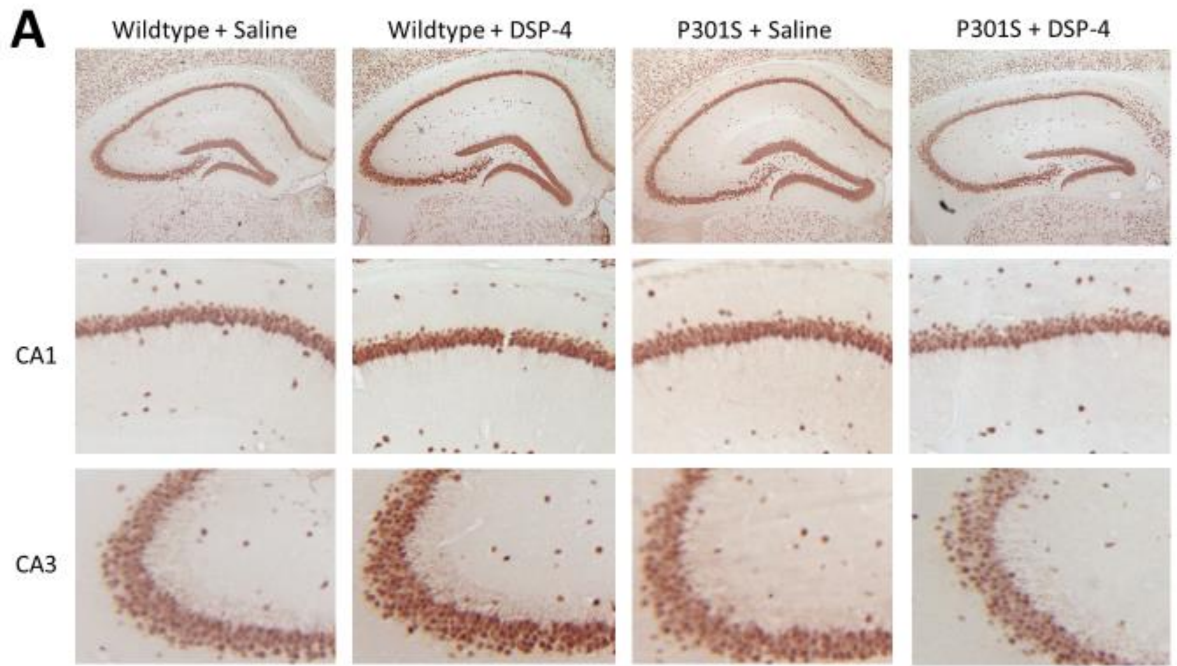
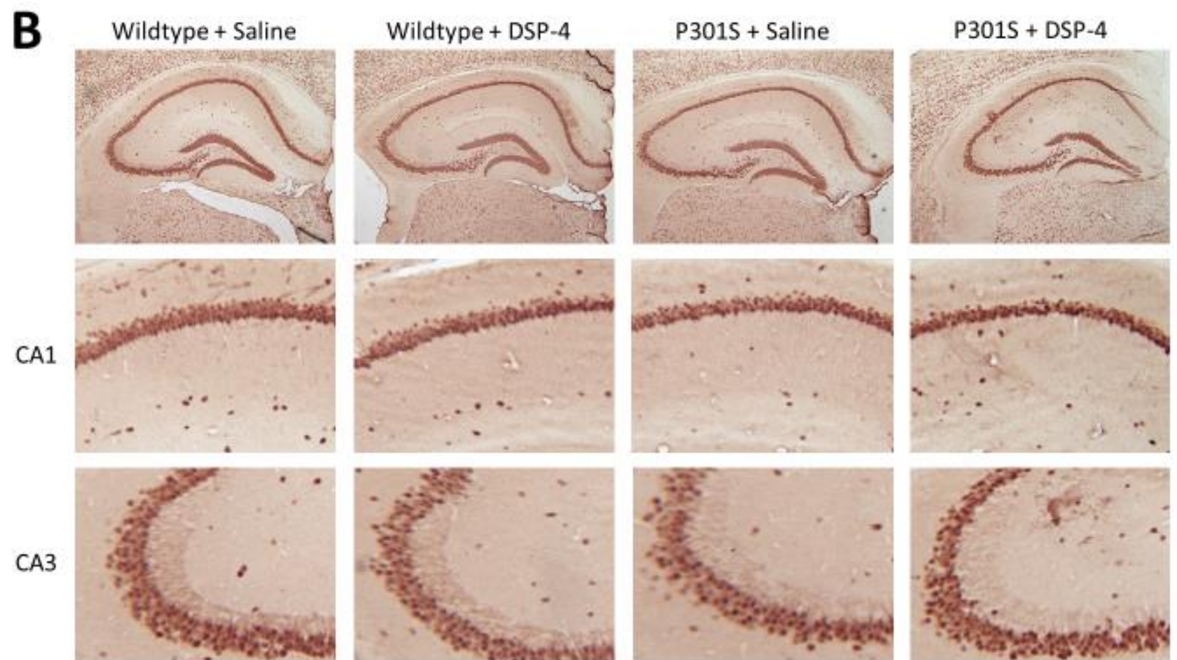
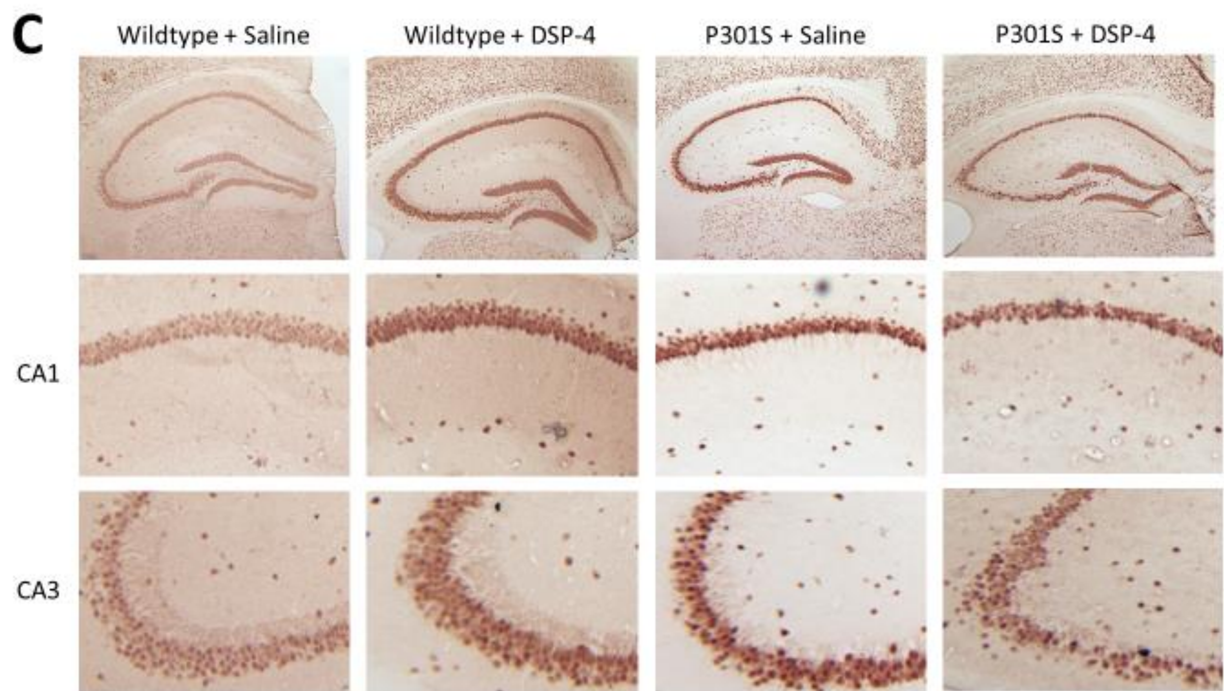


Figure 3.6 *DSP-4 lesions exacerbate paired helical tau pathology in the CA3 region of the hippocampus at 6 months in P301S mice* **(a&b)** Representative images and **(c-e)** quantification of PHF1 immunoreactivity in the hippocampus show significant exacerbation of hyperphosphorylated tau immunoreactivity in the CA3 region with DSP-4 treatment in P301S transgenics at 6 months. (* indicates $p<0.05$) Data shown as mean ± SEM, n = 8 – 12 per group, scale bar = 100 µm.


A
Wildtype + Saline
Wildtype + DSP-4
P301S + Saline
P301S + DSP-4
CA1
CA3


B
Wildtype + Saline
Wildtype + DSP-4
P301S + Saline
P301S + DSP-4
CA1
CA3


C
Wildtype + Saline
Wildtype + DSP-4
P301S + Saline
P301S + DSP-4
CA1
CA3

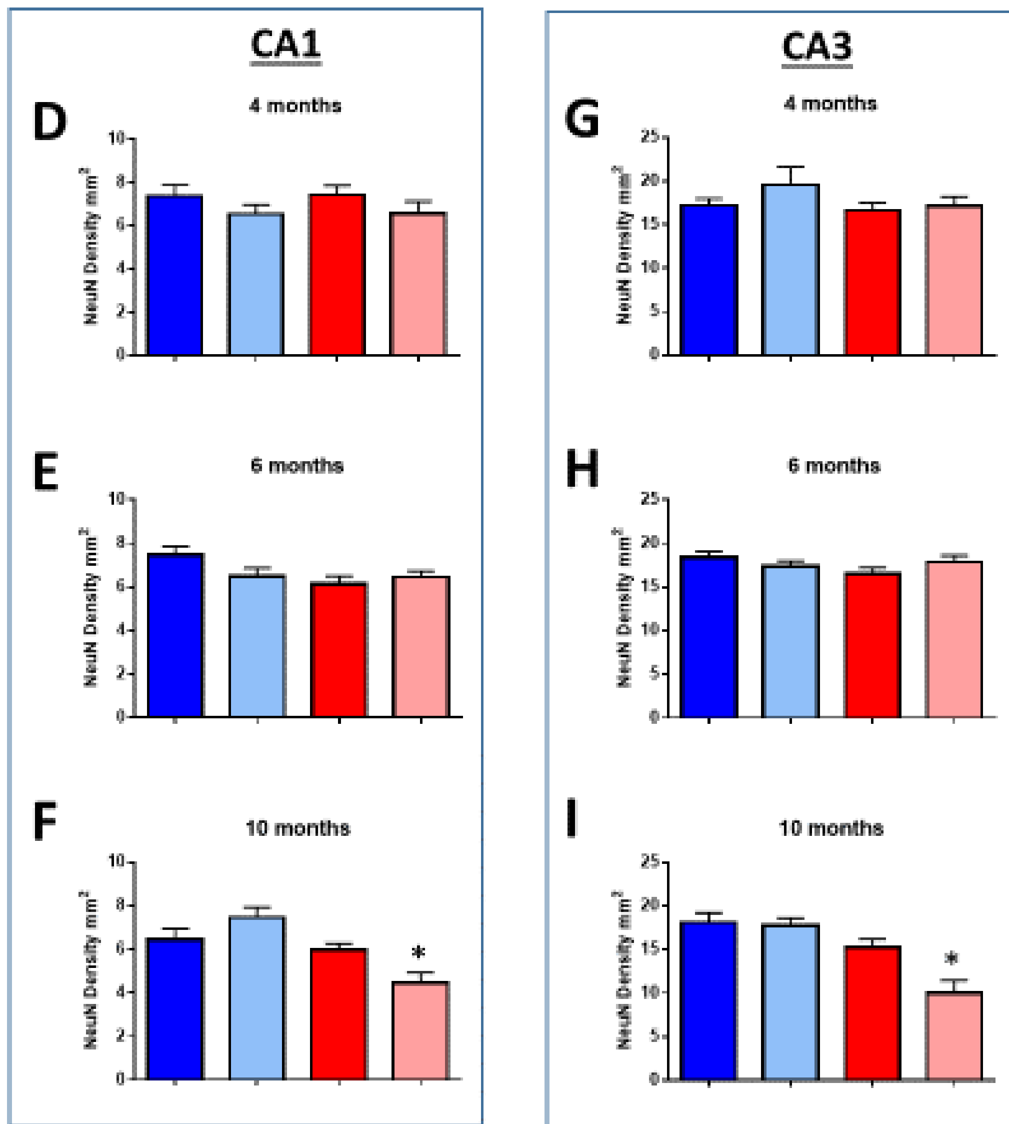
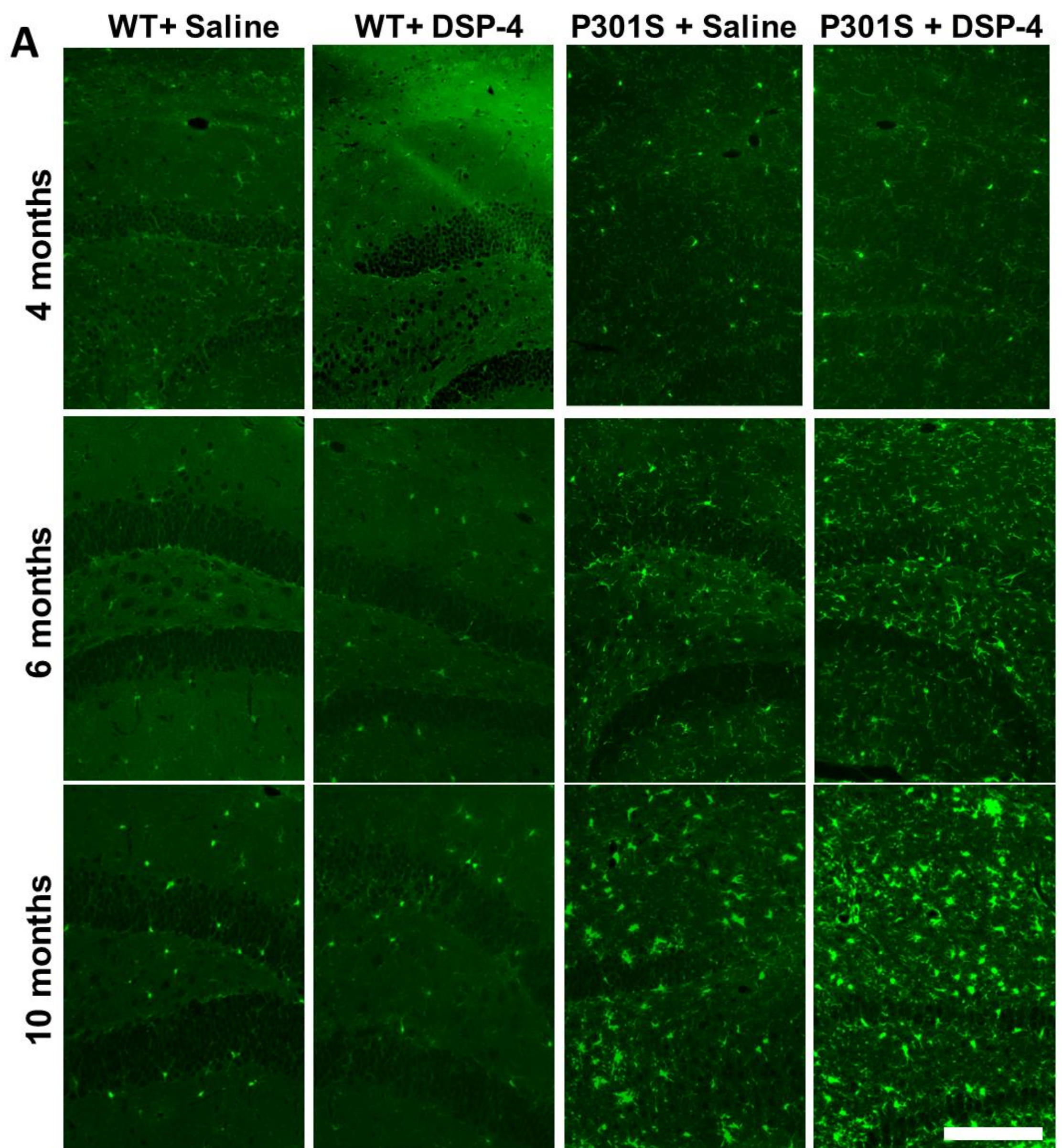


Figure 3.7 *DSP-4 lesions promote neurodegeneration in aged P301S mice***(a-c)** Representative images and quantification of NeuN immunoreactivity in the **(d-f)** CA1 and **(g-i)** CA3 regions of the hippocampus show significant neuronal cell loss in DSP-4 treated P301S transgenics at 10 months. (* denotes $p<0.05$, compared to saline treated P301S). Data shown as mean ± SEM, n = 8 – 12 per group.


A
WT+ Saline
WT+ DSP-4
P301S + Saline
P301S + DSP-4
4 months
6 months
10 months

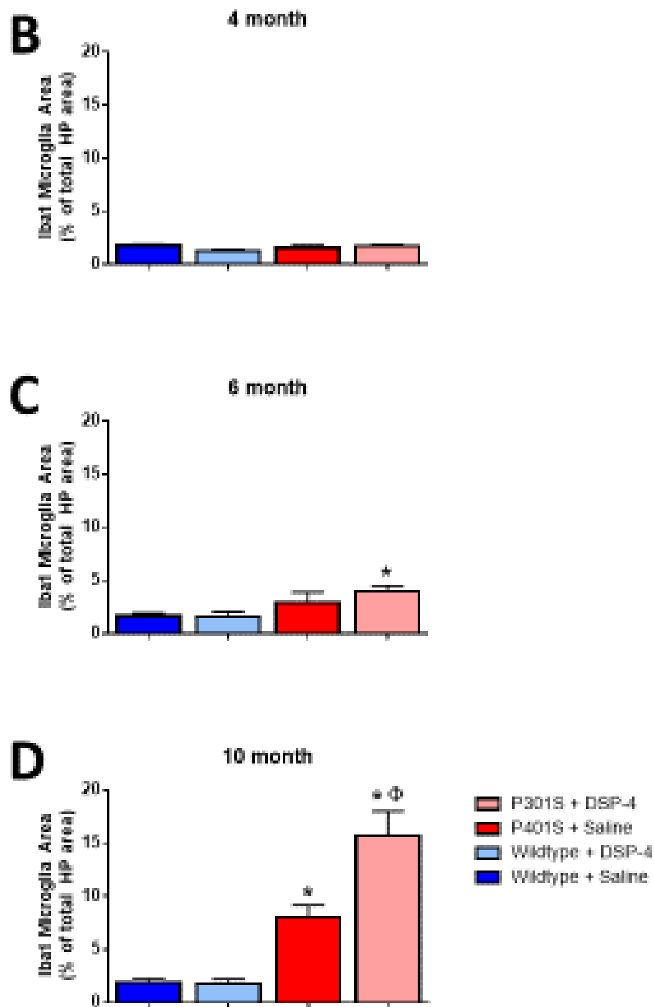
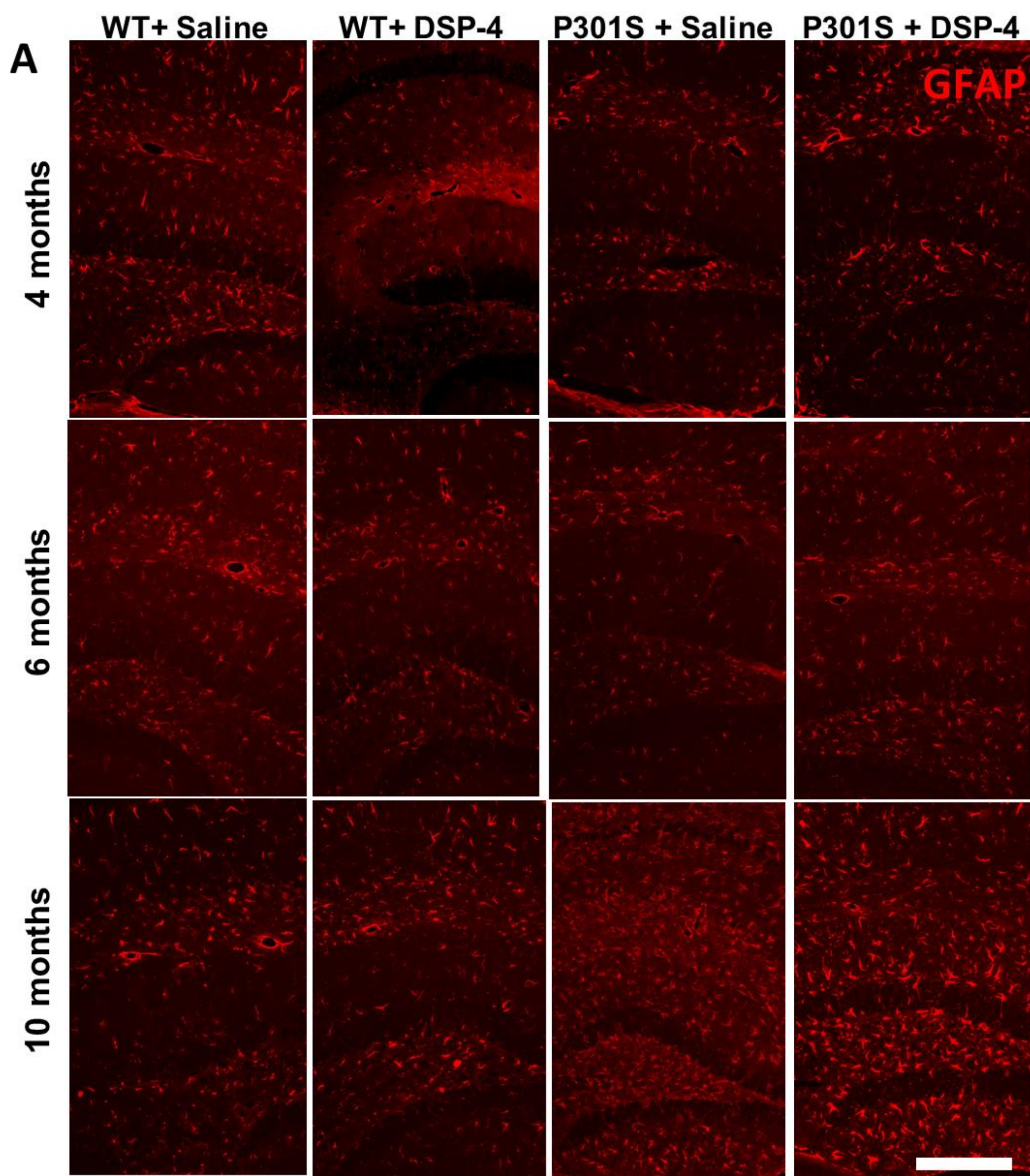


Figure 3.8 *DSP-4 lesions increase activated microglia in P301S mice* **(a)** Representative immunofluorescence images and **(b-d)** quantification of Iba1 immunoreactivity in the hippocampus (* donates p<0.05, comparing transgenics with DSP-4 or saline to wildtypes with saline**,** Φ denotes p<0.05, comparing DSP-4 to saline). Data shown as mean ± SEM, n = 8 – 12 per group, scale bar = 100 µm.


WT+ Saline
WT+ DSP-4
P301S + Saline
P301S + DSP-4
A
GFAP
4 months
6 months
10 months

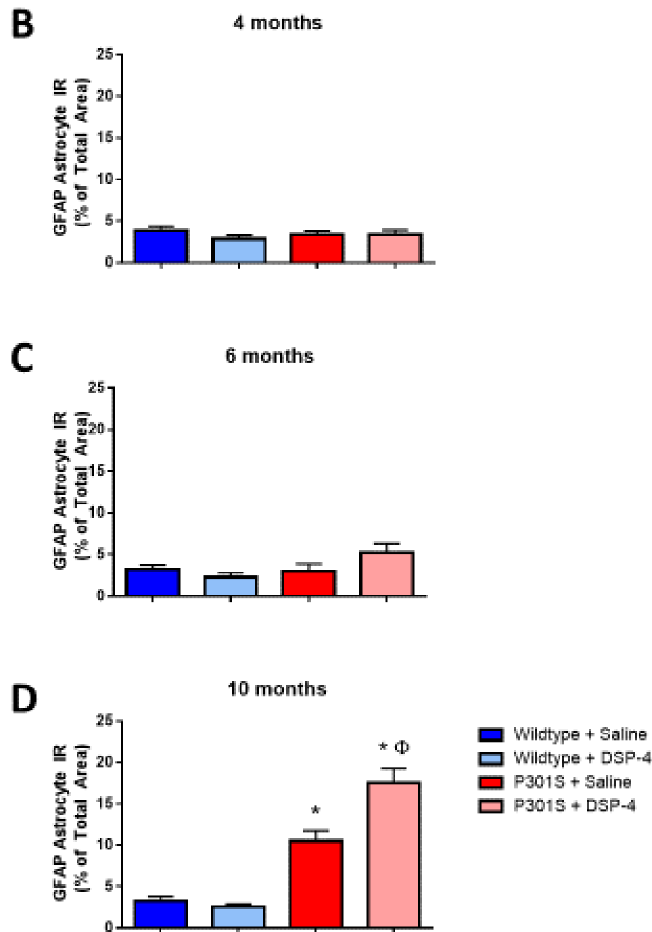


Figure 3.9 *DSP-4 lesions increase activated astrocytes in aged P301S mice***(a)** Representative immunofluorescence images and **(b-d)** quantification of GFAP immunoreactivity in the hippocampus (* donates p<0.05, comparing transgenics with DSP-4 or saline to wildtypes with saline**,** Φ denotes p<0.05, comparing DSP-4 to saline). Data shown as mean ± SEM, n = 8 – 12 per group, scale bar = 100 µm.

LC

		4 months	6 months	10 months
AT8	Saline	*	*	**
	DSP-4	*	**	***
PHF1	Saline		*	**
	DSP-4		*	**

HP

AT8		4 months	6 months	10 months
CA1	Saline	*	**	***
	DSP-4	*	**	***
DG	Saline	*	*	**
	DSP-4	*	**	**
CA3	Saline	*	**	***
	DSP-4	*	**	***
PHF1				
CA1	Saline		*	*
	DSP-4		*	*
DG	Saline		*	*
	DSP-4		*	*
CA3	Saline		*	**
	DSP-4		**	**
NeuN				
CA1	Saline	-	-	-
	DSP-4	-	-	*
CA3	Saline	-	-	-
	DSP-4	-	-	*
Iba1	Saline	-	-	**
	DSP-4	-	*	***
GFAP	Saline	-	-	**
	DSP-4	-	-	***

Figure 3.10 *Summary of neuropathological changes in lesioned and unlesioned P301S mice*

General summary of histological quantification for tau pathology (AT8 and PHF1), neurodegeneration (NeuN), and neuroinflammation (Iba1 and GFAP) at the level of the locus coeruleus (LC) and the CA1 and CA3 regions of the hippocampus (HP) at each time point. * indicate intensity of neuropathology or neurodegeneration in the case of NeuN staining. Empty cells indicate no data acquired for that time point. Bolded boxes highlight where significant differences are present for a stain at a timepoint.

CHAPTER 4: BEHAVIORAL CONSEQUENCES OF LOCUS COERULEUS DEGENERATION IN A MOUSE MODEL OF HYPERPHOSPHORYLATED TAU

4.1 ABSTRACT

Alzheimer's disease is the most common cause of dementia and is defined by two canonical neuropathologies: beta-amyloid plaques and tau neurofibrillary tangles (NFTs). The locus coeruleus (LC) is the main source of norepinephrine (NE) in the forebrain and is implicated in many different cognitive and behavioral functions. Degeneration of the LC is a hallmark of Alzheimer's disease (AD) that correlates well with the plaques, tangles and cognitive impairment. The resulting dysfunction of the noradrenergic system likely contributes to many of the phenotypes of the disease including memory loss and affective abnormalities. Experimental lesions of the LC in transgenic mice overexpressing mutant amyloid precursor protein exacerbate both pathology and cognitive deficits, indicating a potent interaction between LC degeneration and beta-amyloid-mediated disease. Whether a similar interaction exists between LC loss and tau pathology has not been investigated. Here we assessed the behavioral consequences of LC degeneration by lesioning the LC with the noradrenergic neurotoxin, DSP-4, in P301S tau transgenic mice, which produces a form of the protein that is prone to hyper-phosphorylation and aggregation. We have previously shown that lesions of the LC in this model aggravates tau pathology, neuroinflammation and neurodegeneration in the hippocampus, a key learning and memory center in the brain that is compromised in AD. In parallel with the increased hippocampal tau pathology in DSP-4 treated P301S mice at 6 months of age, we found that LC lesions exacerbated hippocampal-dependent cognitive impairments. At 10 months of age, a time when LC degeneration intensified neuroinflammation and neurodegeneration, we observed accelerated mortality in P301S mice. These results suggest that the intact LC protects against tau-mediated cognitive impairment and death, and highlights the deleterious consequences of LC degeneration in AD.

4.2 INTRODUCTION

The locus coeruleus (LC) is the brainstem nucleus responsible for supplying most of the brain and the spinal cord with norepinephrine (NE). The LC undergoes significant dysfunction and degeneration in the brains of patients with Alzheimer's disease (AD). Prior to frank cell body degeneration, the noradrenergic system exhibits shortened dendrites, reduced markers of noradrenergic synthesis and impaired transmission in projection areas like the hippocampus and frontal cortex (Cross, Crow et al. 1981, Bondareff, Mountjoy et al. 1987, Tejani-Butt, Yang et al. 1993, Haglund, Sjobeck et al. 2006, Grudzien, Shaw et al. 2007, Gannon, Che et al. 2015, Theofilas, Ehrenberg et al. 2016). Because the LC and its extensive forebrain projections have integral roles in many behaviors and cognitive functions (e.g. arousal, attention, affective behaviors, learning and memory), damage to this system likely contributes to many of the symptoms of AD, especially some of the earliest changes (Mann 1983, Zweig, Ross et al. 1989, Jalbert, Daiello et al. 2008, Weinshenker 2008, Gannon, Che et al. 2015).

Although AD is most well-known for its memory impairments, there are also many non-cognitive symptoms including sleep disturbances and increased anxiety, irritability, depression or apathy), that occur early and prior to cognitive impairments, which are thought to be regulated in part by early dysfunction of the LC and NE system (Herrmann, Lanctot et al. 2004, Lanari, Amenta et al. 2006). As the disease worsens, changes in cognitive function begin to appear, including memory loss of recent events, difficulties with problem solving and navigation, and confusion, before severe dementia eventually begins to set in. These changes characterizing the later stages of the disease pair well with the timeline of pathological progression for when the LC begins to degenerate (Theofilas, Ehrenberg et al. 2016). It is likely that the LC contributes to modulating the cognitive functions and supporting overall neuronal health and survival, thus, its dysfunction and degeneration may be directly contributing to the dementia and disability seen as patients succumb to the disease in later stages.

AD is characterized by two primary neuropathologies: beta-amyloid plaques and tau neurofibrillary tangles. There is considerable evidence that LC degeneration exacerbates cognitive impairments in transgenic mice expressing mutant human amyloid precursor protein (APP) and/or presenilin-1 (PS1) that increase beta-amyloid production and deposition. Experimental lesions of the LC in APP23, F717F APP, and APP/PS1 mice with the noradrenergic neurotoxin, *N*-(2-chloroethyl)-*N*-ethyl-bromo-benzylamine (DSP-4), leads to a robust exacerbation of neuropathology as well as cognitive and behavioral deficits in the transgenic mice, suggesting that loss of LC neurons synergizes with beta-amyloid pathology to worsen the disease (Heneka, Ramanathan et al. 2006, Kalinin, Gavrilyuk et al. 2007, Heneka, Nadrigny et al. 2010, Rey, Jardanhazi-Kurutz et al. 2012). However, the impact of LC degeneration on tau-mediated deficits has not been investigated.

We have shown that LC lesions exacerbate tau pathology, neuroinflammation and neurodegeneration in the P301S transgenic mice, which express a mutant form of human tau that is prone to hyperphosphorylation and aggregation. LC lesioned P301S mice show accelerated tau pathology in the hippocampus by 6 months of age and increased neurodegeneration, neuroinflammation, and mortality by 10 months. The goal of this study was to determine whether the effects of LC degeneration on neuropathology are physiologically relevant and impact behavior, cognition and physical phenotypes. We find that loss of LC neurons exacerbates hippocampal-dependent learning and memory deficits and reduces survival at ages that correspond with the changes in neuropathology.

4.3 MATERIALS AND METHODS

Animals

Male and female P301S transgenic mice, which express the 4N1R isoform of human microtubule-associated protein tau (MAPT) with the P301S mutation under the mouse prion promoter (Prnp), were used (B6N.Cg-Tg(Prnp-MAPT*P301S)PS19Vle/J, Jackson Laboratory, #024841, for more

detail see *Materials and Methods* from Chapter 2 and 3). These mice manifest cognitive and behavioral impairments concomitantly with the establishment of their neuropathology (Yoshiyama, Higuchi et al. 2007, Takeuchi, Iba et al. 2011). Non-transgenic wild-type littermates (WT) were used as controls.

Lesion of the noradrenergic system

There were four groups of animals within each age group: P301S transgenic mice (P301S) and non-transgenic littermate controls (WT) with either DSP-4 or saline treatment. DSP-4 was dissolved in 0.9% bacteriostatic saline and administered at a dose of 50 mg/kg, i.p. Mice were injected on day 1 and day 7, after which animals received 1 administration per month, with the final treatment given one month prior to behavioral testing at 4, 6, and 10 months.

Circadian Rhythm/Locomotor Activity

Mice were placed in locomotion recording chambers (transparent Plexiglas cages placed into a rack with 7 infrared photobeams spaced 5 cm apart; San Diego Instruments Inc., La Jolla, CA), with bedding, food and water at 9:30AM, and ambulations (consecutive beam breaks) were recorded for 24 hours in 10 min bins.

Open Field

The circular (1 meter in diameter) open field arena was white with grey walls (35 cm high), and was brightly illuminated. The arena was divided into two zones: an inner circle (60 cm in diameter) and the surrounding outside (width 20 cm). Each animal was placed in the center at the start of the trial and given 10 min to freely explore the arena. An automated tracking system (TopScan, CleverSys Inc, Reston, Virginia) recorded the distance traveled, velocity and duration of time spent in the inside and outside zones. The arena was thoroughly cleaned in between trials

with Quatracide (Pharmacal Research Laboratories, Inc., 65020F). The percent of time spent in the inner circle versus the outer circle as well as the latency to escape to the outer ring were used as a measurement of anxiety.

Morris Water Maze

The Morris Water Maze was conducted in a circular tank (52 inch diameter) filled with opaque water at 23°C. In the Northwestern quadrant of the tank, a hidden circular platform (30 cm diameter) was present 1 cm below the water surface. The tank was brightly lit and flanked by white walls on the north and east sides and white curtains on the west and south sides of the tank, all with external cues for spatial references. Over five days, mice were trained to find the platform when released into the tank from randomized start points over 4 trials each day (South, North, East, West). Each trial lasted a maximum of 60 sec; if a mouse did not successfully find the platform in time, they were manually guided to it and allowed to sit on the platform for 10 sec. An automated tracking system was used to record the latency and distance to the platform as well as speed. On the sixth day, a probe trial was conducted where the platform was removed and mice were released from the South start point and allowed to swim for 60 sec. The automated tracking system recorded the duration and distance the mice spent in each quadrant of the maze as well as the number of times they crossed over the previous location of the platform.

Fear Conditioning

Fear conditioning training and contextual and cued fear testing were conducted over three consecutive days. The chamber (7 inches width x 7 inches length x 12 inches height, H10-11M-TC, Coulbourn Instruments, Holliston, Massachusetts, USA) was equipped with a house light, an electric grid shock floor (H10-11M-TC, Coulbourn Instruments) that could be removed and replaced with a non-shock wire mesh floor (H10-11-M-TC, Coulbourn Instruments), a ceiling mounted camera providing a top-down view of the chamber and a speaker. Chambers were

cleaned in between animals with Quatracide. The acquisition trial on Day 1 lasted 7 min and began with a 3-min acclimatization period, followed by 3 tone-shock pairings whereby the tone was present for 20 sec and was co-terminated with a 3 sec, 0.5mA foot shock. Mouse behavior was recorded for 60 sec following tone-shock presentation before the next round. The contextual fear testing on Day 2 was performed in the same chamber as Day 1 and lasted min without any presentation of tone or shock. The cued fear testing on Day 3 was conducted in a different chamber than Day 1 & 2, with a square mesh floor instead of the previous shock grid floor. The cued fear testing trial lasted 9 min, with the tone starting after 3 min and continuing until the end of the trial. Trials were programmed and run using the FreezeFrame software (Coulbourn Instruments) to automatically record the animals' freezing behavior during each trial.

Social Recognition

The social recognition arena consisted of a white Plexiglas rectangular chamber divided into 3 chambers (left, middle, right) with removable sliding walls (each chamber measured: 20 cm x 45 cm x 45 cm). An upside down, metal, wire-mesh pencil holder was used to enclose the stimulus mice, preventing direct contact but allowing for visual, audio and olfactory cues. Stimulus animals were 8-12 week old, gender-matched C57BL/6 mice. At the beginning of the test, the subject mouse was sequestered to the middle chamber of the arena (with sliding walls down) for 5 min to habituate to the chamber. In Trial 1, an empty cup was placed in one of the outer chambers and a cup with a stimulus mouse was placed in the other outer chamber. At the start of the trial, the sliding walls were removed, giving the mouse access to all three chambers for 10 min. The TopScan (CleverSys) automated tracking system was again used to record the duration of time the mouse spent in each of the 3 chambers as well as the duration and number of times the mouse's nose approached within 3 cm of the cups. Following Trial 1, the subject mouse and stimulus mice were returned to their respective home cages for 30 min, and both cups as well as the floors and walls of the arena were thoroughly cleaned with 70% ethanol. In Trial 2, the same

stimulus mouse was placed under a cup in the same chamber from Trial 1, while a novel stimulus mouse was placed under the previously empty cup in the other chamber. The subject mice were returned to the arena and allowed to explore for another 10 min. Identical tracking parameters were used in Trial 2 as with Trial 1. "Sociability" was calculated by dividing the time spent exploring the cup with the mouse by the total time spent investigating both cups in Trial 1. Social memory was calculated by dividing the time spent exploring the cup with the novel mouse by the total time spent investigating both cups (familiar mouse + novel mouse) in Trial 2.

Survival & Physical Phenotypes

Mouse deaths were recorded and plotted in a Kaplan-Meier survival graph. Neurodegenerative physical phenotypes were observed and scored according to previously established protocol (Guyenet, Furrer et al. 2010). Kyphosis is a dorsal curvature of the spine caused by loss of muscle tone in spinal muscles and is a sign of neurodegeneration. To perform the kyphosis test, mice were removed from the cage, observed while walking on a flat surface and scored per the rating scale below (Table 1). Hindlimb clasping is another sign of neurodegeneration. To perform the hindlimb test, mice were firmly grasped by the base of the tail and lifted for 10 sec. Scoring was performed per the rating scale below (Table 1).

Table 1. Scoring system for physical phenotypes

Score	Kyphosis	Hindlimb Clasping
0	Easily straightens spine as it walks without persistent kyphosis	Hindlimbs consistently splayed outwards and away from the abdomen for entire 10 seconds
1	Mild kyphosis while stationary, but able to straighten spine as it walks	**One** hindlimb is retracted towards abdomen for **less** than 50% of the time

2	Mild kyphosis while stationary and unable to straighten spine as it walks	**Both** hindlimbs are retracted towards abdomen for **less** than 50% of the time
3	Severe and pronounce kyphosis while stationary and while walking	**Both** hindlimbs are retracted towards abdomen for **more** than 50% of the time

Statistical Analysis

Data was analyzed via 2-way ANOVA with varying factors depending on the test. Tukey's or Sidak's post-hoc tests were used to for multiple comparisons. Significance was set at $p<0.05$, with 2 tailed variants of tests implemented. Multiplicity adjusted p-value was reported for each comparison. Data are presented as means ± standard error of mean (SEM). Calculations were performed and figures created using GraphPad Prism version 6 for Windows (GraphPad Software, Inc., San Diego, CA, USA).

4.4 RESULTS

DSP-4 does not alter circadian activity, but reduces novelty-induced locomotion in P301S mice

We assessed circadian locomotor activity across 24 h for two reasons. First, AD patients can suffer from sleep disturbances (Bliwise 2004), and circadian activity can be used as a proxy for sleep-wake cycles in mice (Richardson, Moore-Ede et al. 1985, Eckel-Mahan and Sassone-Corsi 2015, Musiek, Xiong et al. 2015, Ray and Reddy 2016). Second, we wanted to account for any motor impairment that could impact performance in other tests and confound assessment of cognition.

Initially, high levels of ambulations were observed in all groups as they explored the novel test environment, which diminished over the next few hours as they habituated and assumed typical low light cycle activity (Fig. 4.1a-c). There was a predictable increase in ambulations after the initiation of the dark cycle at 7:00 pm as the mice became active again, which extended a few hours into the beginning of the next light cycle at 7:00 am before

diminishing again. No significant differences in circadian activity were seen across the groups at any age, although there was a trend towards hypoactivity during the first few hours in the chamber at all ages and dark cycle hyperactivity at 10 months in both lesioned and unlesioned P301S mice compared to their WT counterparts.

Because NE depletion is associated with decreased exploratory activity in a novel environment, we also specifically analyzed the first 10 min immediately after mice were placed into the chambers (Figure 4.1d-f). A two-way ANOVA (genotype x treatment) revealed a main effect of genotype ($F(1, 33) = 4.686$, $p=0.0377$), treatment ($F(1, 33) = 12.18$, $p=0.0014$) and a genotype x treatment interaction ($F(1, 33) = 6.398$) at 10 months. Tukey's post hoc tests showed DSP-4 treated P301 mice had significantly less novelty-induced locomotor activity compared to all other groups (vs WT + saline: -235.7 ambulations ± 61.17, $p=0.0027$; vs WT + DSP-4: +195.7 ambulations ± 59.03, $p=0.0114$; vs P301S + saline: +250.9 ± 64.24, $p=0.0024$). There were no significant effects or differences 4 or 6 months.

Neither P301S nor DSP-4 alter anxiety-like behaviors in the open field task

Another phenotype consistent with NE dysfunction seen in Alzheimer's patients is anxiety. We tested mice in an open field paradigm to probe for anxiety-like behaviors. There were no significant genotype or treatment differences at any age for either the percent time spent in the center vs the outer ring (Figure 4.2a-c) or the latency to escape from the center to the outer ring (Figure 4.2d-e).

DSP-4 exacerbates spatial learning and memory deficits in P301S mice

A salient feature of AD patients is a tendency to get lost and trouble remembering even familiar places (Lithfous, Dufour et al. 2013, Allison, Fagan et al. 2016), largely due to the accumulation of pathology and neurodegeneration in the hippocampus (Leung, Barnes et al. 2010, Nedelska, Andel et al. 2012). To probe for spatial learning and memory deficits, we tested mice in the

Morris Water Maze, a hippocampal-dependent task. Mice were trained with 4 trials daily over 5 consecutive days using surrounding spatial cues to learn the location of a hidden platform in a tank of water, and latency to find the platform and swim speed were recorded. On the sixth day, mice were returned to the maze without the platform, and the amount of time spent swimming in the previous quadrant location of the platform was recorded.

At 4 months, all groups showed significant learning over the 5 training days with significantly lower latencies to reach the platform compared to Day 1 (Figure 4.3a), but P301S mice with LC lesions learned more slowly. Two-way repeated measures ANOVA (time x group) showed a main effect of time ($F(4, 215) = 29.02$, $p<0.0001$) and group ($F(3, 215) = 7.399$, $p<0.0001$). Dunnett's multiple comparisons of latencies showed unlesioned WT and P301S mice had significantly lower latencies to find the platform starting from Day 2 of training compared to Day 1 (-15.03 sec ± 5.292, p=0.0177; -19.64 sec ± 5.292, p=0.001 respectively) with continued improvement in latencies over the rest of the training period. Lesioned WT mice showed significant learning by Day 3 (-18.23 sec ± 5.528 p=0.0043), also with similar improvements on later days, while DSP-4 treated P301S mice did not show significant learning until the final training day (-15.84 sec ± 5.292, p=0.0112).

At 6 months, there were main effects again of time ($F (4, 144) = 27.61$), $p<0.0001$) and group ($F (3, 36) = 6.772$, $p=0.001$) (Figure 4.3b). Compared to Day 1, WT mice showed significant learning by Day 2 (-14.49 sec ± 4.244, p=0.0031), and DSP-4 treated WT mice and unlesioned P301S mice by Day 3 (-13.53 sec ± 4.433, p=0.0099 and -14.03 sec ± 4.901, p=0.0173, respectively), with similar improvements over the remaining training days. However, P301S mice with LC lesions never learned significantly compared to Day 1.

At 10 months, there was a main effect of training day ($F (4, 120) = 10.46$, $p<0.0001$) (Figure 4.3c). Unlesioned and lesioned WT mice still demonstrated learning (Day 3, -14.72 sec ± 4.964, p=0.0132 and Day 2, -13.54 sec ± 4.299, p=0.0076 respectively). However, neither group

of P301S mice showed significant learning over the training period. There were no significant differences in swim speed across the groups at any age (Figure 4.3d-f).

At 4 months, all groups performed similarly in the probe trial and spent over 25% of their time swimming in the correct quadrant (Figure 4.4a), but at 6 months, the lesioned transgenics were impaired. A two-way ANOVA revealed a main effect of genotype ($F(1, 37) = 10.53$, $p = 0.0025$) and treatment ($F(1, 37) = 4.136$, $p=0.0492$) (Figure 4.4b). Sidak's multiple comparison revealed a significant reduction in time spent in the correct quadrant for DSP-4 treated P301S animals compared to unlesioned WT mice (-20.79% ± 5.665, $p=0.0046$), while all other groups were not significantly different from each other. At 10 months, there was a main effect of genotype ($F(1, 33) = 21.94$, $p<0.0001$), and again P301S mice with LC lesions had significantly reduced time spent in the correct quadrant compared to unlesioned (-24.84% ± 5.665, $p=0.0007$) and lesioned (-24.27% ± 5.467, $p=0.0006$) WT mice (Figure 4.4c). Combined, these results indicate a synergistic impairment of both initial learning and memory recall between the presence of aberrant tau and LC degeneration.

DSP-4 blocks contextual fear memory in P301S mice while leaving cued fear memory intact

We assessed the mice in a fear conditioning paradigm for 2 reasons. First, like the Morris water maze, contextual fear conditioning is hippocampal-dependent but is a measure of associative learning rather than spatial learning. Second, cued fear conditioning relies on associative learning but does not require an intact hippocampus. Thus, we could use these paradigms to assess both the categorical and neuroanatomical specificity of our water maze results. Mice were placed in the fear conditioning chamber and given 3 tone-shock pairings on Day 1. The following day, they were returned to the same chamber and contextual freezing was recorded. The next day, they were returned the chamber with different contextual cues, and freezing in response to the tone was assessed (see Figure 4.5a for protocol outline).

For contextual fear conditioning, two-way ANOVA (group x time) showed a main effect of group ($F(3, 318) = 15.31$, $p<0.0001$) (Figure 4.5d)). Tukey's multiple comparisons revealed that LC lesions (both WT and P301S) significantly lowered contextual freezing compared to their unlesioned counterparts ($p=0.0268$; $p=0.0003$ respectively). At 6 months, there was again a main effect of group ($F(3, 255) = 7.579$, $p<0.0001$) (Figure 4.5e), and posthoc tests revealed that DSP-4 treated P301S mice had significantly less contextual freezing compared to all other groups (WT + saline, $p=0.0004$; WT + DSP-4, $p=0.0032$; P301S + saline, $p=0.0001$). At 10 months, there was no significant difference in contextual freezing across the groups for the entire duration of the test (Figure 4.5f). However, when looking at only the second timepoint of the test, there was a significant difference ($F(3, 64) = 3.507$, $p=0.0202$) as lesioned P301S again had significantly less contextual freezing compared to lesioned and unlesioned wildtypes (vs unlesioned wildtype: -31.85% ± 10.71%, $p=0.0268$; vs lesioned wildtype: -29.80% ± 10.71%, $p=0.0268$) as well as trending towards significantly less compared to unlesioned P301S ($p=0.0597$). All groups displayed similarly high levels of freezing with the onset of the cue (Figure 4.5g-i), indicating that cued fear memory retrieval was intact at each of the ages tested.

DSP-4 does not impact sociability, but may interfere with social memory in P301S mice

Among the most devastating consequences of AD patients is the inability to recognize family and friends. We modeled this by using a paradigm that allows for testing of both general sociability (Trial 1) as well as social reference memory (Trial 2) (see Figure 4.6a for protocol outline). Mice were initially habituated to the middle chamber of a 3-chamber Plexiglas arena before gaining full access to the arena for 10 min. During this first trial, the two outer chambers each contained a wire mesh cup, one holding a novel, age- and sex- matched conspecific, while the other was empty. The ratio of time spent investigating the cup holding the mouse out of the total cups investigation time was calculated as the measure of sociability. A ratio of over 0.5 indicated a preference for the social stimulus (mouse) over the empty cup. We found no differences across

the groups at any age point in terms of sociability; there was a small, but consistent preference for the mouse over the empty cup (Figure 4.6b-d).

Following the sociability trial, the mice were removed from the arena and returned to their home cages for 30 min. When they return to the arena for the second 10-min trial, one cup held the same mouse from the first trial, while the second cup held a stranger mouse. The ratio of time spent investigating the cup with the stranger mouse over the total investigation time of both cups was calculated as the measure of novelty preference. A ratio of over 0.5 indicated that the subject mouse, which normally prefers novelty, recognizes the familiar mouse and seeks out the stranger mouse. We observed a similar novelty preference ratio across all animals across ages and near 0.5, indicating either weak recognition of the familiar mouse or a lack of novelty preference (Figure 4.6e-g). At 10 months, a 2-way ANOVA (genotype x treatment) showed a main effect of genotype ($F(1, 31) = 8.102$, $p=0.0078$). Post hoc tests revealed that DSP-4 treated P310S mice had a significantly lower preference ratio compared to unlesioned WT (-0.1815 ± 0.06112, $p=0.0338$) and a trend towards a lower preference ratio compared to lesioned WT as well ($p=0.05898$).

DSP-4 decreases life-span of P301S mice but does not alter physical neurodegenerative phenotypes

Because P301S mice develop motor and postural phenotypes, we were also interested in whether NE depletion would exacerbate these physical manifestations of disease. While no gross locomotor deficits were observed at the beginning of behavioral testing in the oldest group (~10 months) (Figure 1), many of the transgenic mice from the eldest age group began to develop degenerative phenotypes by the end of this period (~11-12 month). All mice were rated per a rating scale for severity of kyphosis (Figure 4.7b-d) and hindlimb clasping (Figure 4.7e-g), both physical phenotypes associated with neurodegeneration, especially in the spinal cord. A two-way ANOVA analysis (genotype x treatment) revealed a main effect of genotype for both hindlimb

clasping ($F(1, 24) = 8.459$, $p=0.0077$) and kyphosis ($F(1, 23) = 12.96$, $p=0.0015$) at 6 months and 10 months ($F(1, 38) = 6.953$, $p=0.0121$; $F(1, 37) = 55.23$, $p<0.0001$), but no effect of treatment.

Because AD is not only a disease of cognitive impairments, but a leading cause of death in the US, and P301S mice have a shorter lifespan than WT animals (Yoshiyama, Higuchi et al. 2007), we also determined whether DSP-4 lesions of the LC impacted the survival of P301S mice (Figure 4.7a). No WT mice in the 10-month group (total of 11 for saline treated and 12 for DSP-4 treated) died by the end of behavioral testing, and only 1 out of 12 saline-treated P301S mouse was euthanized 1 week early (due to pronounced physical degenerative phenotype). By contrast, 5 of the 12 P301S mice with LC lesions were found dead in their cage prior to the end of the experiment or had to either be euthanized prematurely (from one week to 2 months early). Log-rank Mantel-Cox analysis showed that the DSP-4 treated P301S Kaplan Meier survival curve was significantly different from saline-treated P301S mice ($p=0.0401$) as well as both groups of WT animals (both $p=0.0113$).

4.5 DISCUSSION

It is well documented that experimental lesions of the LC exacerbate amyloid-mediated neuropathology and cognitive deficits in APP transgenic mice. These results presented here demonstrate, for the first time, that LC degeneration also accelerates the deleterious effects of aberrant tau. We found that DSP-4 had little effect on its own in WT mice but further impaired spatial and associative learning and memory in P301S mice at 6 months, which was associated with increased hippocampal tau pathology (see Chapter 3). Moreover, LC lesions promoted premature death in older transgenics in parallel with increases in neuroinflammation and neurodegeneration (see Chapter 3). Combined, these studies suggest that loss of noradrenergic neurons may be a key contributor to both beta-amyloid and tau-mediated AD progression.

Locomotor and circadian activity

We found no effect of DSP-4 or the P301S transgene on general circadian activity based on a 24-hour test in a locomotor chamber (Figure 4.1a-c), or on locomotor activity in the open field task (data not shown). These results conflict with earlier reports that P301S mice are hyperactive in the open field task by 6-7 (Takeuchi, Iba et al. 2011) but are consistent with more recent studies that did not observe locomotor differences at similar ages (Jiang, Zhang et al. 2016).

Although the LC noradrenergic system is intricately tied to endogenous pacemaker cells and diurnal rhythms (Linsell, Lightman et al. 1985, Cagampang, Okamura et al. 1994, Moriya, Tahara et al. 2015) and has been implicated in sleep regulation dopamine beta-hydroxylase knockout ($DBH^{-/-}$) mice that lack NE completely have normal circadian rhythms (Hunsley and Palmiter 2003, Swoap, Weinshenker et al. 2004). DSP-4 lesions in WT and beta-amyloid transgenic mice also have inconsistent effects, decreasing locomotion at 4 months (Jardanhazi-Kurutz, Kummer et al. 2010), but increasing it at 10 months (Heneka, Ramanathan et al. 2006).

It is possible that our detection and/or measures were not sensitive enough to capture the nuances of activity that some other studies have seen. Investigating these measures in more detail with smaller time bins or considering different types of locomotor activity (vertical rearing in addition to horizontal ambulations) may provide more insight. We also did not measure sleep directly, but used circadian activity as a proxy. Nevertheless, the grossly normal activity conferred by both the transgene and the lesion effectively ruled out motor deficits as an explanation for phenotypes we observed in our other behavioral tasks.

Novelty-induced locomotor activity

The LC system has been classically implicated in arousal and attention, and NE depletion attenuates exploratory activity. For example, $DBH^{-/-}$ mice and rats with 6-OHDA-induced LC lesions have reduced locomotor activity associated with being placed in a novel environment compared to NE-competent controls with normal levels of NE (Weinshenker, Miller et al. 2002). DSP-4 lesions in rats also reduce exploration in an open field arena (Harro, Oreland et al. 1995).

While we did not observe an effect of the DSP-4 lesion or tau transgene alone, there was a synergistic reduction in ambulations during the first 10 min following exposure to the locomotor chambers (Figure 4.1d-f). Interestingly, this attenuation of exploratory activity in DSP-4 treated P301S mice was not recapitulated in the open field task. It is possible that the larger, open arena of the open field task provided a more stimulating environment that overcame the NE-mediated arousal deficit. It is also unclear exactly how the aberrant tau is contributing to this effect.

Anxiety

The LC is considered an important stress-responsive nucleus that contributes to anxiety, which is also prevalent in the AD population (Gallagher, Coen et al. 2011, Kaiser, Liang et al. 2014, Tagai, Nagata et al. 2014, Zhao, Tan et al. 2016). We did not observe any significant genotype or treatment differences in anxiety-like phenotypes using the open field task. This is again in conflict with some previous reports of a less anxious phenotype in P301S mice by 3 (via increased time in center of open field) (Takeuchi, Iba et al. 2011) and 9 months (via increased time in open arms of elevated plus maze) (Lopez-Gonzalez, Aso et al. 2015). The lack of a DSP-4 effect of also goes against previous reports of its anxiogenic qualities; both WT and APP/PS1 mice with LC lesions showed a decrease in time spent in the center at 12 months (Jardanhazi-Kurutz, Kummer et al. 2010). On the other hand, our data are consistent with normal anxiety-like behavior in the open field, elevated plus maze, and light-dark box in $DBH^{-/-}$ mice (Marino, Bourdelat-Parks et al. 2005, Schank, Liles et al. 2008).

We only assessed two outcome measures in our open field test (percent time in the center vs outside; latency to outside). It may be informative to examine additional measures such as distance travelled and number of fecal boli, as well as breaking the test into smaller time bins to look for any differences in acclimation to the environment over the test period. Further examination of anxiety-like phenotypes via other tests like the elevated plus maze, elevated zero

mazes or light/dark exploration may also help to clarify if the phenotype is present (Bailey and Crawley 2009).

Hippocampal-dependent learning and memory

The hippocampus, which is densely innervated by the LC, plays an integral role in learning and memory and features prominently in the pathophysiology of AD (Kim and Diamond 2002, Harley 2007, Lister and Barnes 2009). Previously, we described an exacerbation of pre-tangle tau pathology in the hippocampus of P301S mice with DSP-4 lesions at 6 months. Development of tau pathology is well correlated with the onset of cognitive impairment in models of aberrant tau, and we hypothesized there would be corresponding cognitive deficits in hippocampal based tasks.

We first tested mice with the Morris Water Maze: a canonical spatial learning and memory task that relies heavily on an intact hippocampus (Morris 1984, D'Hooge and De Deyn 2001). While there were no effects of the transgene or DSP-4 alone at 6 months, the combination impaired both initial learning as well as reference memory recall. By 10 months, P301S mice with intact LCs caught up to their lesioned counterparts and exhibited similar learning deficits, but the synergy was still apparent for recall. This exacerbation of spatial learning and memory matches the increased severity of tau pathology seen in lesioned transgenics compared to their unlesioned counterparts at each age point, suggesting that the tau pathology may be functionally responsible for the cognitive impairment.

The interaction between tau and LC degeneration is not surprising, as both the P301S transgene and DSP-4 lesion are reported to induce spatial and learning deficits in this task. The original study for the P301S mice reported deficits as early as 6 months (Takeuchi, Iba et al. 2011), another didn't see impairment until 7 months (Jiang, Zhang et al. 2016), and a third only observed only a trend towards a deficit by 10 months (Dumont, Stack et al. 2011). Slight variations in Morris Water Maze protocol, such exact maze dimensions, the particular spatial cues in the room, and the inclusion of pre-training with a visible platform may explain variations in the

data. Moreover, the two studies where an earlier and more aggressive development of deficits were seen employed mice that were maintained on the original C3HxC57BL/6 hybrid background (Takeuchi, Iba et al. 2011, Jiang, Zhang et al. 2016), while our study as well as the Dumont et al study which saw later, more subtle deficits were backcrossed to C57BL/6 for multiple generations and thus essentially congenic. The interaction between DSP-4 and the transgene was also reminiscent of that seen with DSP-4 treatment of beta-amyloid transgenic mice; deficits emerged by 6 months in the lesioned transgenics compared to their unlesioned counterparts, and the difference disappeared by 12 months when the unlesioned transgenics caught up (Jardanhazi-Kurutz, Kummer et al. 2010).

We did not see any deleterious effects of DSP-4 in WT mice, as one study has previously reported (Jardanhazi-Kurutz, Kummer et al. 2010). The role of NE in spatial learning and memory is complicated. Many studies of DSP-4 lesions in rats have shown no impairment of spatial learning and memory, especially if there is a substantial recovery period between lesion and testing (Sontag, Hauser et al. 2011, Hauser, Sontag et al. 2012). Some studies suggest NE may be more important for impairing working memory (more related to prefrontal cortex) as opposed to the hippocampal associated reference memory (Sontag, Hauser et al. 2008). It is also possible that DSP-4 lesions require a secondary insult to impair reference memory: a study looking at isolation rearing in combination with DSP-4 lesions found impairments in the Morris Water Maze only when both factors were concurrent (Lapiz, Mateo et al. 2001).

To further probe the functional consequences of the exacerbated tau pathology in the hippocampus of lesioned P301S mice we tested them with a fear conditioning paradigm. Fear conditioning is an associative, rather than spatial learning task, and can be modified to be either hippocampal-dependent (contextual fear memory) or hippocampal-independent (cued fear memory). All 4 groups of mice displayed intact cued fear memory at every age tested, but there were significant differences in performance in contextual fear memory. At 4 months, both WT and P301S mice with LC lesions performed significantly worse than their saline-treated

counterparts, but the effects on WT mice diminished with age, perhaps due to compensatory mechanisms that fail in the presence of tau pathology. By 6 months, when lesioned transgenics showed a significant deficit in the Morris water maze, there was also a selective and synergistic impairment in contextual fear memory in this group. At 10 months, the deficit was somewhat ameliorated and only seen within the first two min of the test, after which the P301S + DSP-4 mice had similar levels of freezing as the other groups. However, it is important to note that the 10-month analysis was confounded because many of the P301S mice were beginning to show motor phenotypes and had reduced overall activity in the fear conditioning arena, which was difficult to parse out from bona fide “freezing”.

Besides the hippocampus, the amygdala is the other critical brain region important for fear conditioning; lesions of this nucleus can attenuate the instinctual freezing behavioral for rodents in response to both contextual and cued fear (Goosens and Maren 2001). P301S mice are reported to develop intense filamentous tau pathology in this area by 6 months (Takeuchi, Iba et al. 2011), but we did not confirm the presence of tau pathology in this area in our experiments. The lack of an anxiety phenotype in our other measures combined with the intact cued fear memory suggests relatively normal amygdala function, but further studies examining AD-like neuropathology in the amygdala are needed to confirm that conclusion.

Sociability and social memory

Deficits in social memory are another prominent and troublesome phenotype in human AD patients. We saw no differences in sociability across genotype or treatment at each time point, and only a modest reduction in social memory was evident in 10-month old P301S mice with LC lesions. In retrospect, it seems likely that the low overall level of social novelty discrimination, even in young, saline-treated WT mice, indicates a sub-optimal social memory protocol. Our protocol was a modification of Crawley’s three chamber social recognition paradigm, where we introduced an additional 30-min inter-trial interval (ITI) between Trial 1 and Trial 2. We

hypothesize that this ITI was too long to allow for even WT test subjects to remember the familiar mouse from Trial 1.

Other explanations are also possible. The most important sensory system for mice is the olfactory system (Rokni, Hemmelder et al. 2014), and mice rely heavily on their sense of smell when engaging in social interactions (Kaidanovich-Beilin, Lipina et al. 2011). Our cups had finer mesh compared to those used in previous studies, which may have impeded the ability of our test subjects to effectively smell and differentiate the stimulus mice. Furthermore, while olfactory dysfunction has not been investigated in our line of P301S mice, it has been reported in another line of P301S tau mice (under the Thy2.1 promoter) beginning at 3 months (Yang, Kuan et al. 2016), which could again impede the ability of our subjects to perform in this task. Improvements in our paradigm or the use of a regular social interaction test where the mice can physically interact may be more effective at elucidating whether a social memory deficit exists.

Neurodegenerative phenotypes and survival

The P301S mouse is characterized by an intense physical neurodegenerative and motor phenotype whereby kyphosis and hindlimb clasping is seen relatively early and leads to premature death as severely impacted mice are unable to move, eat or drink (Takeuchi, Iba et al. 2011). We did not see any enhancement of either physical phenotype with the DSP-4 lesion. There are two separates descending noradrenergic projections to the spinal cord and it only receives some of its noradrenergic input from the LC (primarily the dorsal and intermediate zones) (Fritschy and Grzanna 1989, Lyons, Fritschy et al. 1989). DSP-4 lesions leave the rest of the spinal cord's noradrenergic inputs from the A5 and A7 cell groups intact, which could explain that lack of their impact in our study (Fritschy and Grzanna 1989, Lyons, Fritschy et al. 1989).

However, there was a profound deleterious effect of DSP-4 lesions on the survival of P301S mice, resulting in premature death in almost 50% of those animals by the end of the study

while only 1 of the saline-treated P301S mice expired. We did not determine the exact cause of death, but AD is lethal in humans and thought to be associated with excessive neurodegeneration.

Genetic differences and drift in disease phenotypes of P301S mice

The results reported here were often in conflict with the original paper and other consequent ones regarding the timing of behavioral abnormalities and death in the P301S line. In comparison with some previous reports (e.g. Yoshiyama et al., 2007) cognitive impairment and mortality were significantly delayed in our experiments. We believe two factors may be at the root of these differences: genetic drift and background strain differences.

Genetic drift is a common issue in the maintenance of transgenic lines across multiple research fields (Davis, Maillet et al. 2012). Like all living creatures, mice are intrinsically driven to change over time, and spontaneous mutations can occur that alter gene products and functions (Taft, Davisson et al.). Thus, the initially reported phenotypes that identify a transgenic line can often shift or even change over time (National Centre for the Replacement , Kelmenson 2016). Since its debut in 2007, the P301S (aka PS19) line has already been reported by the developing group itself to have attenuated tau pathology onset (hyperphosphorylated tau inclusions that were prominent at 6 months did not appear until over 12 months of age) (Yoshiyama, Higuchi et al. 2007, Iba, Guo et al. 2013). Similarly, the neuronal death reported at 9 months in the original line was delayed until after 12 months (Zhang, Carroll et al. 2012, Iba, Guo et al. 2013). This drift was evident even though the strain was maintained on the original B6C3 background.

The other potential contributor to the disparity in our results is the mouse strain, which can profoundly influence pathology and behavior. We used P301S mice that had been backcrossed onto C57Bl/6J background. The majority of the mice used in the Yoshiyama et al., 2007 paper which characterized the full behavioral and cognitive profile of the P301S line were congenics (backcrossed to C57Bl/6J) (except for the mice used for the Morris Water Maze test, which were kept on the original B6C3H background). Variations in the number of backcrosses

between the studies, or even the source of the WT C57Bl/6J breeders, may have contributed to the varying degree of pathological and behavioral phenotypes. Importantly, the shift in onset of behavioral phenotypes corresponds to the shift in pathology (see Chapter 3) we observed. Thus, while the exact timing may be different between studies, analogous connections between the emergence of pathology and cognitive deficits remain the same.

Conclusion

We found that DSP-4 induced lesion of the LC constitutes a physiologically relevant event in the context of the P301S mice, and acts synergistically with aberrant tau to disrupt neuronal function. Lesioned P301S mice showed exacerbated cognitive deficits in hippocampal-dependent cognitive tasks, which were time-locked with the aggravated tau pathology seen within the hippocampus. Furthermore, LC lesions accelerated premature death in the P301S mice, which was associated with increased neuroinflammation and neurodegeneration. Our results suggest that targeting the LC noradrenergic system may be an effective therapeutic strategy to combat the progression of neuropathology and cognitive impairment in AD.

FIGURES

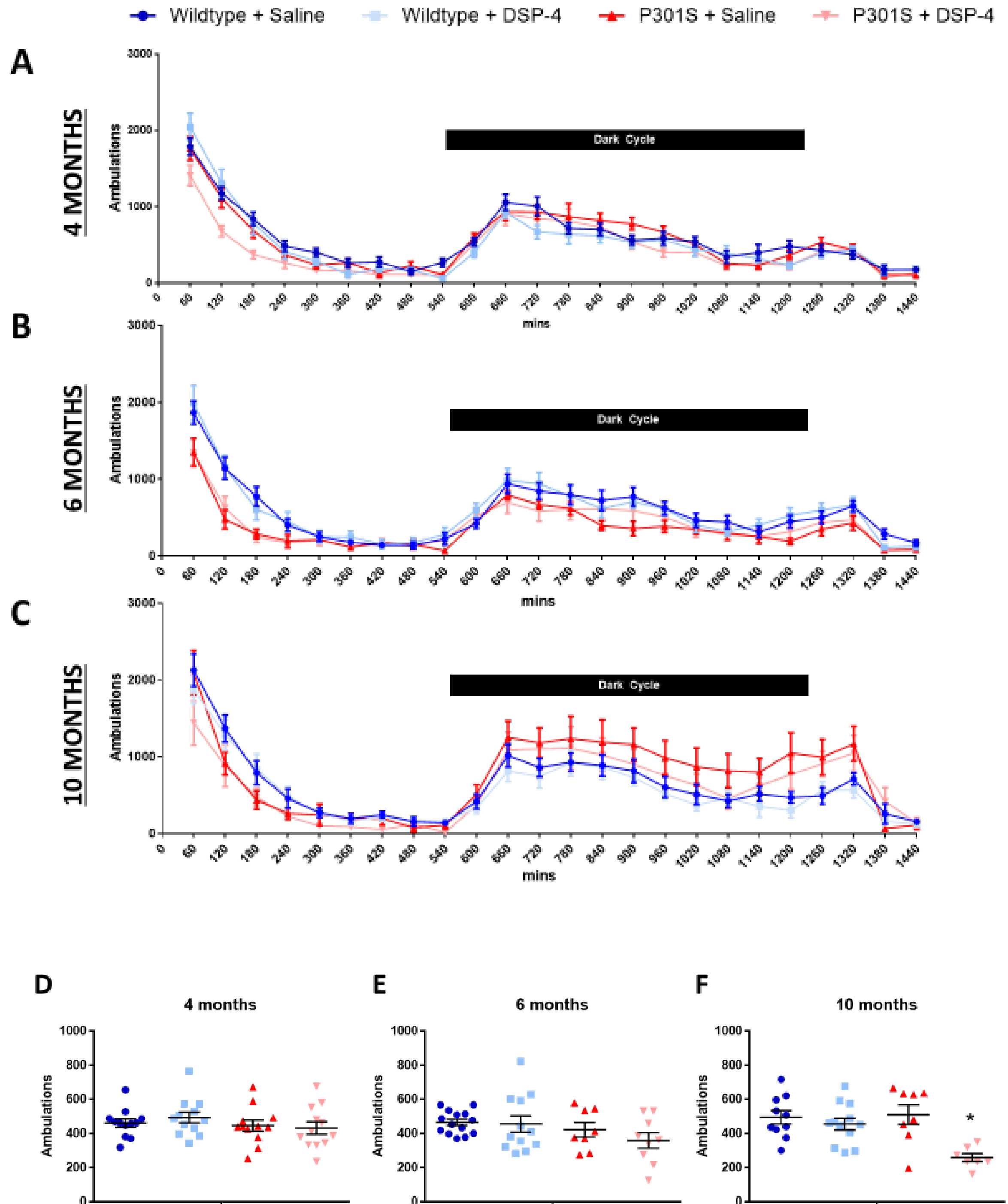


Figure 4.1 *DSP-4 has no effect on circadian activity but reduces novelty-induced locomotion in aged P301S mice.* Mice were placed in a large, clear plastic cage with bedding, food and water for 24 h. Shown are the mean ± SEM ambulations **(a-c)** over the entire 24 hours, and the first 10 min of the test **(d-f)**. *$p \leq 0.05$ compared to all other groups at that age. N=7-12 per group.

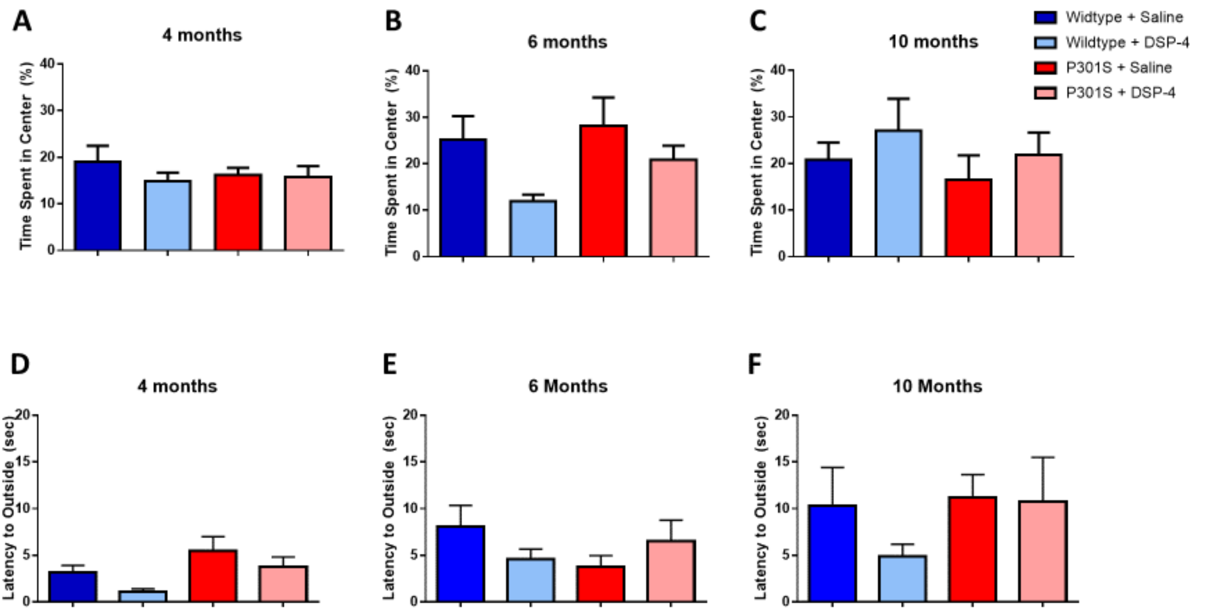


Figure 4.2 *Neither DSP-4 nor P301S impacts anxiety-like behavior in the open field test.* Mice were placed inside an open field arena for 10 min. Shown are the mean ± SEM for **(a-c)** percent time spent in center of the arena, and **(d-f)** latency to leave the center for the outer ring of the arena. N=7-12 per group.

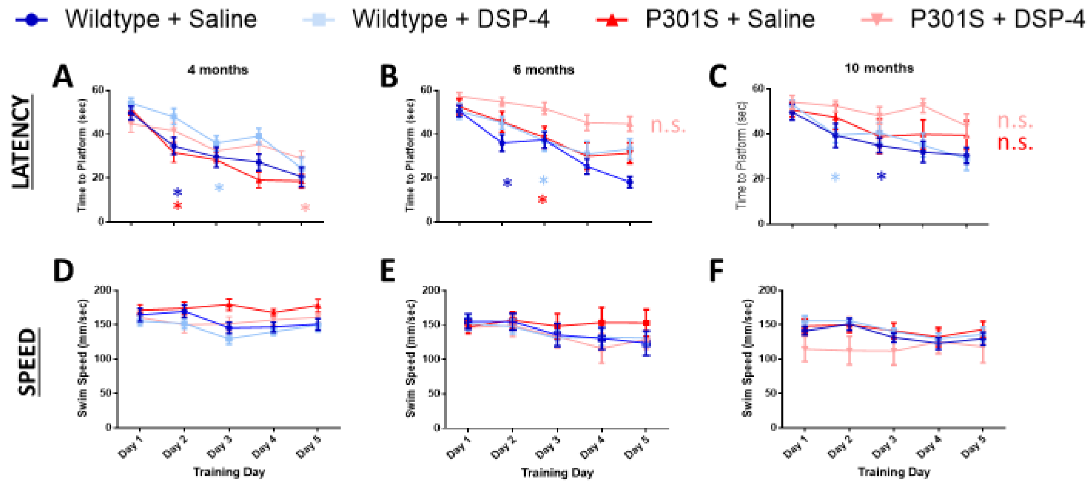


Figure 4.3 *LC lesions aggravate spatial learning deficits in P301S mice in the Morris water maze.*

Mice were trained over 5 days to locate a hidden platform in a tank of water using spatial cues around the room. Shown are the mean ± SEM for **(a-c)** latency to find the platform and **(d-f)** swim speed during trials. * denotes significant difference from Day 1 ($p \leq 0.05$). N=8-12 per group.

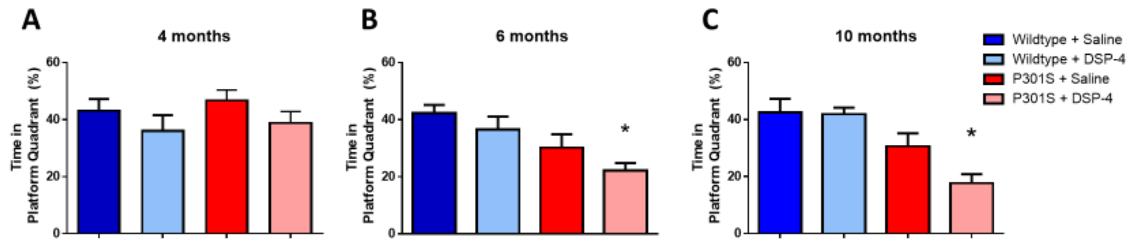


Figure 4.4 *DSP-4 and P301S act synergistically to impair spatial memory recall in the Morris water maze probe trial*

Following 5 days of training to find hidden platform in water maze, mice were returned to the maze on Day 6 with the platform removed. Time spent swimming in the different quadrants were recorded. Shown are the mean ± SEM for **(a-c)** percent time spent swimming in the correct quadrant where the platform had been during training. * denotes $p \leq 0.05$, compared to wildtype + saline. N=8-12 per group.

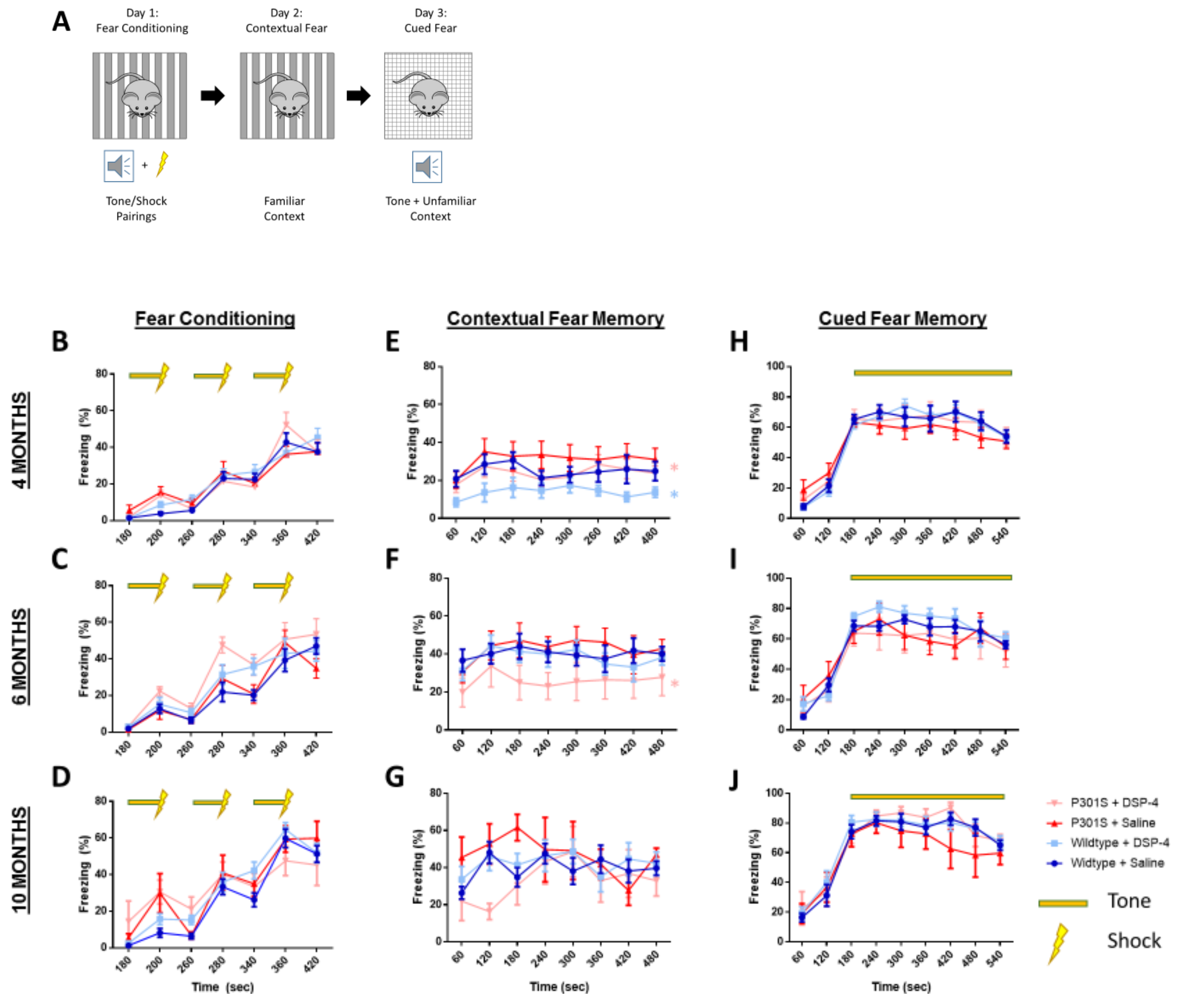


Figure 4.5 *DSP-4 induces contextual fear memory deficits while leaving cued fear memory intact in P301S mice*. Mice were fear conditioned and probed for contextual and cued fear memories. Percent time spent freezing was recorded as fear behavior and shown as mean ± SEM. **(a)** Overall schematic of fear conditioning paradigm. **(b-d)** Fear training on Day 1 followed by testing of **(e-g)** contextual fear memory on Day 2 in the same chamber with no tone and testing of **(h-j)** cued fear memory on Day 3 in a different chamber with tone presented * denotes p≤0.05, N = 8-12.

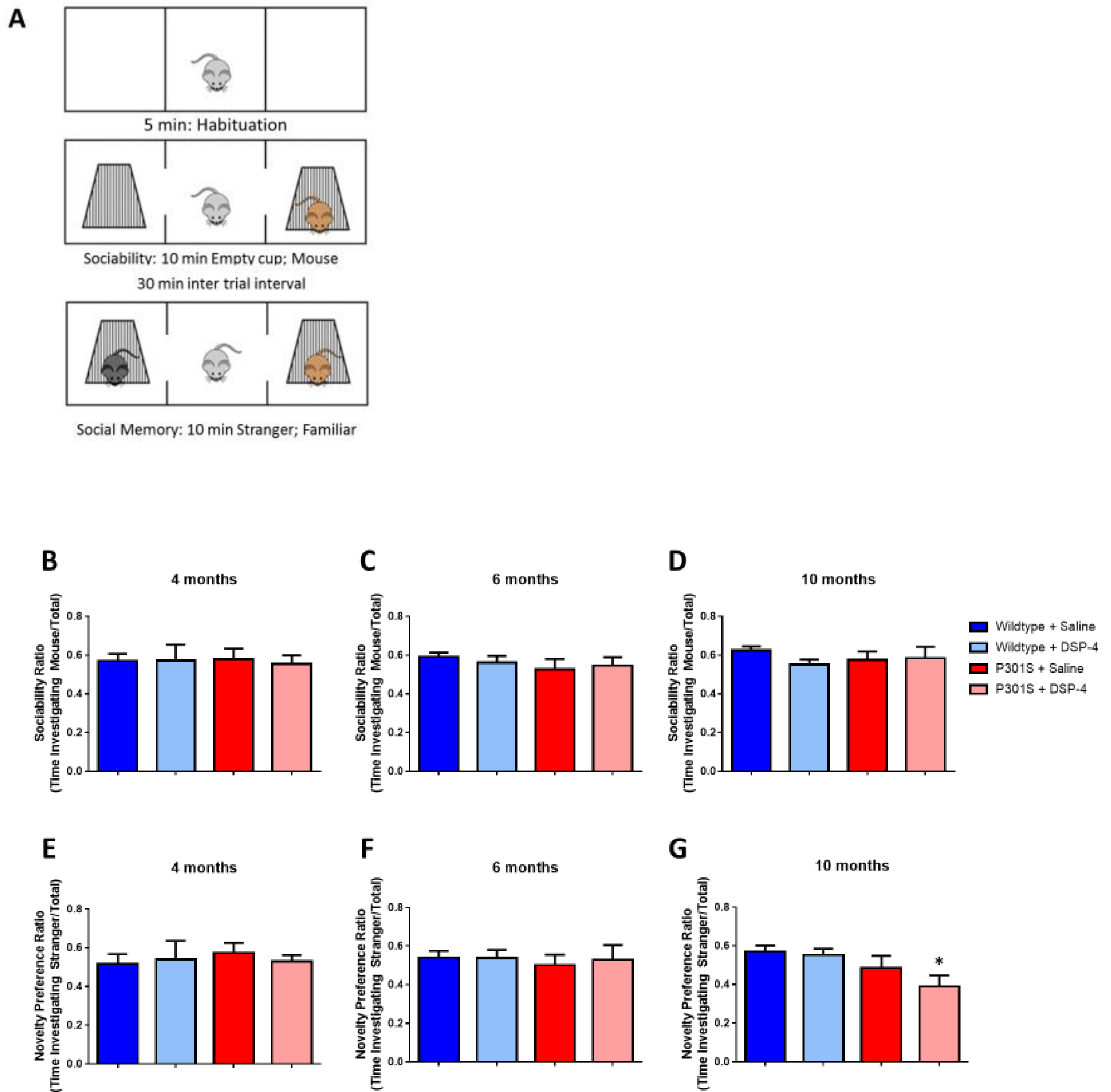


Figure 4.6 *DSP-4 does not impact sociability, but modestly impairs social memory in aged P301S mice.* **(a)** Schematic of social interaction task paradigm. Mice were habituated in the middle chamber of the 3-chamber arena for 5 min before a 10-min sociability trial **(b-d)** where an empty cup and a cup holding a stimulus mouse were placed in the side chambers. Shown are the sociability ratios (time spent investigating cup with mouse over time spent investigating both cups) as mean ± SEM. Mice were then returned to their home cage for 30 min before returning

for a 10-min social memory trial **(e-g)** with the familiar mouse in the same cup and a novel mouse in the previously empty cup. Shown are the novelty preference ratios (time spent investigating cup with novel mouse over time spent investigating both cups). *denotes $p<0.05$ compared to wildtype + saline, N=6-12 per group.

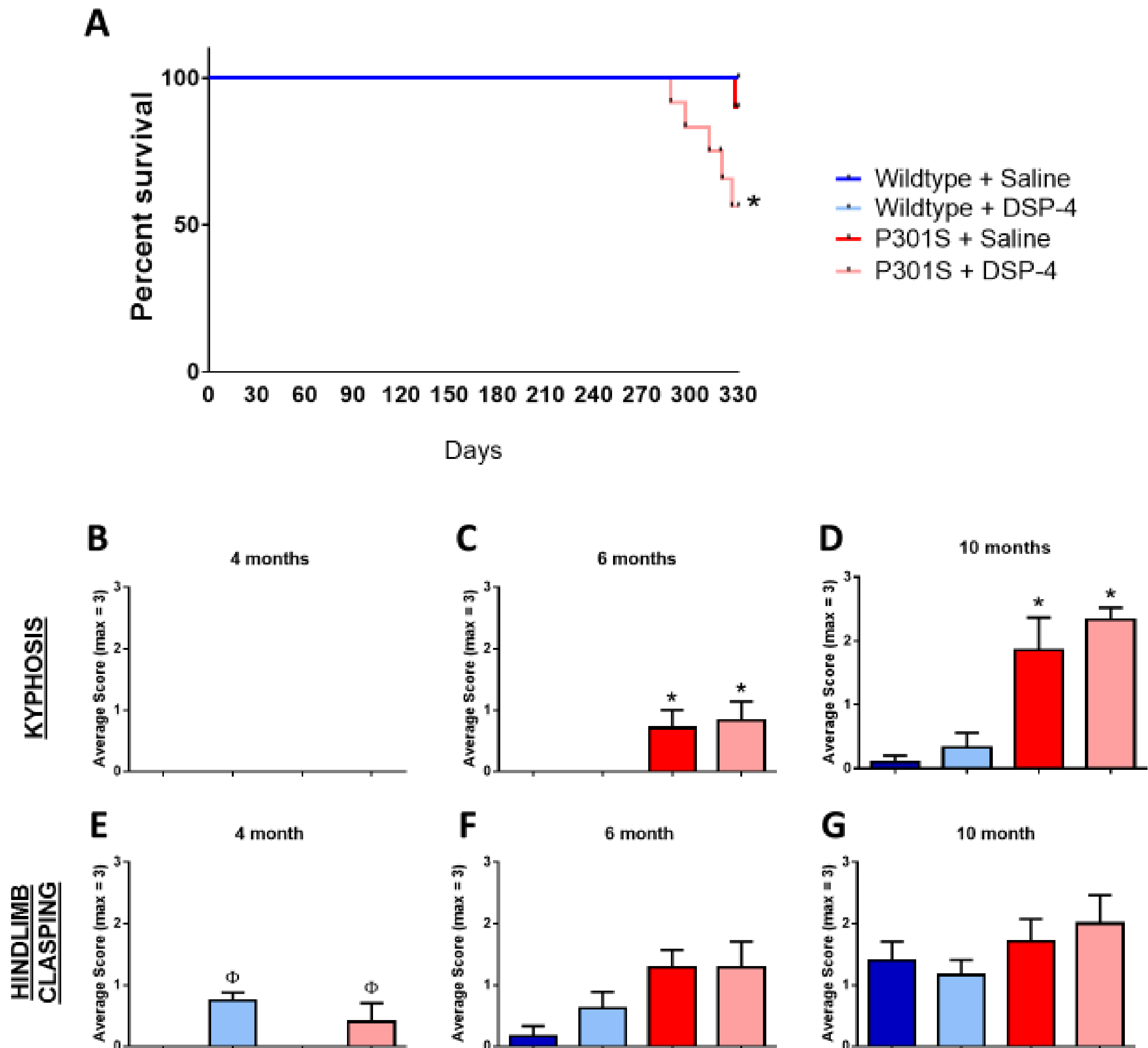


Figure 4.7 *DSP-4 reduces survival in transgenic mice but does not augment physical neurodegenerative phenotypes.* **(a)** Survival curve (12 mice per group). *p<0.05 compared to all other groups. **(b-d)** Kyphosis quantification scores (see Table 1 for scoring system). * denotes p<0.05 compared to wildtype group with same treatment. **(e-g)** Hindlimb clasping (see Table 1 for scoring system). φ denotes p<0.05 compared to unlesioned group of the same genotype. N=6-12 per group.

CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

Portions of this chapter were used verbatim, with permission, from the following publication: Chalermpalanupap, T, Kinkhead, B, Hu, WT, Kummer, MP, Hammerschmidt, T, Heneka, MT, Weinshenker, D, Levey, AI. "Targeting norepinephrine in mild cognitive impairment and Alzheimer's disease" Alzheimers Res Ther. 2013; 5(2): 21.

5.1 SUMMARY

Alzheimer's disease (AD) is an insidious and debilitating disease that has established itself as a formidable foe for science and medicine in our time. Over the last few decades, the disease has quickly become an unsustainable burden both in terms of the number of people it is claiming as well as the burgeoning economic and societal costs (Alzheimer'sAssociation 2016). The lack of a clear understanding of the underlying mechanisms for why we develop the canonical beta-amyloid plaques and tau neurofibrillary tangles (NFT) and how they lead to the temporally and spatially systematic destruction of our brains has greatly hampered both our attempts to diagnose the disease, as well as stop it. As we learn more about the disease, it is clear that identifying and treating the disease early, before the onset of cognitive impairments, will be the key to effectively combating this disease.

In an attempt to clarify the earliest changes that may be contributing to the pathogenesis of this disease, Dr. Heiko Braak and his group have determined that pre-tangle hyperphosphorylated tau in the locus coeruleus (LC) of young, cognitively unimpaired individuals may be the first detectable sign that resembles AD. The discovery of this early deposition of tau in this subcortical nuclei is intriguing as the LC is also a site of major neurodegeneration in AD, and its degeneration correlates well with other markers of disease progression. Remarkably, there has been little investigation into the consequences of these early pre-tangle tau deposits in the LC since their discovery by Braak in 2011, and equally little is known about the role of LC degeneration in tau pathogenesis. These were the knowledge gaps we aimed to fill with our studies.

In the second chapter, we described a new protocol to culture and compare LC cells expressing aberrant, hyper-phosphorylation prone tau (P301S tau) to control and establish their dysfunctional structural phenotype and conferred sensitivity to a secondary insult. We found the aberrant tau negatively impacted LC cells: significantly reducing their neurite lengths by nearly

half. Interestingly, despite this striking effect on the LC's structural phenotype, there was no significant cell death even after over a week growing in culture. However, when the P301S tau-bearing neurons were challenged with a secondary toxin like the noradrenergic toxin DSP-4, they were more susceptible to cell death with a lower, sub-threshold concentrations that normally did not kill control LC cells. These results suggest a synergy between aberrant tau and a secondary insult. This has powerful implications for our understanding of the role of early hyperphosphorylated tau in the LC and the interaction between the pathology that emerges early in our brains and the environmental triggers that may push the brain over disease threshold.

We then investigated the impact of an induced LC lesion and loss of cortical norepinephrine on neuropathology and cognition in a mouse model of aberrant tau. At an early age, we demonstrated that lesioned transgenic mice have exacerbated early tau pathology in the hippocampus as well as corresponding deficits in hippocampus-based cognitive tasks such as the Morris water maze and contextual fear conditioning. At a later age, we found that these differences in tau pathology leveled out and instead there was an exacerbation of neurodegeneration and neuroinflammation in lesioned animals that corresponded to increased mortality. This is, to our knowledge, the first study demonstrating the effect of LC lesions and loss of the NE system in a mouse model of tau.

Our experiments have elucidated the important interplay between tau pathology and the integrity of the LC in AD: both at the early pre-clinical stage where pre-tangle tau is depositing in the LC as well as later in the disease when the LC degenerates and contributes to exacerbating tau pathology and cognitive impairments. This interconnectedness between the LC and tau provides further evidence that AD pathogenesis is likely not a linear cascade of events, but more likely a disruption of a highly interconnected network that results in paralleling and reciprocal dysfunction across the brain as the disease progresses.

The LC's susceptibility to early tau pathology dysfunction

Braak's epidemiological studies propose that the LC may be the initiating site of AD pathogenesis, specifically the first place to accumulate pretangle tau pathology. Our study touched on how the early tau pathology seen in the LC of brains from young, cognitively unimpaired individuals may be impacting the nuclei, but a question still left unanswered is what causes the LC to accumulate these pretangle tau pathologies in the first place.

The few hypotheses that have been suggested for why the LC could be vulnerable are primarily based on its unique structural, neurochemical and functional characteristics within the brain (Mravec, Lejavova et al. 2014, Mather and Harley 2016). As the main source of NE, the LC's projection axons are some of the longest in the brain; thus, an abundance of tau is likely needed within these neurons to maintain axonal stability. It is not yet clear what mechanism are involved when tau pathologically hyper-phosphorylates, but theoretically if the LC were to be exposed to and succumb to such mechanisms, it seems there would be a large pool of tau available to corrupt.

Aside from the large abundance of tau-filled neurites, the LC in general is also quite vulnerable to dysfunction (Mather and Harley 2016). Its long, thin axons are, like most other monoaminergic systems, unmyelinated, leaving them exposed and vulnerable to toxins (Pamphlett 2014, Mather and Harley 2016). The nucleus' proximal location to the fourth ventricle could also mean higher exposure to toxins or deleterious compounds lurking in the cerebrospinal fluid (Mravec, Lejavova et al. 2014).

Finally, its tireless role providing NE to the majority of the brain despite its relatively tiny size likely means that the LC is an energetically voracious nucleus with high mitochondrial demands. Such demands have already been shown to induce high mitochondrial oxidative stress that puts the nucleus at a greater risk for degeneration (Cho 2014).

Potential mechanism of tau and locus coeruleus involvement in AD pathogenesis

What causes the two hallmark proteins, beta-amyloid and tau, to aggregate into the canonical plaques and tangles also remains an important question. Our study, together with those from groups studying the role of LC and the NE system in beta-amyloid-depositing transgenic mice, certainly emphasize its importance in AD pathogenesis. More work is still needed to clarify the underlying mechanisms of this role.

We focused the discussion within our studies on neuroinflammation as a probable link between LC dysfunction and AD pathogenesis, but it is more likely that a variety of systems are impacted by the loss of this nucleus and its noradrenergic pathways. For instance, the LC and NE have known modulatory effects for neuroplasticity and neuronal survival. NE has been shown to regulate synaptic plasticity by facilitating long-term potentiation throughout the hippocampus (Neuman and Harley 1983, Katsuki, Izumi et al. 1997). Its activation of β-receptors also stimulates the production of nerve growth factor and brain-derived neurotrophic factor, both integral to neuronal survival (Counts and Mufson 2010).

The LC is also involved in maintaining the integrity of the blood brain barrier (BBB), which acts as the interface between the vascular system and the central nervous system and is integral to the preservation of the delicate environment of the brain. Lesions of the LC result in leaks (Harik and McGunigal 1984) and disrupt the activity of the sodium/potassium pumps (ATPase) crucial for the regulation of homeostasis across the BBB (Harik 1986). In addition to affecting permeability and function, lesions of the LC can also result in the physical deterioration of the BBB: reducing the number of tight junctions lining the cerebral blood vessels that act as its foundation (Kalinin, Feinstein et al. 2006). It is very likely, then, that dysfunction of the LC contributes to the increased permeability of the BBB seen in AD brains (Claudio 1996, Zipser, Johanson et al. 2007, Marques, Sousa et al. 2013).

This is nowhere near an exhaustive list of the potential means by which LC degeneration can promote AD pathogenesis. It is our hope that, as the LC's role in AD pathogenesis becomes

more clear, future research will focus on this system and on developing new tools and techniques to facilitate its investigation.

5.2 FUTURE DIRECTIONS

As the role of the LC in AD becomes more prominent, even more questions are sure to arise. For example, AD is a uniquely human disease (while some aged non-human primates develop extensive plaque pathology, extensive NFTs and cognitive impairments are missing) (Heuer, Rosen et al. 2012, Perez, Sherwood et al. 2016), thus, if the LC is integral to the pathogenesis of AD, are there differences between the physiology, morphology or function of the LC/NE system in humans versus other animals? So far, comparative studies of the anatomy of the LC in humans versus non-human primates show significantly higher number of cells in human LC, however, this is believed to be primarily a result of anatomical scaling, rather than intrinsic differences in neuronal density (Sharma, Xu et al. 2010). More in depth stereological investigations could provide information on whether there are differences in the size and distribution of LC neurons and the neuronal makeup of the nucleus (small unipolar versus larger multipolar cells). Investigation of phylogenetic differences in NE signaling (pharmacology, distribution, function) between humans and other species may also provide interesting insight into why we are the only ones to develop this disease.

A similar question arises when thinking about the epidemiology of AD, as it is thought to be more prevalent in women than men (Alzheimer'sAssociation 2016, Mazure and Swendsen 2016). Sexual differences in the LC/noradrenergic system exist (extensively reviewed in (Bangasser, Wiersielis et al. 2016). Rat LCs are sexually dimorphic: with larger volumes, more neurons and denser and more branched dendrites in females than in males (Guillamon, de Blas et al. 1988, Pinos, Collado et al. 2001, Bangasser, Zhang et al. 2011). LC size is also reported to be significantly different in human males and females, again with a higher number of neurons in women (Ohm, Busch et al. 1997). Estrogen has also been show to increase NE production and

decrease NE degradation (Vathy and Etgen 1988). While these dimorphisms exist, we did not see any sex differences across our groups and different behaviors in our studies (data not shown). We have not looked at the differences at the level of pathology, which may be more sensitive to any modulation from the sexual dimorphism and will be an interesting next step.

Finally, a very important remaining question is whether tau is actually capable of spreading from the LC to the rest of the brain, as shown for other areas of the brain like the hippocampus, to mimic the hypothesized progression seen with Braak staging (Clavaguera, Bolmont et al. 2009, Braak, Thal et al. 2011, de Calignon, Polydoro et al. 2012, Lasagna-Reeves, Castillo-Carranza et al. 2012). While Braak staging has been amended to include the subcortical LC tau pathology found in the brains of young, cognitively healthy individuals without any precursor or intermediate forms as the first AD-like pathology that develops, it is still a great area of debate whether this staging represents spread or a snapshot of selective vulnerability of different areas of the brain. To test whether tau can spread from the LC like it can from other areas of the brain, our lab will be employing both brain homogenate and viral expression to "plant" tau in the LC before watching to see whether it spread over time to other areas of the brain in the recognizable Braak pattern.

5.3 THE FUTURE OF DIAGNOSTICS AND THERAPEUTICS

Despite many unanswered questions, our results support the push to focus on the LC and tau (possibly both together) as potential areas to invest in as promising areas for the development of new diagnostic and therapeutic avenues.

Developments in diagnostics to target tau and the locus coeruleus

The effectiveness in detecting beta-amyloid load via imaging techniques in living patients has vastly improved in recent years. While the ability to detect tau in a similar manner is still in its early stages comparatively, it may prove to be a more useful diagnostic tool, especially in light of

the hypothesized earlier deposition of tau pathology relative to beta-amyloid, as well as the strong correlation of tauopathy with dementia.

Improvements in the spatial resolution of imaging techniques may also allow for detection of early, subtle changes in LC size or shape. Conventional magnetic resonance imaging techniques were ineffective at identify the LC, but recent improvements using high structural contrast ratios comparing the nuclei to their surrounding tissue have been more successful (Sasaki, Shibata et al. 2006, Keren, Lozar et al. 2009). This structural contrast is a result of the neuromelanin that is present in LC cells as a byproduct of NE and has demonstrated high reproducibility as a marker (Keren, Taheri et al. 2015, Langley, Huddleston et al. 2016). Recently, this neuromelanin-based technique has been used to successfully used to discriminate patients with AD or mild cognitive impairment (MCI) from healthy controls (Takahashi, Shibata et al. 2015).

While this highlights the great potential for this line of diagnostics, it is important to note that it is still in early development: the MRI contrast ratio was not able to predict progression from MCI to AD (there was no difference in contrast ratios between patients with MCI who did or did not go on to convert to full AD) (Takahashi, Shibata et al. 2015). Nevertheless, such developments provide an exciting avenue to pursue in early detection, and the utility of the technique expands to other neurodegenerative diseases as well: LC contrast ratios have also been successfully used to differentiate LC sizes in Parkinson's disease, schizophrenia and depression (Sasaki, Shibata et al. 2006, Shibata, Sasaki et al. 2007, Shibata, Sasaki et al. 2008).

Potential therapies that target tau and the locus coeruleus

While the bulk of therapeutics have been focused on targeting the beta-amyloid system, the consistent failures experienced, especially in recent years, have encouraged many researchers to turn to other avenues. Tau-modifying therapies are primarily aimed at interfering with tau aggregation or inhibiting tau hyperphosphorylation (reviewed in (Jiang, Yu et al. 2012,

Yiannopoulou and Papageorgiou 2013)) and are emerging as a greater area of interest as amyloid-modifying therapies continue to fail in clinical trials.

Also interesting is the pharmaceutical targeting of the LC and its noradrenergic system. To date, most clinical studies using noradrenergic pharmacotherapy have been primarily focused on treating the aggression and other behavioral disturbances that occur in many late-stage AD patients. β-adrenergic receptor antagonists (i.e. propranolol) are somewhat effective in the treatment of aggression and agitation, which may be caused by NE overstimulation (Greendyke, Kanter et al. 1986, Shankle, Nielson et al. 1995), while antidepressants inhibiting NE reuptake, such as the tricyclic imipramine, have been used to treat depression, which may be caused by NE deficiency (Reifler, Teri et al. 1989). Although the idea of increasing NE to treat cognitive impairment in AD is still in its infancy, there are some tantalizing pieces of data in support of this approach. For example, clonidine, which suppresses NE release by activating the α2-adrenergic autoreceptor, impairs short-term recognition memory in patients (Riekkinen, Laakso et al. 1999), suggesting that facilitating NE release may be beneficial. However, the same group also determined that clonidine could also enhance spatial working memory in AD patients (Riekkinen and Riekkinen 1999), highlighting the complexity of these processes.

Multiple clinical studies looking at hypertension in humans have also proposed similar therapeutic effects with β-blockers against inflammation as well as dementia. Use of β-blockers to treat elderly patients with hypertension has shown positive trends towards lowering the incident of dementia (Khachaturian, Zandi et al. 2006) as well as slowing the annual rate of functional decline in AD patients (Hajjar, Catoe et al. 2005, Rosenberg, Mielke et al. 2008). Nevibolol and another β1-blocker, metoprolol, have been shown to attenuate the release of atherosclerotic inflammatory markers such as ICAM-1 (soluble intercellular adhesion molecule-1) in humans after a year of treatment (Serg, Kampus et al. 2012).

Although NE pharmacotherapies are widely used in medicine, drugs that regulate NE transmission in brain could have complicated effects in AD. The integrity of LC and

pharmacological responsiveness in prodromal stages of AD are poorly understood. While preclinical studies suggest potential for NE-enhancing therapies to reduce neuro-inflammation, amyloid burden, and ameliorate cognitive impairment, clinical observations in AD patients also suggest the potential to impact non-cognitive symptoms of AD including mood, apathy, disinhibition, sleep, agitation and aggression (Marsh, Biglan et al. 2009, Koppel, Goldberg et al. 2012).

5.4 CONCLUSION

Our results indicate that irregularities in tau or LC function have deleterious effects on each other. The *in vitro* data from Chapter 2 highlights that mutated human tau prone to hyperphosphorylation reduces LC neurite length and sensitizes the neurons to a lower threshold of toxins. In turn, our *in* vivo data from Chapters 3 and 4 show that LC degeneration has pronounced effects on tau pathology and cognitive functions in a tau mouse model. These reciprocal effects have great implications for the role of the LC in AD. The important role of the LC in AD pathogenesis underscores the need to investigate early changes in the disease. Further elucidating the role of the LC could also allow clinicians to adapt and apply already approved therapeutics to help combat the dysfunction from the loss of the noradrenergic system. Together, these studies suggest that the locus coeruleus and the early accumulation of tau pathology within it may be a strong, novel target for both diagnostics and therapeutics that could pave the way towards alleviating the AD crisis.

REFERENCES

Abbott, A. and E. Dolgin (2016). "Failed Alzheimer's trial does not kill leading theory of disease." Nature **540**(7631): 15-16.

Allen, B., E. Ingram, M. Takao, M. J. Smith, R. Jakes, K. Virdee, H. Yoshida, M. Holzer, M. Craxton, P. C. Emson, C. Atzori, A. Migheli, R. A. Crowther, B. Ghetti, M. G. Spillantini and M. Goedert (2002). "Abundant tau filaments and nonapoptotic neurodegeneration in transgenic mice expressing human P301S tau protein." J Neurosci **22**(21): 9340-9351.

Allison, S. L., A. M. Fagan, J. C. Morris and D. Head (2016). "Spatial Navigation in Preclinical Alzheimer's Disease." J Alzheimers Dis **52**(1): 77-90.

Alonso, A. C., B. Li, I. Grundke-Iqbal and K. Iqbal (2008). "Mechanism of tau-induced neurodegeneration in Alzheimer disease and related tauopathies." Curr Alzheimer Res **5**(4): 375-384.

Alonso, A. D., J. Di Clerico, B. Li, C. P. Corbo, M. E. Alaniz, I. Grundke-Iqbal and K. Iqbal (2010). "Phosphorylation of tau at Thr212, Thr231, and Ser262 combined causes neurodegeneration." J Biol Chem **285**(40): 30851-30860.

Alzheimer'sAssociation (2016). 2016 Alzheimer's Disease Facts and Figures. **12**.

Alzheimer'sAssociation. (2016). "Diagnostic Procedures." from http://www.alz.org/professionals_and_researchers_diagnostic_procedures.asp.

Alzheimer'sAssociation. (2016). "What Is Dementia?" Retrieved 8/26/16, 2016, from http://www.alz.org/what-is-dementia.asp.

Alzheimer, A., R. A. Stelzmann, H. N. Schnitzlein and F. R. Murtagh (1995). "An English translation of Alzheimer's 1907 paper, "Uber eine eigenartige Erkankung der Hirnrinde"." Clin Anat **8**(6): 429-431.

Amaral, D. G. and J. A. Foss (1975). "Locus coeruleus lesions and learning." Science **188**(4186): 377-378.

Amaral, D. G. and H. M. Sinnamon (1977). "The locus coeruleus: neurobiology of a central noradrenergic nucleus." Prog Neurobiol **9**(3): 147-196.

Archer, T., G. Jonsson and S. B. Ross (1984). "A parametric study of the effects of the noradrenaline neurotoxin DSP4 on avoidance acquisition and noradrenaline neurones in the CNS of the rat." Br J Pharmacol **82**(1): 249-257.

Arriagada, P. V., J. H. Growdon, E. T. Hedley-Whyte and B. T. Hyman (1992). "Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease." Neurology **42**(3 Pt 1): 631-639.

Attems, J., D. R. Thal and K. A. Jellinger (2012). "The relationship between subcortical tau pathology and Alzheimer's disease." Biochem Soc Trans **40**(4): 711-715.

Bailey, K. R. and J. N. Crawley (2009). Anxiety-Related Behaviors in Mice. Methods of Behavior Analysis in Neuroscience. J. J. Buccafusco. Boca Raton (FL).

Ballatore, C., K. R. Brunden, D. M. Huryn, J. Q. Trojanowski, V. M. Lee and A. B. Smith, 3rd (2012). "Microtubule stabilizing agents as potential treatment for Alzheimer's disease and related neurodegenerative tauopathies." J Med Chem **55**(21): 8979-8996.

Bancher, C., C. Brunner, H. Lassmann, H. Budka, K. Jellinger, G. Wiche, F. Seitelberger, I. Grundke-Iqbal, K. Iqbal and H. M. Wisniewski (1989). "Accumulation of abnormally phosphorylated τ precedes the formation of neurofibrillary tangles in Alzheimer's disease." Brain Research **477**(1-2): 90-99.

Bangasser, D. A., K. R. Wiersielis and S. Khantsis (2016). "Sex differences in the locus coeruleus-norepinephrine system and its regulation by stress." Brain Res **1641**(Pt B): 177-188.

Bangasser, D. A., X. Zhang, V. Garachh, E. Hanhauser and R. J. Valentino (2011). "Sexual dimorphism in locus coeruleus dendritic morphology: a structural basis for sex differences in emotional arousal." Physiol Behav **103**(3-4): 342-351.

Bardou, I., R. M. Kaercher, H. M. Brothers, S. C. Hopp, S. Royer and G. L. Wenk (2014). "Age and duration of inflammatory environment differentially affect the neuroimmune response and catecholaminergic neurons in the midbrain and brainstem." Neurobiol Aging **35**(5): 1065-1073.

Barker, W. W., C. A. Luis, A. Kashuba, M. Luis, D. G. Harwood, D. Loewenstein, C. Waters, P. Jimison, E. Shepherd, S. Sevush, N. Graff-Radford, D. Newland, M. Todd, B. Miller, M. Gold, K. Heilman, L. Doty, I. Goodman, B. Robinson, G. Pearl, D. Dickson and R. Duara (2002). "Relative frequencies of Alzheimer disease, Lewy body, vascular and frontotemporal dementia, and hippocampal sclerosis in the State of Florida Brain Bank." Alzheimer Dis Assoc Disord **16**(4): 203-212.

Bateman, R. J., P. S. Aisen, B. De Strooper, N. C. Fox, C. A. Lemere, J. M. Ringman, S. Salloway, R. A. Sperling, M. Windisch and C. Xiong (2011). "Autosomal-dominant Alzheimer's disease: a review and proposal for the prevention of Alzheimer's disease." Alzheimers Res Ther **3**(1): 1.

Benarroch, E. E. (2009). "The locus ceruleus norepinephrine system: functional organization and potential clinical significance." Neurology **73**(20): 1699-1704.

Benilova, I., E. Karran and B. De Strooper (2012). "The toxic Abeta oligomer and Alzheimer's disease: an emperor in need of clothes." Nat Neurosci **15**(3): 349-357.

Benitez-King, G., L. Ortiz-Lopez, S. Morales-Mulia, G. Jimenez-Rubio, G. Ramirez-Rodriguez and I. Meza (2006). "Phosphorylation-dephosphorylation imbalance of cytoskeletal associated proteins in neurodegenerative diseases." Recent Pat CNS Drug Discov **1**(2): 219-230.

Berridge, C. W., B. E. Schmeichel and R. A. Espana (2012). "Noradrenergic modulation of wakefulness/arousal." Sleep Med Rev **16**(2): 187-197.

Berridge, C. W. and B. D. Waterhouse (2003). "The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes." Brain Res Brain Res Rev **42**(1): 33-84.

Bird, T. D. (1998). Alzheimer Disease Overview.

Blennow, K. (2004). "Cerebrospinal fluid protein biomarkers for Alzheimer's disease." NeuroRx **1**(2): 213-225.

Blennow, K., M. J. de Leon and H. Zetterberg (2006). "Alzheimer's disease." Lancet **368**(9533): 387-403.

Blennow, K. and H. Hampel (2003). "CSF markers for incipient Alzheimer's disease." Lancet Neurol **2**(10): 605-613.

Bliwise, D. L. (2004). "Sleep disorders in Alzheimer's disease and other dementias." Clin Cornerstone **6 Suppl 1A**: S16-28.

Boksa, P., D. Aitken and M. Meaney (1989). "Effects of the catecholaminergic neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) on adrenal chromaffin cells in culture." Biochem Pharmacol **38**(9): 1491-1498.

Bondareff, W., C. Q. Mountjoy, M. Roth, M. N. Rossor, L. L. Iversen, G. P. Reynolds and D. L. Hauser (1987). "Neuronal degeneration in locus ceruleus and cortical correlates of Alzheimer disease." Alzheimer Dis Assoc Disord **1**(4): 256-262.

Borsody, M. K. and J. M. Weiss (2002). "Peripheral endotoxin causes long-lasting changes in locus coeruleus activity via IL-1 in the brain." Acta Neuropsychiatr **14**(6): 303-321.

Borsody, M. K. and J. M. Weiss (2004). "The effects of endogenous interleukin-1 bioactivity on locus coeruleus neurons in response to bacterial and viral substances." Brain Res **1007**(1-2): 39-56.

Braak, H. and E. Braak (1995). "Staging of Alzheimer's disease-related neurofibrillary changes." Neurobiol Aging **16**(3): 271-278; discussion 278-284.

Braak, H. and K. Del Tredici (2011). "Alzheimer's pathogenesis: is there neuron-to-neuron propagation?" Acta Neuropathol **121**(5): 589-595.

Braak, H. and K. Del Tredici (2011). "The pathological process underlying Alzheimer's disease in individuals under thirty." Acta Neuropathol **121**(2): 171-181.

Braak, H., D. R. Thal, E. Ghebremedhin and K. Del Tredici (2011). "Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years." J Neuropathol Exp Neurol **70**(11): 960-969.

Braithwaite, S. P., J. B. Stock, P. J. Lombroso and A. C. Nairn (2012). "Protein phosphatases and Alzheimer's disease." Prog Mol Biol Transl Sci **106**: 343-379.

Bretteville, A., F. Marcouiller, C. Julien, N. B. El Khoury, F. R. Petry, I. Poitras, D. Mouginot, G. Levesque, S. S. Hebert and E. Planel (2012). "Hypothermia-induced hyperphosphorylation: a new model to study tau kinase inhibitors." Sci Rep **2**: 480.

Buerger, K., M. Ewers, T. Pirttilä, R. Zinkowski, I. Alafuzoff, S. J. Teipel, J. DeBernardis, D. Kerkman, C. McCulloch, H. Soininen and H. Hampel (2006). "CSF phosphorylated tau protein correlates with neocortical neurofibrillary pathology in Alzheimer's disease." Brain **129**(11): 3035-3041.

Bugiani, O. (2000). "FTDP-17: phenotypical heterogeneity within P301S." Ann Neurol **48**(1): 126.

Bugiani, O., J. R. Murrell, G. Giaccone, M. Hasegawa, G. Ghigo, M. Tabaton, M. Morbin, A. Primavera, F. Carella, C. Solaro, M. Grisoli, M. Savoiardo, M. G. Spillantini, F. Tagliavini, M. Goedert and B. Ghetti (1999). "Frontotemporal dementia and corticobasal degeneration in a family with a P301S mutation in tau." J Neuropathol Exp Neurol **58**(6): 667-677.

Bullido, M. J., M. C. Ramos, A. Ruiz-Gomez, A. S. Tutor, I. Sastre, A. Frank, F. Coria, P. Gil, F. Mayor, Jr. and F. Valdivieso (2004). "Polymorphism in genes involved in adrenergic signaling associated with Alzheimer's." Neurobiol Aging **25**(7): 853-859.

Butterfield, D. A. (2002). "Amyloid beta-peptide (1-42)-induced oxidative stress and neurotoxicity: implications for neurodegeneration in Alzheimer's disease brain. A review." Free Radic Res **36**(12): 1307-1313.

Cagampang, F. R., H. Okamura and S. Inouye (1994). "Circadian rhythms of norepinephrine in the rat suprachiasmatic nucleus." Neurosci Lett **173**(1-2): 185-188.

Cantero, J. L., E. Hita-Yanez, B. Moreno-Lopez, F. Portillo, A. Rubio and J. Avila (2010). "Tau protein role in sleep-wake cycle." J Alzheimers Dis **21**(2): 411-421.

Carrettiero, D. C., F. E. Santiago, A. C. P. Motzko-Soares and M. C. Almeida (2015). "Temperature and toxic Tau in Alzheimer's disease: new insights." Temperature: Multidisciplinary Biomedical Journal **2**(4): 491-498.

Casey, D. A., D. Antimisiaris and J. O'Brien (2010). "Drugs for Alzheimer's Disease: Are They Effective?" Pharmacy and Therapeutics **35**(4): 208-211.

Castellano, J. M., J. Kim, F. R. Stewart, H. Jiang, R. B. DeMattos, B. W. Patterson, A. M. Fagan, J. C. Morris, K. G. Mawuenyega, C. Cruchaga, A. M. Goate, K. R. Bales, S. M. Paul, R. J. Bateman and D. M. Holtzman (2011). "Human apoE isoforms differentially regulate brain amyloid-beta peptide clearance." Sci Transl Med **3**(89): 89ra57.

Catafau, A. M. and S. Bullich (2015). "Amyloid PET imaging: applications beyond Alzheimer's disease." Clin Transl Imaging **3**(1): 39-55.

Cerf, E., A. Gustot, E. Goormaghtigh, J. M. Ruysschaert and V. Raussens (2011). "High ability of apolipoprotein E4 to stabilize amyloid-beta peptide oligomers, the pathological entities responsible for Alzheimer's disease." FASEB J **25**(5): 1585-1595.

Chan-Palay, V. and E. Asan (1989). "Alterations in catecholamine neurons of the locus coeruleus in senile dementia of the Alzheimer type and in Parkinson's disease with and without dementia and depression." J Comp Neurol **287**(3): 373-392.

Chaunu, M. P., V. Deramecourt, V. Buee-Scherrer, I. Le Ber, A. Brice, N. Ehrle, K. El Hachimi, M. Pluot, C. A. Maurage, S. Bakchine and L. Buee (2013). "Juvenile frontotemporal

dementia with parkinsonism associated with tau mutation G389R." J Alzheimers Dis **37**(4): 769-776.

Cho, M. (2014). "Pacemaker's burden." Nat Neurosci **17**(6): 755.

Citron, M. (2010). "Alzheimer's disease: strategies for disease modification." Nat Rev Drug Discov **9**(5): 387-398.

Claudio, L. (1996). "Ultrastructural features of the blood-brain barrier in biopsy tissue from Alzheimer's disease patients." Acta Neuropathol **91**(1): 6-14.

Clavaguera, F., T. Bolmont, R. A. Crowther, D. Abramowski, S. Frank, A. Probst, G. Fraser, A. K. Stalder, M. Beibel, M. Staufenbiel, M. Jucker, M. Goedert and M. Tolnay (2009). "Transmission and spreading of tauopathy in transgenic mouse brain." Nat Cell Biol **11**(7): 909-913.

Cohen, R. M., K. Rezai-Zadeh, T. M. Weitz, A. Rentsendorj, D. Gate, I. Spivak, Y. Bholat, V. Vasilevko, C. G. Glabe, J. J. Breunig, P. Rakic, H. Davtyan, M. G. Agadjanyan, V. Kepe, J. R. Barrio, S. Bannykh, C. A. Szekely, R. N. Pechnick and T. Town (2013). "A transgenic Alzheimer rat with plaques, tau pathology, behavioral impairment, oligomeric abeta, and frank neuronal loss." J Neurosci **33**(15): 6245-6256.

Combarros, O., D. R. Warden, N. Hammond, M. Cortina-Borja, O. Belbin, M. G. Lehmann, G. K. Wilcock, K. Brown, P. G. Kehoe, R. Barber, E. Coto, V. Alvarez, P. Deloukas, R. Gwilliam, R. Heun, H. Kolsch, I. Mateo, A. Oulhaj, A. Arias-Vasquez, M. Schuur, Y. S. Aulchenko, M. A. Ikram, M. M. Breteler, C. M. van Duijn, K. Morgan, A. D. Smith and D. J. Lehmann (2010). "The dopamine beta-hydroxylase -1021C/T polymorphism is associated with the risk of Alzheimer's disease in the Epistasis Project." BMC Med Genet **11**: 162.

Counts, S. E. and E. J. Mufson (2010). "Noradrenaline activation of neurotrophic pathways protects against neuronal amyloid toxicity." J Neurochem **113**(3): 649-660.

Cross, A. J., T. J. Crow, E. K. Perry, R. H. Perry, G. Blessed and B. E. Tomlinson (1981). "Reduced dopamine-beta-hydroxylase activity in Alzheimer's disease." British Medical Journal (Clinical research ed.) **282**(6258): 93-94.

Cruchaga, C., J. S. Kauwe, O. Harari, S. C. Jin, Y. Cai, C. M. Karch, B. A. Benitez, A. T. Jeng, T. Skorupa, D. Carrell, S. Bertelsen, M. Bailey, D. McKean, J. M. Shulman, P. L. De Jager, L. Chibnik, D. A. Bennett, S. E. Arnold, D. Harold, R. Sims, A. Gerrish, J. Williams, V. M. Van Deerlin, V. M. Lee, L. M. Shaw, J. Q. Trojanowski, J. L. Haines, R. Mayeux, M. A. Pericak-Vance, L. A. Farrer, G. D. Schellenberg, E. R. Peskind, D. Galasko, A. M. Fagan, D. M. Holtzman, J. C. Morris and A. M. Goate (2013). "GWAS of cerebrospinal fluid tau levels identifies risk variants for Alzheimer's disease." Neuron **78**(2): 256-268.

Cummings, J. L. and D. Kaufer (1996). "Neuropsychiatric aspects of Alzheimer's disease: the cholinergic hypothesis revisited." Neurology **47**(4): 876-883.

D'Hooge, R. and P. P. De Deyn (2001). "Applications of the Morris water maze in the study of learning and memory." Brain Res Brain Res Rev **36**(1): 60-90.

Davis, J., M. Maillet, J. M. Miano and J. D. Molkentin (2012). "Lost in Transgenesis: A Users guide for Genetically Manipulating the Mouse in Cardiac Research." Circulation research **111**(6): 761-777.

Dawson, H. N., A. Ferreira, M. V. Eyster, N. Ghoshal, L. I. Binder and M. P. Vitek (2001). "Inhibition of neuronal maturation in primary hippocampal neurons from τ deficient mice." Journal of cell science **114**(6): 1179-1187.

de Calignon, A., M. Polydoro, M. Suarez-Calvet, C. William, D. H. Adamowicz, K. J. Kopeikina, R. Pitstick, N. Sahara, K. H. Ashe, G. A. Carlson, T. L. Spires-Jones and B. T. Hyman (2012). "Propagation of tau pathology in a model of early Alzheimer's disease." Neuron **73**(4): 685-697.

Delacourte, A. and A. Defossez (1986). "Alzheimer's disease: Tau proteins, the promoting factors of microtubule assembly, are major components of paired helical filaments." J Neurol Sci **76**(2-3): 173-186.

Dolan, P. J. and G. V. W. Johnson (2010). "The role of tau kinases in Alzheimer's disease." Curr Opin Drug Discov Devel **13**(5): 595-603.

Doody, R. S., R. G. Thomas, M. Farlow, T. Iwatsubo, B. Vellas, S. Joffe, K. Kieburtz, R. Raman, X. Sun, P. S. Aisen, E. Siemers, H. Liu-Seifert and R. Mohs (2014). "Phase 3 trials of solanezumab for mild-to-moderate Alzheimer's disease." N Engl J Med **370**(4): 311-321.

Drechsel, D. N., A. A. Hyman, M. H. Cobb and M. W. Kirschner (1992). "Modulation of the dynamic instability of tubulin assembly by the microtubule-associated protein tau." Mol Biol Cell **3**(10): 1141-1154.

Drewes, G., B. Trinczek, S. Illenberger, J. Biernat, G. Schmitt-Ulms, H. E. Meyer, E. M. Mandelkow and E. Mandelkow (1995). "Microtubule-associated protein/microtubule affinity-regulating kinase (p110mark). A novel protein kinase that regulates tau-microtubule interactions and dynamic instability by phosphorylation at the Alzheimer-specific site serine 262." J Biol Chem **270**(13): 7679-7688.

Dudley, M. W., B. D. Howard and A. K. Cho (1990). "The interaction of the beta-haloethyl benzylamines, xylamine, and DSP-4 with catecholaminergic neurons." Annu Rev Pharmacol Toxicol **30**: 387-403.

Dujardin, S., M. Colin and L. Buee (2015). "Invited review: Animal models of tauopathies and their implications for research/translation into the clinic." Neuropathol Appl Neurobiol **41**(1): 59-80.

Dumont, M., C. Stack, C. Elipenahli, S. Jainuddin, M. Gerges, N. N. Starkova, L. Yang, A. A. Starkov and F. Beal (2011). "Behavioral deficit, oxidative stress, and mitochondrial dysfunction precede tau pathology in P301S transgenic mice." Faseb j **25**(11): 4063-4072.

Eckel-Mahan, K. and P. Sassone-Corsi (2015). "Phenotyping Circadian Rhythms in Mice." Curr Protoc Mouse Biol **5**(3): 271-281.

Eiden, L. E., M. K. Schafer, E. Weihe and B. Schutz (2004). "The vesicular amine transporter family (SLC18): amine/proton antiporters required for vesicular accumulation and regulated exocytotic secretion of monoamines and acetylcholine." Pflugers Arch **447**(5): 636-640.

Eisenhofer, G., I. J. Kopin and D. S. Goldstein (2004). "Catecholamine metabolism: a contemporary view with implications for physiology and medicine." Pharmacol Rev **56**(3): 331-349.

Elobeid, A., H. Soininen and I. Alafuzoff (2012). "Hyperphosphorylated tau in young and middle-aged subjects." Acta Neuropathol **123**(1): 97-104.

Elrod, R., E. R. Peskind, L. DiGiacomo, K. I. Brodkin, R. C. Veith and M. A. Raskind (1997). "Effects of Alzheimer's disease severity on cerebrospinal fluid norepinephrine concentration." Am J Psychiatry **154**(1): 25-30.

Fá, M., D. Puzzo, R. Piacentini, A. Staniszewski, H. Zhang, M. A. Baltrons, D. D. Li Puma, I. Chatterjee, J. Li, F. Saeed, H. L. Berman, C. Ripoli, W. Gulisano, J. Gonzalez, H. Tian, J. A. Costa, P. Lopez, E. Davidowitz, W. H. Yu, V. Haroutunian, L. M. Brown, A. Palmeri, E. M. Sigurdsson, K. E. Duff, A. F. Teich, L. S. Honig, M. Sierks, J. G. Moe, L. D'Adamio, C. Grassi, N. M. Kanaan, P. E. Fraser and O. Arancio (2016). "Extracellular Tau Oligomers Produce An Immediate Impairment of LTP and Memory." Scientific Reports **6**: 19393.

Fagan, A. M., M. A. Mintun, R. H. Mach, S.-Y. Lee, C. S. Dence, A. R. Shah, G. N. LaRossa, M. L. Spinner, W. E. Klunk, C. A. Mathis, S. T. DeKosky, J. C. Morris and D. M. Holtzman (2006). "Inverse relation between in vivo amyloid imaging load and cerebrospinal fluid Aβ42 in humans." Annals of Neurology **59**(3): 512-519.

Feinstein, D. L., M. T. Heneka, V. Gavrilyuk, C. Dello Russo, G. Weinberg and E. Galea (2002). "Noradrenergic regulation of inflammatory gene expression in brain." Neurochem Int **41**(5): 357-365.

Feinstein, D. L., S. Kalinin and D. Braun (2016). "Causes, consequences, and cures for neuroinflammation mediated via the locus coeruleus: noradrenergic signaling system." J Neurochem **139 Suppl 2**: 154-178.

Ferreira, T. A., A. V. Blackman, J. Oyrer, S. Jayabal, A. J. Chung, A. J. Watt, P. J. Sjostrom and D. J. van Meyel (2014). "Neuronal morphometry directly from bitmap images." Nat Meth **11**(10): 982-984.

Foote, S. L., F. E. Bloom and G. Aston-Jones (1983). "Nucleus locus ceruleus: new evidence of anatomical and physiological specificity." Physiol Rev **63**(3): 844-914.

Forno, L. (1966). "Pathology of Parkinsonism." J Neurosurgery **2**: 266-271.

Forstl, H., A. Burns, R. Levy and N. Cairns (1994). "Neuropathological correlates of psychotic phenomena in confirmed Alzheimer's disease." Br J Psychiatry **165**(2): 53-59.

Forstl, H., R. Levy, A. Burns, P. Luthert and N. Cairns (1994). "Disproportionate loss of noradrenergic and cholinergic neurons as cause of depression in Alzheimer's disease--a hypothesis." Pharmacopsychiatry **27**(1): 11-15.

Freedman, R., S. L. Foote and F. E. Bloom (1975). "Histochemical characterization of a neocortical projection of the nucleus locus coeruleus in the squirrel monkey." J Comp Neurol **164**(2): 209-231.

Fritschy, J. M. and R. Grzanna (1989). "Immunohistochemical analysis of the neurotoxic effects of DSP-4 identifies two populations of noradrenergic axon terminals." Neuroscience **30**(1): 181-197.

Fritschy, J. M. and R. Grzanna (1990). "Demonstration of two separate descending noradrenergic pathways to the rat spinal cord: evidence for an intragriseal trajectory of locus coeruleus axons in the superficial layers of the dorsal horn." J Comp Neurol **291**(4): 553-582.

Fritschy, J. M. and R. Grzanna (1991). "Selective effects of DSP-4 on locus coeruleus axons: are there pharmacologically different types of noradrenergic axons in the central nervous system?" Prog Brain Res **88**: 257-268.

Fujio, K., M. Sato, T. Uemura, T. Sato, R. Sato-Harada and A. Harada (2007). "14-3-3 proteins and protein phosphatases are not reduced in tau-deficient mice." Neuroreport **18**(10): 1049-1052.

Gallagher, D., R. Coen, D. Kilroy, K. Belinski, I. Bruce, D. Coakley, B. Walsh, C. Cunningham and B. A. Lawlor (2011). "Anxiety and behavioural disturbance as markers of prodromal Alzheimer's disease in patients with mild cognitive impairment." Int J Geriatr Psychiatry **26**(2): 166-172.

Gannon, M., P. Che, Y. Chen, K. Jiao, E. D. Roberson and Q. Wang (2015). "Noradrenergic dysfunction in Alzheimer's disease." Frontiers in Neuroscience **9**: 220.

Gardner, L. E., J. D. White, M. J. Eimerbrink, G. W. Boehm and M. J. Chumley (2016). "Imatinib methanesulfonate reduces hyperphosphorylation of tau following repeated peripheral exposure to lipopolysaccharide." Neuroscience **331**: 72-77.

Georganopoulou, D. G., L. Chang, J. M. Nam, C. S. Thaxton, E. J. Mufson, W. L. Klein and C. A. Mirkin (2005). "Nanoparticle-based detection in cerebral spinal fluid of a soluble pathogenic biomarker for Alzheimer's disease." Proc Natl Acad Sci U S A **102**(7): 2273-2276.

German, D. C., K. F. Manaye, C. L. White, 3rd, D. J. Woodward, D. D. McIntire, W. K. Smith, R. N. Kalaria and D. M. Mann (1992). "Disease-specific patterns of locus coeruleus cell loss." Ann Neurol **32**(5): 667-676.

German, D. C., O. Nelson, F. Liang, C.-L. Liang and D. Games (2005). "The PDAPP mouse model of Alzheimer's disease: Locus coeruleus neuronal shrinkage." The Journal of Comparative Neurology **492**(4): 469-476.

Giannakopoulos, P., F. R. Herrmann, T. Bussiere, C. Bouras, E. Kovari, D. P. Perl, J. H. Morrison, G. Gold and P. R. Hof (2003). "Tangle and neuron numbers, but not amyloid load, predict cognitive status in Alzheimer's disease." Neurology **60**(9): 1495-1500.

Gibbs, M. E., D. Maksel, Z. Gibbs, X. Hou, R. J. Summers and D. H. Small (2010). "Memory loss caused by beta-amyloid protein is rescued by a beta(3)-adrenoceptor agonist." Neurobiol Aging **31**(4): 614-624.

Gilsbach, R. and L. Hein (2008). "Presynaptic metabotropic receptors for acetylcholine and adrenaline/noradrenaline." Handb Exp Pharmacol(184): 261-288.

Glenner, G. G. and C. W. Wong (1984). "Alzheimer's disease and Down's syndrome: sharing of a unique cerebrovascular amyloid fibril protein." Biochem Biophys Res Commun **122**(3): 1131-1135.

Goedert, M., A. Klug and R. A. Crowther (2006). "Tau protein, the paired helical filament and Alzheimer's disease." J Alzheimers Dis **9**(3 Suppl): 195-207.

Goedert, M. and M. G. Spillantini (2000). "Tau mutations in frontotemporal dementia FTDP-17 and their relevance for Alzheimer's disease." Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease **1502**(1): 110-121.

Goedert, M. and M. G. Spillantini (2006). "A century of Alzheimer's disease." Science **314**(5800): 777-781.

Goedert, M., M. G. Spillantini, M. C. Potier, J. Ulrich and R. A. Crowther (1989). "Cloning and sequencing of the cDNA encoding an isoform of microtubule-associated protein tau containing four tandem repeats: differential expression of tau protein mRNAs in human brain." Embo j **8**(2): 393-399.

Goedert, M., C. M. Wischik, R. A. Crowther, J. E. Walker and A. Klug (1988). "Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: identification as the microtubule-associated protein tau." Proc Natl Acad Sci U S A **85**(11): 4051-4055.

Goosens, K. A. and S. Maren (2001). "Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats." Learn Mem **8**(3): 148-155.

Greendyke, R. M., D. R. Kanter, D. B. Schuster, S. Verstreate and J. Wootton (1986). "Propranolol treatment of assaultive patients with organic brain disease. A double-blind crossover, placebo-controlled study." J Nerv Ment Dis **174**(5): 290-294.

Grudzien, A., P. Shaw, S. Weintraub, E. Bigio, D. C. Mash and M. M. Mesulam (2007). "Locus coeruleus neurofibrillary degeneration in aging, mild cognitive impairment and early Alzheimer's disease." Neurobiol Aging **28**(3): 327-335.

Guerin, D., J. Sacquet, N. Mandairon, F. Jourdan and A. Didier (2009). "Early locus coeruleus degeneration and olfactory dysfunctions in Tg2576 mice." Neurobiol Aging **30**(2): 272-283.

Guillamon, A., M. R. de Blas and S. Segovia (1988). "Effects of sex steroids on the development of the locus coeruleus in the rat." Brain Res **468**(2): 306-310.

Gulyas, B., D. Brockschnieder, S. Nag, E. Pavlova, P. Kasa, Z. Beliczai, A. Legradi, K. Gulya, A. Thiele, T. Dyrks and C. Halldin (2010). "The norepinephrine transporter (NET) radioligand (S,S)-[18F]FMeNER-D2 shows significant decreases in NET density in the human brain in Alzheimer's disease: a post-mortem autoradiographic study." Neurochem Int **56**(6-7): 789-798.

Guyenet, S. J., S. A. Furrer, V. M. Damian, T. D. Baughan, A. R. La Spada and G. A. Garden (2010). "A simple composite phenotype scoring system for evaluating mouse models of cerebellar ataxia." J Vis Exp(39).

Haass, C., M. G. Schlossmacher, A. Y. Hung, C. Vigo-Pelfrey, A. Mellon, B. L. Ostaszewski, I. Lieberburg, E. H. Koo, D. Schenk, D. B. Teplow and et al. (1992). "Amyloid beta-peptide is produced by cultured cells during normal metabolism." Nature **359**(6393): 322-325.

Haglund, M., M. Sjobeck and E. Englund (2006). "Locus ceruleus degeneration is ubiquitous in Alzheimer's disease: possible implications for diagnosis and treatment." Neuropathology **26**(6): 528-532.

Hajjar, I., H. Catoe, S. Sixta, R. Boland, D. Johnson, V. Hirth, D. Wieland and P. Eleazer (2005). "Cross-sectional and longitudinal association between antihypertensive medications and cognitive impairment in an elderly population." J Gerontol A Biol Sci Med Sci **60**(1): 67-73.

Hammerschmidt, T., M. P. Kummer, D. Terwel, A. Martinez, A. Gorji, H. C. Pape, K. S. Rommelfanger, J. P. Schroeder, M. Stoll, J. Schultze, D. Weinshenker and M. T. Heneka (2012). "Selective Loss of Noradrenaline Exacerbates Early Cognitive Dysfunction and Synaptic Deficits in APP/PS1 Mice." Biol Psychiatry.

Hammerschmidt, T., M. P. Kummer, D. Terwel, A. Martinez, A. Gorji, H. C. Pape, K. S. Rommelfanger, J. P. Schroeder, M. Stoll, J. Schultze, D. Weinshenker and M. T. Heneka (2013). "Selective loss of noradrenaline exacerbates early cognitive dysfunction and synaptic deficits in APP/PS1 mice." Biol Psychiatry **73**(5): 454-463.

Hampton, D. W., D. J. Webber, B. Bilican, M. Goedert, M. G. Spillantini and S. Chandran (2010). "Cell-mediated neuroprotection in a mouse model of human tauopathy." J Neurosci **30**(30): 9973-9983.

Hanger, D. P., B. H. Anderton and W. Noble (2009). "Tau phosphorylation: the therapeutic challenge for neurodegenerative disease." Trends Mol Med **15**(3): 112-119.

Harada, A., K. Oguchi, S. Okabe, J. Kuno, S. Terada, T. Ohshima, R. Sato-Yoshitake, Y. Takei, T. Noda and N. Hirokawa (1994). "Altered microtubule organization in small-calibre axons of mice lacking tau protein." Nature **369**(6480): 488-491.

Hardy, J. and D. J. Selkoe (2002). "The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics." Science **297**(5580): 353-356.

Harik, S. I. (1986). "Blood--brain barrier sodium/potassium pump: modulation by central noradrenergic innervation." Proc Natl Acad Sci U S A **83**(11): 4067-4070.

Harik, S. I. and T. McGunigal, Jr. (1984). "The protective influence of the locus ceruleus on the blood-brain barrier." Ann Neurol **15**(6): 568-574.

Harley, C. W. (2007). "Norepinephrine and the dentate gyrus." Prog Brain Res **163**: 299-318.

Harro, J., L. Oreland, E. Vasar and J. Bradwejn (1995). "Impaired exploratory behaviour after DSP-4 treatment in rats: implications for the increased anxiety after noradrenergic denervation." Eur Neuropsychopharmacol **5**(4): 447-455.

Hart, N. J., Y. Koronyo, K. L. Black and M. Koronyo-Hamaoui (2016). "Ocular indicators of Alzheimer's: exploring disease in the retina." Acta Neuropathol.

Hauser, J., T. A. Sontag, O. Tucha and K. W. Lange (2012). "The effects of the neurotoxin DSP4 on spatial learning and memory in Wistar rats." Atten Defic Hyperact Disord **4**(2): 93-99.

Heaton, G. R., B. M. Davis, L. A. Turner and M. F. Cordeiro (2015). "Ocular biomarkers of Alzheimer's disease." Cent Nerv Syst Agents Med Chem **15**(2): 117-125.

Hebert, L. E., L. A. Beckett, P. A. Scherr and D. A. Evans (2001). "Annual incidence of Alzheimer disease in the United States projected to the years 2000 through 2050." Alzheimer Dis Assoc Disord **15**(4): 169-173.

Hebert, L. E., J. Weuve, P. A. Scherr and D. A. Evans (2013). "Alzheimer disease in the United States (2010-2050) estimated using the 2010 census." Neurology **80**(19): 1778-1783.

Heeba, G. H., M. E. Mahmoud and A. A. El Hanafy (2012). "Anti-inflammatory potential of curcumin and quercetin in rats: Role of oxidative stress, heme oxygenase-1 and TNF-alpha." Toxicol Ind Health.

Hendrie, H. C. (1998). "Epidemiology of dementia and Alzheimer's disease." Am J Geriatr Psychiatry **6**(2 Suppl 1): S3-18.

Heneka, M. T., M. J. Carson, J. El Khoury, G. E. Landreth, F. Brosseron, D. L. Feinstein, A. H. Jacobs, T. Wyss-Coray, J. Vitorica, R. M. Ransohoff, K. Herrup, S. A. Frautschy, B.

Finsen, G. C. Brown, A. Verkhratsky, K. Yamanaka, J. Koistinaho, E. Latz, A. Halle, G. C. Petzold, T. Town, D. Morgan, M. L. Shinohara, V. H. Perry, C. Holmes, N. G. Bazan, D. J. Brooks, S. Hunot, B. Joseph, N. Deigendesch, O. Garaschuk, E. Boddeke, C. A. Dinarello, J. C. Breitner, G. M. Cole, D. T. Golenbock and M. P. Kummer (2015). "Neuroinflammation in Alzheimer's disease." Lancet Neurol **14**(4): 388-405.

Heneka, M. T., M. J. Carson, J. E. Khoury, G. E. Landreth, F. Brosseron, D. L. Feinstein, A. H. Jacobs, T. Wyss-Coray, J. Vitorica, R. M. Ransohoff, K. Herrup, S. A. Frautschy, B. Finsen, G. C. Brown, A. Verkhratsky, K. Yamanaka, J. Koistinaho, E. Latz, A. Halle, G. C. Petzold, T. Town, D. Morgan, M. L. Shinohara, V. H. Perry, C. Holmes, N. G. Bazan, D. J. Brooks, S. Hunot, B. Joseph, N. Deigendesch, O. Garaschuk, E. Boddeke, C. A. Dinarello, J. C. Breitner, G. M. Cole, D. T. Golenbock and M. P. Kummer "Neuroinflammation in Alzheimer's disease." The Lancet Neurology **14**(4): 388-405.

Heneka, M. T., E. Galea, V. Gavriluyk, L. Dumitrescu-Ozimek, J. Daeschner, M. K. O'Banion, G. Weinberg, T. Klockgether and D. L. Feinstein (2002). "Noradrenergic depletion potentiates beta -amyloid-induced cortical inflammation: implications for Alzheimer's disease." J Neurosci **22**(7): 2434-2442.

Heneka, M. T., F. Nadrigny, T. Regen, A. Martinez-Hernandez, L. Dumitrescu-Ozimek, D. Terwel, D. Jardanhazi-Kurutz, J. Walter, F. Kirchhoff, U. K. Hanisch and M. P. Kummer (2010). "Locus ceruleus controls Alzheimer's disease pathology by modulating microglial functions through norepinephrine." Proc Natl Acad Sci U S A **107**(13): 6058-6063.

Heneka, M. T., M. Ramanathan, A. H. Jacobs, L. Dumitrescu-Ozimek, A. Bilkei-Gorzo, T. Debeir, M. Sastre, N. Galldiks, A. Zimmer, M. Hoehn, W. D. Heiss, T. Klockgether and M. Staufenbiel (2006). "Locus ceruleus degeneration promotes Alzheimer pathogenesis in amyloid precursor protein 23 transgenic mice." J Neurosci **26**(5): 1343-1354.

Heppner, F. L., R. M. Ransohoff and B. Becher (2015). "Immune attack: the role of inflammation in Alzheimer disease." Nat Rev Neurosci **16**(6): 358-372.

Herrmann, N., K. L. Lanctot and L. R. Khan (2004). "The role of norepinephrine in the behavioral and psychological symptoms of dementia." J Neuropsychiatry Clin Neurosci **16**(3): 261-276.

Heuer, E., R. F. Rosen, A. Cintron and L. C. Walker (2012). "Nonhuman primate models of Alzheimer-like cerebral proteopathy." Curr Pharm Des **18**(8): 1159-1169.

Ho, S. Y., C. Y. Chao, H. L. Huang, T. W. Chiu, P. Charoenkwan and E. Hwang (2011). "NeurphologyJ: an automatic neuronal morphology quantification method and its application in pharmacological discovery." BMC Bioinformatics **12**: 230.

Holsinger, R. M., J. S. Lee, A. Boyd, C. L. Masters and S. J. Collins (2006). "CSF BACE1 activity is increased in CJD and Alzheimer disease versus [corrected] other dementias." Neurology **67**(4): 710-712.

Holtzman, D. M., A. Goate, J. Kelly and R. Sperling (2011). "Mapping the road forward in Alzheimer's disease." Sci Transl Med **3**(114): 114ps148.

Holtzman, D. M., J. C. Morris and A. M. Goate (2011). "Alzheimer's disease: the challenge of the second century." Sci Transl Med **3**(77): 77sr71.

Howlett, D. R. and J. C. Richardson (2009). "The pathology of APP transgenic mice: a model of Alzheimer's disease or simply overexpression of APP?" Histol Histopathol **24**(1): 83-100.

Hunsley, M. S. and R. D. Palmiter (2003). "Norepinephrine-deficient mice exhibit normal sleep-wake states but have shorter sleep latency after mild stress and low doses of amphetamine." Sleep **26**(5): 521-526.

Hutton, M., C. L. Lendon, P. Rizzu, M. Baker, S. Froelich, H. Houlden, S. Pickering-Brown, S. Chakraverty, A. Isaacs, A. Grover, J. Hackett, J. Adamson, S. Lincoln, D. Dickson, P. Davies, R. C. Petersen, M. Stevens, E. de Graaff, E. Wauters, J. van Baren, M. Hillebrand, M. Joosse, J. M. Kwon, P. Nowotny, L. K. Che, J. Norton, J. C. Morris, L. A. Reed, J. Trojanowski, H. Basun, L. Lannfelt, M. Neystat, S. Fahn, F. Dark, T. Tannenberg, P. R. Dodd, N. Hayward, J. B. Kwok, P. R. Schofield, A. Andreadis, J.

Snowden, D. Craufurd, D. Neary, F. Owen, B. A. Oostra, J. Hardy, A. Goate, J. van Swieten, D. Mann, T. Lynch and P. Heutink (1998). "Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17." Nature **393**(6686): 702-705.

Iba, M., J. L. Guo, J. D. McBride, B. Zhang, J. Q. Trojanowski and V. M. Lee (2013). "Synthetic tau fibrils mediate transmission of neurofibrillary tangles in a transgenic mouse model of Alzheimer's-like tauopathy." J Neurosci **33**(3): 1024-1037.

Ikegami, S., A. Harada and N. Hirokawa (2000). "Muscle weakness, hyperactivity, and impairment in fear conditioning in tau-deficient mice." Neurosci Lett **279**(3): 129-132.

Iqbal, K., C. Alonso Adel, S. Chen, M. O. Chohan, E. El-Akkad, C. X. Gong, S. Khatoon, B. Li, F. Liu, A. Rahman, H. Tanimukai and I. Grundke-Iqbal (2005). "Tau pathology in Alzheimer disease and other tauopathies." Biochim Biophys Acta **1739**(2-3): 198-210.

Irie, K., K. Murakami, Y. Masuda, A. Morimoto, H. Ohigashi, H. Hara, R. Ohashi, K. Takegoshi, H. Fukuda, M. Nagao, T. Shimizu and T. Shirasawa (2007). "The toxic conformation of the 42-residue amyloid beta peptide and its relevance to oxidative stress in Alzheimer's disease." Mini Rev Med Chem **7**(10): 1001-1008.

Ittner, L. M., Y. D. Ke, F. Delerue, M. Bi, A. Gladbach, J. van Eersel, H. Wölfing, B. C. Chieng, M. J. Christie, I. A. Napier, A. Eckert, M. Staufenbiel, E. Hardeman and J. Götz (2010). "Dendritic Function of Tau Mediates Amyloid-β Toxicity in Alzheimer's Disease Mouse Models." Cell **142**(3): 387-397.

Iversen, L. L., M. N. Rossor, G. P. Reynolds, R. Hills, M. Roth, C. Q. Mountjoy, S. L. Foote, J. H. Morrison and F. E. Bloom (1983). "Loss of pigmented dopamine-beta-hydroxylase positive cells from locus coeruleus in senile dementia of Alzheimer's type." Neurosci Lett **39**(1): 95-100.

Jalbert, J. J., L. A. Daiello and K. L. Lapane (2008). "Dementia of the Alzheimer type." Epidemiol Rev **30**: 15-34.

James, O. G., P. M. Doraiswamy and S. Borges-Neto (2015). "PET Imaging of Tau Pathology in Alzheimer’s Disease and Tauopathies." Frontiers in Neurology **6**: 38.

Jardanhazi-Kurutz, D., M. P. Kummer, D. Terwel, K. Vogel, T. Dyrks, A. Thiele and M. T. Heneka (2010). "Induced LC degeneration in APP/PS1 transgenic mice accelerates early cerebral amyloidosis and cognitive deficits." Neurochem Int **57**(4): 375-382.

Jardanhazi-Kurutz, D., M. P. Kummer, D. Terwel, K. Vogel, A. Thiele and M. T. Heneka (2011). "Distinct adrenergic system changes and neuroinflammation in response to induced locus ceruleus degeneration in APP/PS1 transgenic mice." Neuroscience **176**: 396-407.

Jiang, T., L. Tan, X. C. Zhu, J. S. Zhou, L. Cao, M. S. Tan, H. F. Wang, Q. Chen, Y. D. Zhang and J. T. Yu (2015). "Silencing of TREM2 exacerbates tau pathology, neurodegenerative changes, and spatial learning deficits in P301S tau transgenic mice." Neurobiol Aging **36**(12): 3176-3186.

Jiang, T., J. T. Yu and L. Tan (2012). "Novel disease-modifying therapies for Alzheimer's disease." J Alzheimers Dis **31**(3): 475-492.

Jiang, T., Y. D. Zhang, Q. Chen, Q. Gao, X. C. Zhu, J. S. Zhou, J. Q. Shi, H. Lu, L. Tan and J. T. Yu (2016). "TREM2 modifies microglial phenotype and provides neuroprotection in P301S tau transgenic mice." Neuropharmacology **105**: 196-206.

Joachim, C. L. and D. J. Selkoe (1992). "The seminal role of beta-amyloid in the pathogenesis of Alzheimer disease." Alzheimer Dis Assoc Disord **6**(1): 7-34.

Johnson, G. V. and W. H. Stoothoff (2004). "Tau phosphorylation in neuronal cell function and dysfunction." J Cell Sci **117**(Pt 24): 5721-5729.

Johnson, J. D., J. Campisi, C. M. Sharkey, S. L. Kennedy, M. Nickerson, B. N. Greenwood and M. Fleshner (2005). "Catecholamines mediate stress-induced increases in peripheral and central inflammatory cytokines." Neuroscience **135**(4): 1295-1307.

Johnson, J. D., V. Cortez, S. L. Kennedy, T. E. Foley, H. Hanson, 3rd and M. Fleshner (2008). "Role of central beta-adrenergic receptors in regulating proinflammatory cytokine responses to a peripheral bacterial challenge." Brain Behav Immun **22**(7): 1078-1086.

Jonsson, T., J. K. Atwal, S. Steinberg, J. Snaedal, P. V. Jonsson, S. Bjornsson, H. Stefansson, P. Sulem, D. Gudbjartsson, J. Maloney, K. Hoyte, A. Gustafson, Y. Liu, Y. Lu, T. Bhangale, R. R. Graham, J. Huttenlocher, G. Bjornsdottir, O. A. Andreassen, E. G. Jonsson, A. Palotie, T. W. Behrens, O. T. Magnusson, A. Kong, U. Thorsteinsdottir, R. J. Watts and K. Stefansson (2012). "A mutation in APP protects against Alzheimer's disease and age-related cognitive decline." Nature **488**(7409): 96-99.

Kaidanovich-Beilin, O., T. Lipina, I. Vukobradovic, J. Roder and J. R. Woodgett (2011). "Assessment of social interaction behaviors." J Vis Exp(48).

Kaiser, N. C., L. J. Liang, R. J. Melrose, S. S. Wilkins, D. L. Sultzer and M. F. Mendez (2014). "Differences in anxiety among patients with early- versus late-onset Alzheimer's disease." J Neuropsychiatry Clin Neurosci **26**(1): 73-80.

Kalinin, S., D. L. Feinstein, H. L. Xu, G. Huesa, D. A. Pelligrino and E. Galea (2006). "Degeneration of noradrenergic fibres from the locus coeruleus causes tight-junction disorganisation in the rat brain." Eur J Neurosci **24**(12): 3393-3400.

Kalinin, S., V. Gavrilyuk, P. E. Polak, R. Vasser, J. Zhao, M. T. Heneka and D. L. Feinstein (2007). "Noradrenaline deficiency in brain increases beta-amyloid plaque burden in an animal model of Alzheimer's disease." Neurobiol Aging **28**(8): 1206-1214.

Kalinin, S., P. E. Polak, S. X. Lin, A. J. Sakharkar, S. C. Pandey and D. L. Feinstein (2012). "The noradrenaline precursor L-DOPS reduces pathology in a mouse model of Alzheimer's disease." Neurobiol Aging **33**(8): 1651-1663.

Kato, T., Y. Inui, A. Nakamura and K. Ito (2016). "Brain fluorodeoxyglucose (FDG) PET in dementia." Ageing Res Rev **30**: 73-84.

Katsuki, H., Y. Izumi and C. F. Zorumski (1997). "Noradrenergic regulation of synaptic plasticity in the hippocampal CA1 region." J Neurophysiol **77**(6): 3013-3020.

Kawas, C. H. and M. M. Corrada (2006). "Alzheimer's and dementia in the oldest-old: a century of challenges." Curr Alzheimer Res **3**(5): 411-419.

Kayed, R. and C. A. Lasagna-Reeves (2013). "Molecular mechanisms of amyloid oligomers toxicity." J Alzheimers Dis **33 Suppl 1**: S67-78.

Kelmenson, P. (2016). "Maybe it's not you, maybe it's your mice!" JAX Blog https://www.jax.org/news-and-insights/jax-blog/2016/august/maybe-its-not-you 2016.

Keren, N. I., C. T. Lozar, K. C. Harris, P. S. Morgan and M. A. Eckert (2009). "In vivo mapping of the human locus coeruleus." Neuroimage **47**(4): 1261-1267.

Keren, N. I., S. Taheri, E. M. Vazey, P. S. Morgan, A.-C. E. Granholm, G. S. Aston-Jones and M. A. Eckert (2015). "Histologic validation of locus coeruleus MRI contrast in post-mortem tissue." NeuroImage **113**: 235-245.

Khachaturian, A. S., P. P. Zandi, C. G. Lyketsos, K. M. Hayden, I. Skoog, M. C. Norton, J. T. Tschanz, L. S. Mayer, K. A. Welsh-Bohmer and J. C. Breitner (2006). "Antihypertensive medication use and incident Alzheimer disease: the Cache County Study." Arch Neurol **63**(5): 686-692.

Khan, S. S. and G. S. Bloom (2016). "Tau: The Center of a Signaling Nexus in Alzheimer's Disease." Front Neurosci **10**: 31.

Kidd, M. (1963). "Paired helical filaments in electron microscopy of Alzheimer's disease." Nature **197**: 192-193.

Kim, J. J. and D. M. Diamond (2002). "The stressed hippocampus, synaptic plasticity and lost memories." Nat Rev Neurosci **3**(6): 453-462.

Kong, Y., L. Ruan, L. Qian, X. Liu and Y. Le (2010). "Norepinephrine promotes microglia to uptake and degrade amyloid beta peptide through upregulation of mouse formyl peptide receptor 2 and induction of insulin-degrading enzyme." J Neurosci **30**(35): 11848-11857.

Koppel, J., T. E. Goldberg, M. L. Gordon, E. Huey, P. Davies, L. Keehlisen, S. Huet, E. Christen and B. S. Greenwald (2012). "Relationships between behavioral syndromes and cognitive domains in Alzheimer disease: the impact of mood and psychosis." Am J Geriatr Psychiatry **20**(11): 994-1000.

Kosik, K. S., C. L. Joachim and D. J. Selkoe (1986). "Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease." Proc Natl Acad Sci U S A **83**(11): 4044-4048.

Kosik, K. S., L. D. Orecchio, S. Bakalis and R. L. Neve (1989). "Developmentally regulated expression of specific tau sequences." Neuron **2**(4): 1389-1397.

Kummer, M. P., T. Hammerschmidt, A. Martinez, D. Terwel, G. Eichele, A. Witten, S. Figura, M. Stoll, S. Schwartz, H. C. Pape, J. L. Schultze, D. Weinshenker, M. T. Heneka and I. Urban (2014). "Ear2 deletion causes early memory and learning deficits in APP/PS1 mice." J Neurosci **34**(26): 8845-8854.

Lanari, A., F. Amenta, G. Silvestrelli, D. Tomassoni and L. Parnetti (2006). "Neurotransmitter deficits in behavioural and psychological symptoms of Alzheimer's disease." Mech Ageing Dev **127**(2): 158-165.

Langley, J., D. E. Huddleston, C. J. Liu and X. Hu (2016). "Reproducibility of locus coeruleus and substantia nigra imaging with neuromelanin sensitive MRI." Magnetic Resonance Materials in Physics, Biology and Medicine: 1-5.

Lapiz, M. D., Y. Mateo, S. Durkin, T. Parker and C. A. Marsden (2001). "Effects of central noradrenaline depletion by the selective neurotoxin DSP-4 on the behaviour of the isolated rat in the elevated plus maze and water maze." Psychopharmacology (Berl) **155**(3): 251-259.

Larson, M. E. and S. E. Lesne (2012). "Soluble Abeta oligomer production and toxicity." J Neurochem **120 Suppl 1**: 125-139.

Lasagna-Reeves, C. A., D. L. Castillo-Carranza, U. Sengupta, M. J. Guerrero-Munoz, T. Kiritoshi, V. Neugebauer, G. R. Jackson and R. Kayed (2012). "Alzheimer brain-derived tau oligomers propagate pathology from endogenous tau." Sci Rep **2**: 700.

Lawson, V. A., H. M. Klemm, J. M. Welton, C. L. Masters, P. Crouch, R. Cappai and G. D. Ciccotosto (2011). "Gene knockout of tau expression does not contribute to the pathogenesis of prion disease." J Neuropathol Exp Neurol **70**(11): 1036-1045.

Lei, P., S. Ayton, D. I. Finkelstein, L. Spoerri, G. D. Ciccotosto, D. K. Wright, B. X. W. Wong, P. A. Adlard, R. A. Cherny, L. Q. Lam, B. R. Roberts, I. Volitakis, G. F. Egan, C. A. McLean, R. Cappai, J. A. Duce and A. I. Bush (2012). "Tau deficiency induces parkinsonism with dementia by impairing APP-mediated iron export." Nat Med **18**(2): 291-295.

Leung, K. K., J. Barnes, G. R. Ridgway, J. W. Bartlett, M. J. Clarkson, K. Macdonald, N. Schuff, N. C. Fox and S. Ourselin (2010). "Automated cross-sectional and longitudinal hippocampal volume measurement in mild cognitive impairment and Alzheimer's disease." Neuroimage **51**(4): 1345-1359.

Lill, C. M., A. Rengmark, L. Pihlstrom, I. Fogh, A. Shatunov, P. M. Sleiman, L. S. Wang, T. Liu, C. F. Lassen, E. Meissner, P. Alexopoulos, A. Calvo, A. Chio, N. Dizdar, F. Faltraco, L. Forsgren, J. Kirchheiner, A. Kurz, J. P. Larsen, M. Liebsch, J. Linder, K. E. Morrison, H. Nissbrandt, M. Otto, J. Pahnke, A. Partch, G. Restagno, D. Rujescu, C. Schnack, C. E. Shaw, P. J. Shaw, H. Tumani, O. B. Tysnes, O. Valladares, V. Silani, L. H. van den Berg, W. van Rheenen, J. H. Veldink, U. Lindenberger, E. Steinhagen-Thiessen, S. Teipel, R. Perneczky, H. Hakonarson, H. Hampel, C. A. von Arnim, J. H. Olsen, V. M. Van Deerlin, A. Al-Chalabi, M. Toft, B. Ritz and L. Bertram (2015). "The role of TREM2 R47H as a risk factor for Alzheimer's disease, frontotemporal lobar degeneration, amyotrophic lateral sclerosis, and Parkinson's disease." Alzheimers Dement **11**(12): 1407-1416.

Lindsay, J., D. Laurin, R. Verreault, R. Hébert, B. Helliwell, G. B. Hill and I. McDowell (2002). "Risk Factors for Alzheimer's Disease: A Prospective Analysis from the Canadian Study of Health and Aging." American Journal of Epidemiology **156**(5): 445-453.

Ling, Y., K. Morgan and N. Kalsheker (2003). "Amyloid precursor protein (APP) and the biology of proteolytic processing: relevance to Alzheimer's disease." Int J Biochem Cell Biol **35**(11): 1505-1535.

Linsell, C. R., S. L. Lightman, P. E. Mullen, M. J. Brown and R. C. Causon (1985). "Circadian rhythms of epinephrine and norepinephrine in man." J Clin Endocrinol Metab **60**(6): 1210-1215.

Lister, J. P. and C. A. Barnes (2009). "Neurobiological changes in the hippocampus during normative aging." Arch Neurol **66**(7): 829-833.

Lithfous, S., A. Dufour and O. Despres (2013). "Spatial navigation in normal aging and the prodromal stage of Alzheimer's disease: insights from imaging and behavioral studies." Ageing Res Rev **12**(1): 201-213.

Lithner, C. U., M. M. Hedberg and A. Nordberg (2011). "Transgenic mice as a model for Alzheimer's disease." Curr Alzheimer Res **8**(8): 818-831.

Liu, J., D. Wang, S. Q. Li, Y. Yu and R. D. Ye (2016). "Suppression of LPS-induced tau hyperphosphorylation by serum amyloid A." J Neuroinflammation **13**: 28.

Liu, L., S. Luo, L. Zeng, W. Wang, L. Yuan and X. Jian (2013). "Degenerative alterations in noradrenergic neurons of the locus coeruleus in Alzheimer's disease." Neural Regeneration Research **8**(24): 2249-2255.

Liu, Y., M. J. Yoo, A. Savonenko, W. Stirling, D. L. Price, D. R. Borchelt, L. Mamounas, W. E. Lyons, M. E. Blue and M. K. Lee (2008). "Amyloid pathology is associated with progressive monoaminergic neurodegeneration in a transgenic mouse model of Alzheimer's disease." J Neurosci **28**(51): 13805-13814.

Lopes, S., A. Lopes, V. Pinto, M. R. Guimaraes, V. M. Sardinha, S. Duarte-Silva, S. Pinheiro, J. Pizarro, J. F. Oliveira, N. Sousa, H. Leite-Almeida and I. Sotiropoulos (2016). "Absence of Tau triggers age-dependent sciatic nerve morphofunctional deficits and motor impairment." Aging Cell **15**(2): 208-216.

Lopez-Gonzalez, I., E. Aso, M. Carmona, M. Armand-Ugon, R. Blanco, A. Naudi, R. Cabre, M. Portero-Otin, R. Pamplona and I. Ferrer (2015). "Neuroinflammatory Gene Regulation, Mitochondrial Function, Oxidative Stress, and Brain Lipid Modifications With Disease Progression in Tau P301S Transgenic Mice as a Model of Frontotemporal Lobar Degeneration-Tau." J Neuropathol Exp Neurol **74**(10): 975-999.

Lossos, A., A. Reches, A. Gal, J. P. Newman, D. Soffer, J. M. Gomori, M. Boher, D. Ekstein, I. Biran, Z. Meiner, O. Abramsky and H. Rosenmann (2003). "Frontotemporal dementia and parkinsonism with the P301S tau gene mutation in a Jewish family." J Neurol **250**(6): 733-740.

Lue, L. F., C. T. Schmitz, G. Serrano, L. I. Sue, T. G. Beach and D. G. Walker (2015). "TREM2 Protein Expression Changes Correlate with Alzheimer's Disease Neurodegenerative Pathologies in Post-Mortem Temporal Cortices." Brain Pathol **25**(4): 469-480.

Luo, W., W. Liu, X. Hu, M. Hanna, A. Caravaca and S. M. Paul (2015). "Microglial internalization and degradation of pathological tau is enhanced by an anti-tau monoclonal antibody." Sci Rep **5**: 11161.

Lyness, S. A., C. Zarow and H. C. Chui (2003). "Neuron loss in key cholinergic and aminergic nuclei in Alzheimer disease: a meta-analysis." Neurobiol Aging **24**(1): 1-23.

Lyons, W. E., J. M. Fritschy and R. Grzanna (1989). "The noradrenergic neurotoxin DSP-4 eliminates the coeruleospinal projection but spares projections of the A5 and A7 groups to the ventral horn of the rat spinal cord." J Neurosci **9**(5): 1481-1489.

Ma, Q. L., X. Zuo, F. Yang, O. J. Ubeda, D. J. Gant, M. Alaverdyan, N. C. Kiosea, S. Nazari, P. P. Chen, F. Nothias, P. Chan, E. Teng, S. A. Frautschy and G. M. Cole (2014). "Loss of

MAP function leads to hippocampal synapse loss and deficits in the Morris Water Maze with aging." J Neurosci **34**(21): 7124-7136.

Madrigal, J. L., B. Garcia-Bueno, A. E. Hinojosa, P. Polak, D. L. Feinstein and J. C. Leza (2010). "Regulation of MCP-1 production in brain by stress and noradrenaline-modulating drugs." J Neurochem **113**(2): 543-551.

Maeda, T. (2000). "The locus coeruleus: history." J Chem Neuroanat **18**(1-2): 57-64.

Mangialasche, F., A. Solomon, B. Winblad, P. Mecocci and M. Kivipelto "Alzheimer's disease: clinical trials and drug development." The Lancet Neurology **9**(7): 702-716.

Mann, D. M. (1983). "The locus coeruleus and its possible role in ageing and degenerative disease of the human central nervous system." Mech Ageing Dev **23**(1): 73-94.

Mann, D. M., J. Lincoln, P. O. Yates, J. E. Stamp and S. Toper (1980). "Changes in the monoamine containing neurones of the human CNS in senile dementia." Br J Psychiatry **136**: 533-541.

Maphis, N., G. Xu, O. N. Kokiko-Cochran, A. E. Cardona, R. M. Ransohoff, B. T. Lamb and K. Bhaskar (2015). "Loss of tau rescues inflammation-mediated neurodegeneration." Front Neurosci **9**: 196.

Marien, M. R., F. C. Colpaert and A. C. Rosenquist (2004). "Noradrenergic mechanisms in neurodegenerative diseases: a theory." Brain Res Brain Res Rev **45**(1): 38-78.

Marino, M. D., B. N. Bourdelat-Parks, L. Cameron Liles and D. Weinshenker (2005). "Genetic reduction of noradrenergic function alters social memory and reduces aggression in mice." Behav Brain Res **161**(2): 197-203.

Marques, F., J. C. Sousa, N. Sousa and J. A. Palha (2013). "Blood-brain-barriers in aging and in Alzheimer's disease." Mol Neurodegener **8**: 38.

Marsh, L., K. Biglan, M. Gerstenhaber and J. R. Williams (2009). "Atomoxetine for the treatment of executive dysfunction in Parkinson's disease: a pilot open-label study." Mov Disord **24**(2): 277-282.

Martin, L., X. Latypova and F. Terro (2011). "Post-translational modifications of tau protein: implications for Alzheimer's disease." Neurochem Int **58**(4): 458-471.

Martin, L., X. Latypova, C. M. Wilson, A. Magnaudeix, M. L. Perrin and F. Terro (2013). "Tau protein phosphatases in Alzheimer's disease: the leading role of PP2A." Ageing Res Rev **12**(1): 39-49.

Maruta, E., K. Yabuuchi, A. Nishiyori, S. Takami, M. Minami and M. Satoh (1997). "Beta2-adrenoceptors on the glial cells mediate the induction of interleukin-1beta mRNA in the rat brain." Brain Res Mol Brain Res **49**(1-2): 291-294.

Marzo, A., J. Bai and S. Otani (2009). "Neuroplasticity regulation by noradrenaline in mammalian brain." Curr Neuropharmacol **7**(4): 286-295.

Maskri, L., X. Zhu, S. Fritzen, K. Kuhn, C. Ullmer, P. Engels, M. Andriske, C. C. Stichel and H. Lubbert (2004). "Influence of different promoters on the expression pattern of mutated human alpha-synuclein in transgenic mice." Neurodegener Dis **1**(6): 255-265.

Masuko, S., Y. Nakajima, S. Nakajima and K. Yamaguchi (1986). "Noradrenergic neurons from the locus ceruleus in dissociated cell culture: culture methods, morphology, and electrophysiology." J Neurosci **6**(11): 3229-3241.

Mateo, I., J. Infante, E. Rodriguez, J. Berciano, O. Combarros and J. Llorca (2006). "Interaction between dopamine beta-hydroxylase and interleukin genes increases Alzheimer's disease risk." J Neurol Neurosurg Psychiatry **77**(2): 278-279.

Mateo, I., J. Infante, E. Rodríguez, J. Berciano, O. Combarros and J. Llorca (2006). "Interaction between dopamine β-hydroxylase and interleukin genes increases Alzheimer's disease risk." J Neurol Neurosurg Psychiatry **77**(2): 278-279.

Mather, M. and C. W. Harley (2016). "The Locus Coeruleus: Essential for Maintaining Cognitive Function and the Aging Brain." Trends Cogn Sci **20**(3): 214-226.

Matsushita, N., H. Okada, Y. Yasoshima, K. Takahashi, K. Kiuchi and K. Kobayashi (2002). "Dynamics of tyrosine hydroxylase promoter activity during midbrain dopaminergic neuron development." J Neurochem **82**(2): 295-304.

Maurer, K., S. Volk and H. Gerbaldo (1997). "Auguste D and Alzheimer's disease." Lancet **349**(9064): 1546-1549.

Mawuenyega, K. G., W. Sigurdson, V. Ovod, L. Munsell, T. Kasten, J. C. Morris, K. E. Yarasheski and R. J. Bateman (2010). "Decreased clearance of CNS beta-amyloid in Alzheimer's disease." Science **330**(6012): 1774.

Mazure, C. M. and J. Swendsen (2016). "Sex differences in Alzheimer's disease and other dementias." The Lancet. Neurology **15**(5): 451-452.

McKhann, G. M., D. S. Knopman, H. Chertkow, B. T. Hyman, C. R. Jack, Jr., C. H. Kawas, W. E. Klunk, W. J. Koroshetz, J. J. Manly, R. Mayeux, R. C. Mohs, J. C. Morris, M. N. Rossor, P. Scheltens, M. C. Carrillo, B. Thies, S. Weintraub and C. H. Phelps (2011). "The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease." Alzheimers Dement **7**(3): 263-269.

Medina, M., F. Hernandez and J. Avila (2016). "New Features about Tau Function and Dysfunction." Biomolecules **6**(2).

Menéndez-González, M. (2014). "Routine lumbar puncture for the early diagnosis of Alzheimer's disease. Is it safe?" Front Aging Neurosci **6**.

Moriya, S., Y. Tahara, H. Sasaki, J. Ishigooka and S. Shibata (2015). "Phase-delay in the light-dark cycle impairs clock gene expression and levels of serotonin, norepinephrine, and their metabolites in the mouse hippocampus and amygdala." Sleep Med **16**(11): 1352-1359.

Morris, M., A. Koyama, E. Masliah and L. Mucke (2011). "Tau reduction does not prevent motor deficits in two mouse models of Parkinson's disease." PLoS One **6**(12): e29257.

Morris, R. (1984). "Developments of a water-maze procedure for studying spatial learning in the rat." J Neurosci Methods **11**(1): 47-60.

Mouton, P. R., B. Pakkenberg, H. J. Gundersen and D. L. Price (1994). "Absolute number and size of pigmented locus coeruleus neurons in young and aged individuals." J Chem Neuroanat **7**(3): 185-190.

Mravec, B., K. Lejavova and V. Cubinkova (2014). "Locus (coeruleus) minoris resistentiae in pathogenesis of Alzheimer's disease." Curr Alzheimer Res **11**(10): 992-1001.

Musiek, E. S., D. D. Xiong and D. M. Holtzman (2015). "Sleep, circadian rhythms, and the pathogenesis of Alzheimer Disease." Experimental & Molecular Medicine **47**(3): e148.

National Centre for the Replacement, R. R. o. A. i. R. "Get my (genetic) drift." Retrieved 12/4/16, 2016, from https://www.nc3rs.org.uk/get-my-genetic-drift.

Nedelska, Z., R. Andel, J. Laczo, K. Vlcek, D. Horinek, J. Lisy, K. Sheardova, J. Bures and J. Hort (2012). "Spatial navigation impairment is proportional to right hippocampal volume." Proc Natl Acad Sci U S A **109**(7): 2590-2594.

Neuman, R. S. and C. W. Harley (1983). "Long-lasting potentiation of the dentate gyrus population spike by norepinephrine." Brain Res **273**(1): 162-165.

Nilson, A. N., K. C. English, J. E. Gerson, T. Barton Whittle, C. Nicolas Crain, J. Xue, U. Sengupta, D. L. Castillo-Carranza, W. Zhang, P. Gupta and R. Kayed (2016). "Tau Oligomers Associate with Inflammation in the Brain and Retina of Tauopathy Mice and in Neurodegenerative Diseases." J Alzheimers Dis **55**(3): 1083-1099.

Ohm, T. G., C. Busch and J. Bohl (1997). "Unbiased estimation of neuronal numbers in the human nucleus coeruleus during aging." Neurobiol Aging **18**(4): 393-399.

Olsson, A., K. Hoglund, M. Sjogren, N. Andreasen, L. Minthon, L. Lannfelt, K. Buerger, H. J. Moller, H. Hampel, P. Davidsson and K. Blennow (2003). "Measurement of alpha- and beta-secretase cleaved amyloid precursor protein in cerebrospinal fluid from Alzheimer patients." Exp Neurol **183**(1): 74-80.

Olsson, F., S. Schmidt, V. Althoff, L. M. Munter, S. Jin, S. Rosqvist, U. Lendahl, G. Multhaup and J. Lundkvist (2014). "Characterization of intermediate steps in amyloid beta (Abeta) production under near-native conditions." J Biol Chem **289**(3): 1540-1550.

Ortman JM, V. V., Hogan H. (2014). An Aging Nation: The Older

Population in the United States, Current Population Reports. U.S. Census Bureau. Washington, D.C.**:** 25-1140.

Pallo, S. P. and G. V. Johnson (2015). "Tau facilitates Abeta-induced loss of mitochondrial membrane potential independent of cytosolic calcium fluxes in mouse cortical neurons." Neurosci Lett **597**: 32-37.

Pamphlett, R. (2014). "Uptake of environmental toxicants by the locus ceruleus: a potential trigger for neurodegenerative, demyelinating and psychiatric disorders." Med Hypotheses **82**(1): 97-104.

Perez, S. E., C. C. Sherwood, M. R. Cranfield, J. M. Erwin, A. Mudakikwa, P. R. Hof and E. J. Mufson (2016). "Early Alzheimer's disease-type pathology in the frontal cortex of wild mountain gorillas (Gorilla beringei beringei)." Neurobiol Aging **39**: 195-201.

Peskind, E. R., D. Wingerson, S. Murray, M. Pascualy, D. J. Dobie, P. Le Corre, R. Le Verge, R. C. Veith and M. A. Raskind (1995). "Effects of Alzheimer's disease and normal aging on cerebrospinal fluid norepinephrine responses to yohimbine and clonidine." Arch Gen Psychiatry **52**(9): 774-782.

Pinos, H., P. Collado, M. Rodriguez-Zafra, C. Rodriguez, S. Segovia and A. Guillamon (2001). "The development of sex differences in the locus coeruleus of the rat." Brain Res Bull **56**(1): 73-78.

Planel, E., K. E. Richter, C. E. Nolan, J. E. Finley, L. Liu, Y. Wen, P. Krishnamurthy, M. Herman, L. Wang, J. B. Schachter, R. B. Nelson, L. F. Lau and K. E. Duff (2007).

"Anesthesia leads to tau hyperphosphorylation through inhibition of phosphatase activity by hypothermia." J Neurosci **27**(12): 3090-3097.

Pool, M., J. Thiemann, A. Bar-Or and A. E. Fournier (2008). "NeuriteTracer: a novel ImageJ plugin for automated quantification of neurite outgrowth." J Neurosci Methods **168**(1): 134-139.

Povova, J., P. Ambroz, M. Bar, V. Pavukova, O. Sery, H. Tomaskova and V. Janout (2012). "Epidemiological of and risk factors for Alzheimer's disease: a review." Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub **156**(2): 108-114.

Qin, Y., Y. Liu, W. Hao, Y. Decker, I. Tomic, M. D. Menger, C. Liu and K. Fassbender (2016). "Stimulation of TLR4 Attenuates Alzheimer's Disease-Related Symptoms and Pathology in Tau-Transgenic Mice." J Immunol.

Querfurth, H. W. and F. M. LaFerla (2010). "Alzheimer's disease." N Engl J Med **362**(4): 329-344.

Ramos, B. P. and A. F. Arnsten (2007). "Adrenergic pharmacology and cognition: focus on the prefrontal cortex." Pharmacol Ther **113**(3): 523-536.

Rang, H. R., JM; FLower, R; Henderson, F (2014). Noradrenergic Transmission. Rang & Dale's Pharmacology, Elseiver Healther Sciences**:** 177-196.

Rapoport, M., H. N. Dawson, L. I. Binder, M. P. Vitek and A. Ferreira (2002). "Tau is essential to beta -amyloid-induced neurotoxicity." Proc Natl Acad Sci U S A **99**(9): 6364-6369.

Raskind, M. A. and E. R. Peskind (1994). "Neurobiologic bases of noncognitive behavioral problems in Alzheimer disease." Alzheimer Dis Assoc Disord **8 Suppl 3**: 54-60.

Raskind, M. A., E. R. Peskind, J. B. Halter and D. C. Jimerson (1984). "Norepinephrine and MHPG levels in CSF and plasma in Alzheimer's disease." Arch Gen Psychiatry **41**(4): 343-346.

Raudino, F. (2013). "Non-cognitive symptoms and related conditions in the Alzheimer's disease: a literature review." Neurol Sci **34**(8): 1275-1282.

Ray, S. and A. B. Reddy (2016). "Cross-talk between circadian clocks, sleep-wake cycles, and metabolic networks: Dispelling the darkness." Bioessays **38**(4): 394-405.

Reed, B. T., F. Behar-Cohen and S. Krantic (2016). "Seeing early signs of Alzheimer's Disease through the lens of the eye." Curr Alzheimer Res.

Reed, L. A., T. J. Grabowski, M. L. Schmidt, J. C. Morris, A. Goate, A. Solodkin, G. W. Van Hoesen, R. L. Schelper, C. J. Talbot, M. A. Wragg and J. Q. Trojanowski (1997). "Autosomal dominant dementia with widespread neurofibrillary tangles." Ann Neurol **42**(4): 564-572.

Regan, P., T. Piers, J. H. Yi, D. H. Kim, S. Huh, S. J. Park, J. H. Ryu, D. J. Whitcomb and K. Cho (2015). "Tau phosphorylation at serine 396 residue is required for hippocampal LTD." J Neurosci **35**(12): 4804-4812.

Reifler, B. V., L. Teri, M. Raskind, R. Veith, R. Barnes, E. White and P. McLean (1989). "Double-blind trial of imipramine in Alzheimer's disease patients with and without depression." Am J Psychiatry **146**(1): 45-49.

Ressler, K. J. and C. B. Nemeroff (1999). "Role of norepinephrine in the pathophysiology and treatment of mood disorders." Biol Psychiatry **46**(9): 1219-1233.

Rey, N. L., D. Jardanhazi-Kurutz, D. Terwel, M. P. Kummer, F. Jourdan, A. Didier and M. T. Heneka (2012). "Locus coeruleus degeneration exacerbates olfactory deficits in APP/PS1 transgenic mice." Neurobiol Aging **33**(2): 426 e421-411.

Rey, N. L., D. Jardanhazi-Kurutz, D. Terwel, M. P. Kummer, F. Jourdan, A. Didier and M. T. Heneka (2012). "Locus coeruleus degeneration exacerbates olfactory deficits in APP/PS1 transgenic mice." Neurobiology of Aging **33**(2): 426.e421-426.e411.

Richardson, G. S., M. C. Moore-Ede, C. A. Czeisler and W. C. Dement (1985). "Circadian rhythms of sleep and wakefulness in mice: analysis using long-term automated recording of sleep." Am J Physiol **248**(3 Pt 2): R320-330.

Riekkinen, M., M. P. Laakso and P. Jakala (1999). "Clonidine impairs sustained attention and memory in Alzheimer's disease." Neuroscience **92**(3): 975-982.

Riekkinen, P., Jr. and M. Riekkinen (1999). "THA improves word priming and clonidine enhances fluency and working memory in Alzheimer's disease." Neuropsychopharmacology **20**(4): 357-364.

Ringman, J. M., L. J. Liang, Y. Zhou, S. Vangala, E. Teng, S. Kremen, D. Wharton, A. Goate, D. S. Marcus, M. Farlow, B. Ghetti, E. McDade, C. L. Masters, R. P. Mayeux, M. Rossor, S. Salloway, P. R. Schofield, J. L. Cummings, V. Buckles, R. Bateman and J. C. Morris (2015). "Early behavioural changes in familial Alzheimer's disease in the Dominantly Inherited Alzheimer Network." Brain **138**(Pt 4): 1036-1045.

Ritchie, K., I. Carriere, C. Berr, H. Amieva, J. F. Dartigues, M. L. Ancelin and C. W. Ritchie (2016). "The clinical picture of Alzheimer's disease in the decade before diagnosis: clinical and biomarker trajectories." J Clin Psychiatry **77**(3): e305-311.

Robbins, T. W. and A. F. Arnsten (2009). "The neuropsychopharmacology of fronto-executive function: monoaminergic modulation." Annu Rev Neurosci **32**: 267-287.

Roberson, E. D., B. Halabisky, J. W. Yoo, J. Yao, J. Chin, F. Yan, T. Wu, P. Hamto, N. Devidze, G. Q. Yu, J. J. Palop, J. L. Noebels and L. Mucke (2011). "Amyloid-beta/Fyn-induced synaptic, network, and cognitive impairments depend on tau levels in multiple mouse models of Alzheimer's disease." J Neurosci **31**(2): 700-711.

Roberson, E. D., K. Scearce-Levie, J. J. Palop, F. Yan, I. H. Cheng, T. Wu, H. Gerstein, G. Q. Yu and L. Mucke (2007). "Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model." Science **316**(5825): 750-754.

Rodriguez-Martin, T., I. Cuchillo-Ibanez, W. Noble, F. Nyenya, B. H. Anderton and D. P. Hanger (2013). "Tau phosphorylation affects its axonal transport and degradation." Neurobiol Aging **34**(9): 2146-2157.

Rokni, D., V. Hemmelder, V. Kapoor and V. N. Murthy (2014). "An olfactory cocktail party: figure-ground segregation of odorants in rodents." Nat Neurosci **17**(9): 1225-1232.

Rosenberg, P. B., M. M. Mielke, J. Tschanz, L. Cook, C. Corcoran, K. M. Hayden, M. Norton, P. V. Rabins, R. C. Green, K. A. Welsh-Bohmer, J. C. Breitner, R. Munger and C. G. Lyketsos (2008). "Effects of cardiovascular medications on rate of functional decline in Alzheimer disease." Am J Geriatr Psychiatry **16**(11): 883-892.

Ross, S. B. and C. Stenfors (2015). "DSP4, a selective neurotoxin for the locus coeruleus noradrenergic system. A review of its mode of action." Neurotox Res **27**(1): 15-30.

Rossner, S., M. Sastre, K. Bourne and S. F. Lichtenthaler (2006). "Transcriptional and translational regulation of BACE1 expression--implications for Alzheimer's disease." Prog Neurobiol **79**(2): 95-111.

Rowe, C. C. and V. L. Villemagne (2011). "Brain amyloid imaging." J Nucl Med **52**(11): 1733-1740.

Sahara, N. and J. Avila (2014). "“Tau Oligomers,” What We Know and What We Don’t Know." Frontiers in Neurology **5**(1).

Salehi, A., M. Faizi, D. Colas, J. Valletta, J. Laguna, R. Takimoto-Kimura, A. Kleschevnikov, S. L. Wagner, P. Aisen, M. Shamloo and W. C. Mobley (2009). "Restoration of norepinephrine-modulated contextual memory in a mouse model of Down syndrome." Sci Transl Med **1**(7): 7ra17.

Salloway, S., R. Sperling, N. C. Fox, K. Blennow, W. Klunk, M. Raskind, M. Sabbagh, L. S. Honig, A. P. Porsteinsson, S. Ferris, M. Reichert, N. Ketter, B. Nejadnik, V. Guenzler, M. Miloslavsky, D. Wang, Y. Lu, J. Lull, I. C. Tudor, E. Liu, M. Grundman, E. Yuen, R. Black and H. R. Brashear (2014). "Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease." N Engl J Med **370**(4): 322-333.

Sanchez-Catasus, C. A., G. N. Stormezand, P. J. van Laar, P. P. De Deyn, M. A. Sanchez and R. A. Dierckx (2016). "FDG-PET for Prediction of AD Dementia in Mild Cognitive

Impairment. A Review of the State of the Art with Particular Emphasis on the Comparison with Other Neuroimaging modalities (MRI and Perfusion SPECT)." Curr Alzheimer Res.

Sara, S. J. (2009). "The locus coeruleus and noradrenergic modulation of cognition." Nat Rev Neurosci **10**(3): 211-223.

Sasaki, M., E. Shibata, K. Tohyama, J. Takahashi, K. Otsuka, K. Tsuchiya, S. Takahashi, S. Ehara, Y. Terayama and A. Sakai (2006). "Neuromelanin magnetic resonance imaging of locus ceruleus and substantia nigra in Parkinson's disease." Neuroreport **17**(11): 1215-1218.

Savitz, S. I., J. A. Erhardt, J. V. Anthony, G. Gupta, X. Li, F. C. Barone and D. M. Rosenbaum (2000). "The novel beta-blocker, carvedilol, provides neuroprotection in transient focal stroke." J Cereb Blood Flow Metab **20**(8): 1197-1204.

Scarano, S., S. Lisi, C. Ravelet, E. Peyrin and M. Minunni (2016). "Detecting Alzheimer's disease biomarkers: From antibodies to new bio-mimetic receptors and their application to established and emerging bioanalytical platforms - A critical review." Anal Chim Acta **940**: 21-37.

Schank, J. R., L. C. Liles and D. Weinshenker (2008). "Norepinephrine signaling through beta-adrenergic receptors is critical for expression of cocaine-induced anxiety." Biol Psychiatry **63**(11): 1007-1012.

Scheuner, D., C. Eckman and M. Jensen (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." Nat Med **2**: 864-870.

Schmidt, K. T. and D. Weinshenker (2014). "Adrenaline Rush: The Role of Adrenergic Receptors in Stimulant-Induced Behaviors." Molecular Pharmacology **85**(4): 640-650.

Scholl, M., S. N. Lockhart, D. R. Schonhaut, J. P. O'Neil, M. Janabi, R. Ossenkoppele, S. L. Baker, J. W. Vogel, J. Faria, H. D. Schwimmer, G. D. Rabinovici and W. J. Jagust

(2016). "PET Imaging of Tau Deposition in the Aging Human Brain." Neuron **89**(5): 971-982.

Schutsky, K., M. Ouyang, C. B. Castelino, L. Zhang and S. A. Thomas (2011). "Stress and glucocorticoids impair memory retrieval via beta2-adrenergic, Gi/o-coupled suppression of cAMP signaling." J Neurosci **31**(40): 14172-14181.

Schutsky, K., M. Ouyang and S. A. Thomas (2011). "Xamoterol impairs hippocampus-dependent emotional memory retrieval via Gi/o-coupled beta2-adrenergic signaling." Learn Mem **18**(9): 598-604.

Schwarz, A. J., P. Yu, B. B. Miller, S. Shcherbinin, J. Dickson, M. Navitsky, A. D. Joshi, M. D. Devous, Sr. and M. S. Mintun (2016). "Regional profiles of the candidate tau PET ligand 18F-AV-1451 recapitulate key features of Braak histopathological stages." Brain **139**(Pt 5): 1539-1550.

Scullion, G. A., D. A. Kendall, C. A. Marsden, D. Sunter and M. C. Pardon (2011). "Chronic treatment with the alpha2-adrenoceptor antagonist fluparoxan prevents age-related deficits in spatial working memory in APPxPS1 transgenic mice without altering beta-amyloid plaque load or astrocytosis." Neuropharmacology **60**(2-3): 223-234.

Selkoe, D. J. and J. Hardy (2016). "The amyloid hypothesis of Alzheimer's disease at 25 years." EMBO Mol Med **8**(6): 595-608.

Sengupta, A., J. Kabat, M. Novak, Q. Wu, I. Grundke-Iqbal and K. Iqbal (1998). "Phosphorylation of tau at both Thr 231 and Ser 262 is required for maximal inhibition of its binding to microtubules." Arch Biochem Biophys **357**(2): 299-309.

Serg, M., P. Kampus, J. Kals, M. Zagura, M. Zilmer, K. Zilmer, T. Kullisaar and J. Eha (2012). "Nebivolol and metoprolol: long-term effects on inflammation and oxidative stress in essential hypertension." Scandinavian Journal of Clinical & Laboratory Investigation **72**(5): 427-432.

Sevigny, J., P. Chiao, T. Bussiere, P. H. Weinreb, L. Williams, M. Maier, R. Dunstan, S. Salloway, T. Chen, Y. Ling, J. O'Gorman, F. Qian, M. Arastu, M. Li, S. Chollate, M. S. Brennan, O. Quintero-Monzon, R. H. Scannevin, H. M. Arnold, T. Engber, K. Rhodes, J. Ferrero, Y. Hang, A. Mikulskis, J. Grimm, C. Hock, R. M. Nitsch and A. Sandrock (2016). "The antibody aducanumab reduces Abeta plaques in Alzheimer's disease." Nature **537**(7618): 50-56.

Shankle, W. R., K. A. Nielson and C. W. Cotman (1995). "Low-dose propranolol reduces aggression and agitation resembling that associated with orbitofrontal dysfunction in elderly demented patients." Alzheimer Dis Assoc Disord **9**(4): 233-237.

Sharma, Y., T. Xu, W. M. Graf, A. Fobbs, C. C. Sherwood, P. R. Hof, J. M. Allman and K. F. Manaye (2010). "Comparative anatomy of the locus coeruleus in humans and nonhuman primates." J Comp Neurol **518**(7): 963-971.

Shiarli, A. M., R. Jennings, J. Shi, K. Bailey, Y. Davidson, J. Tian, E. H. Bigio, B. Ghetti, J. R. Murrell, M. B. Delisle, S. Mirra, B. Crain, P. Zolo, K. Arima, E. Iseki, S. Murayama, H. Kretzschmar, M. Neumann, C. Lippa, G. Halliday, J. Mackenzie, N. Khan, R. Ravid, D. Dickson, Z. Wszolek, T. Iwatsubo, S. M. Pickering-Brown and D. M. Mann (2006). "Comparison of extent of tau pathology in patients with frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17), frontotemporal lobar degeneration with Pick bodies and early onset Alzheimer's disease." Neuropathol Appl Neurobiol **32**(4): 374-387.

Shibata, E., M. Sasaki, K. Tohyama, K. Otsuka, J. Endoh, Y. Terayama and A. Sakai (2008). "Use of neuromelanin-sensitive MRI to distinguish schizophrenic and depressive patients and healthy individuals based on signal alterations in the substantia nigra and locus ceruleus." Biol Psychiatry **64**(5): 401-406.

Shibata, E., M. Sasaki, K. Tohyama, K. Otsuka and A. Sakai (2007). "Reduced signal of locus ceruleus in depression in quantitative neuromelanin magnetic resonance imaging." Neuroreport **18**(5): 415-418.

Shipton, O. A., J. R. Leitz, J. Dworzak, C. E. Acton, E. M. Tunbridge, F. Denk, H. N. Dawson, M. P. Vitek, R. Wade-Martins, O. Paulsen and M. Vargas-Caballero (2011). "Tau protein is required for amyloid {beta}-induced impairment of hippocampal long-term potentiation." J Neurosci **31**(5): 1688-1692.

Small, G. W. (2006). "Diagnostic issues in dementia: neuroimaging as a surrogate marker of disease." J Geriatr Psychiatry Neurol **19**(3): 180-185.

Sontag, T. A., J. Hauser, I. Kaunzinger, M. Gerlach, O. Tucha and K. W. Lange (2008). "Effects of the noradrenergic neurotoxin DSP4 on spatial memory in the rat." J Neural Transm (Vienna) **115**(2): 299-303.

Sontag, T. A., J. Hauser, O. Tucha and K. W. Lange (2011). "Effects of DSP4 and methylphenidate on spatial memory performance in rats." Atten Defic Hyperact Disord **3**(4): 351-358.

Sperfeld, A. D., M. B. Collatz, H. Baier, M. Palmbach, A. Storch, J. Schwarz, K. Tatsch, S. Reske, M. Joosse, P. Heutink and A. C. Ludolph (1999). "FTDP-17: an early-onset phenotype with parkinsonism and epileptic seizures caused by a novel mutation." Ann Neurol **46**(5): 708-715.

Spillantini, M. G., R. A. Crowther and M. Goedert (1996). "Comparison of the neurofibrillary pathology in Alzheimer's disease and familial presenile dementia with tangles." Acta Neuropathol **92**(1): 42-48.

Spires-Jones, T. L., W. H. Stoothoff, A. de Calignon, P. B. Jones and B. T. Hyman (2009). "Tau pathophysiology in neurodegeneration: a tangled issue." Trends Neurosci **32**(3): 150-159.

Stratmann, K., H. Heinsen, H.-W. Korf, D. Del Turco, E. Ghebremedhin, K. Seidel, M. Bouzrou, L. T. Grinberg, J. Bohl, S. B. Wharton, W. den Dunnen and U. Rüb (2016). "Precortical

Phase of Alzheimer's Disease (AD)-Related Tau Cytoskeletal Pathology." Brain Pathology **26**(3): 371-386.

Strozyk, D., K. Blennow, L. R. White and L. J. Launer (2003). "CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study." Neurology **60**(4): 652-656.

Suh, Y. H. and F. Checler (2002). "Amyloid precursor protein, presenilins, and alpha-synuclein: molecular pathogenesis and pharmacological applications in Alzheimer's disease." Pharmacol Rev **54**(3): 469-525.

Swoap, S. J., D. Weinshenker, R. D. Palmiter and G. Garber (2004). "Dbh(-/-) mice are hypotensive, have altered circadian rhythms, and have abnormal responses to dieting and stress." Am J Physiol Regul Integr Comp Physiol **286**(1): R108-113.

Sydow, A., A. Van der Jeugd, F. Zheng, T. Ahmed, D. Balschun, O. Petrova, D. Drexler, L. Zhou, G. Rune, E. Mandelkow, R. D'Hooge, C. Alzheimer and E. M. Mandelkow (2011). "Tau-induced defects in synaptic plasticity, learning, and memory are reversible in transgenic mice after switching off the toxic Tau mutant." J Neurosci **31**(7): 2511-2525.

Szot, P. (2012). "Common factors among Alzheimer's disease, Parkinson's disease, and epilepsy: possible role of the noradrenergic nervous system." Epilepsia **53 Suppl 1**: 61-66.

Szot, P., S. S. White, J. L. Greenup, J. B. Leverenz, E. R. Peskind and M. A. Raskind (2006). "Compensatory changes in the noradrenergic nervous system in the locus ceruleus and hippocampus of postmortem subjects with Alzheimer's disease and dementia with Lewy bodies." J Neurosci **26**(2): 467-478.

Taft, R. A., M. Davisson and M. V. Wiles "Know thy mouse." Trends in Genetics **22**(12): 649-653.

Tagai, K., T. Nagata, S. Shinagawa, K. Nemoto, K. Inamura, N. Tsuno and K. Nakayama (2014). "Correlation between both morphologic and functional changes and anxiety in Alzheimer's disease." Dement Geriatr Cogn Disord **38**(3-4): 153-160.

Takahashi, J., T. Shibata, M. Sasaki, M. Kudo, H. Yanezawa, S. Obara, K. Kudo, K. Ito, F. Yamashita and Y. Terayama (2015). "Detection of changes in the locus coeruleus in patients with mild cognitive impairment and Alzheimer's disease: high-resolution fast spin-echo T1-weighted imaging." Geriatr Gerontol Int **15**(3): 334-340.

Takei, Y., J. Teng, A. Harada and N. Hirokawa (2000). "<div xmlns="http://www.w3.org/1999/xhtml">Defects in Axonal Elongation and Neuronal Migration in Mice with Disrupted tau and map1b Genes</div>." The Journal of Cell Biology **150**(5): 989-1000.

Takeuchi, H., M. Iba, H. Inoue, M. Higuchi, K. Takao, K. Tsukita, Y. Karatsu, Y. Iwamoto, T. Miyakawa, T. Suhara, J. Q. Trojanowski, V. M. Lee and R. Takahashi (2011). "P301S mutant human tau transgenic mice manifest early symptoms of human tauopathies with dementia and altered sensorimotor gating." PLoS One **6**(6): e21050.

Tan, K. S., A. G. Nackley, K. Satterfield, W. Maixner, L. Diatchenko and P. M. Flood (2007). "Beta2 adrenergic receptor activation stimulates pro-inflammatory cytokine production in macrophages via PKA- and NF-kappaB-independent mechanisms." Cell Signal **19**(2): 251-260.

Tejani-Butt, S. M., J. Yang and H. Zaffar (1993). "Norepinephrine transporter sites are decreased in the locus coeruleus in Alzheimer's disease." Brain Res **631**(1): 147-150.

Thal, D. R., U. Rub, C. Schultz, I. Sassin, E. Ghebremedhin, K. Del Tredici, E. Braak and H. Braak (2000). "Sequence of Abeta-protein deposition in the human medial temporal lobe." J Neuropathol Exp Neurol **59**(8): 733-748.

Theofilas, P., A. J. Ehrenberg, S. Dunlop, A. T. Di Lorenzo Alho, A. Nguy, R. E. Leite, R. D. Rodriguez, M. B. Mejia, C. K. Suemoto, R. E. Ferretti-Rebustini, L. Polichiso, C. F. Nascimento, W. W. Seeley, R. Nitrini, C. A. Pasqualucci, W. Jacob Filho, U. Rueb, J. Neuhaus, H. Heinsen and L. T. Grinberg (2016). "Locus coeruleus volume and cell population changes during Alzheimer's disease progression: A stereological study in

human postmortem brains with potential implication for early-stage biomarker discovery." Alzheimers Dement.

Tomlinson, B. E., G. Blessed and M. Roth (1970). "Observations on the brains of demented old people." J Neurol Sci **11**(3): 205-242.

Tomlinson, B. E., D. Irving and G. Blessed (1981). "Cell loss in the locus coeruleus in senile dementia of Alzheimer type." J Neurol Sci **49**(3): 419-428.

Tomozawa, Y., K. Yabuuchi, T. Inoue and M. Satoh (1995). "Participation of cAMP and cAMP-dependent protein kinase in beta-adrenoceptor-mediated interleukin-1 beta mRNA induction in cultured microglia." Neurosci Res **22**(4): 399-409.

Tremblay, P., E. Bouzamondo-Bernstein, C. Heinrich, S. B. Prusiner and S. J. DeArmond (2007). "Developmental expression of PrP in the post-implantation embryo." Brain research **1139**: 60-67.

Turner, R. S. (2006). "Alzheimer's disease." Semin Neurol **26**(5): 499-506.

Vanderstichele, H., E. Van Kerschaver, C. Hesse, P. Davidsson, M. A. Buyse, N. Andreasen, L. Minthon, A. Wallin, K. Blennow and E. Vanmechelen (2000). "Standardization of measurement of beta-amyloid(1-42) in cerebrospinal fluid and plasma." Amyloid **7**(4): 245-258.

Vathy, I. and A. M. Etgen (1988). "Ovarian steroids and hypothalamic norepinephrine release: studies using in vivo brain microdialysis." Life Sci **43**(19): 1493-1499.

Verheijen, J. H., L. G. Huisman, N. van Lent, U. Neumann, P. Paganetti, C. E. Hack, F. Bouwman, J. Lindeman, E. L. Bollen and R. Hanemaaijer (2006). "Detection of a soluble form of BACE-1 in human cerebrospinal fluid by a sensitive activity assay." Clin Chem **52**(6): 1168-1174.

Villemagne, V. L., K. E. Pike, G. Chetelat, K. A. Ellis, R. S. Mulligan, P. Bourgeat, U. Ackermann, G. Jones, C. Szoeke, O. Salvado, R. Martins, G. O'Keefe, C. A. Mathis, W.

E. Klunk, D. Ames, C. L. Masters and C. C. Rowe (2011). "Longitudinal assessment of Abeta and cognition in aging and Alzheimer disease." Ann Neurol **69**(1): 181-192.

Vossel, K. A., K. Zhang, J. Brodbeck, A. C. Daub, P. Sharma, S. Finkbeiner, B. Cui and L. Mucke (2010). "Tau reduction prevents Abeta-induced defects in axonal transport." Science **330**(6001): 198.

Wachterman, M., D. K. Kiely and S. L. Mitchell (2008). "Reporting dementia on the death certificates of nursing home residents dying with end-stage dementia." JAMA **300**(22): 2608-2610.

Wahle, T., D. R. Thal, M. Sastre, A. Rentmeister, N. Bogdanovic, M. Famulok, M. T. Heneka and J. Walter (2006). "GGA1 is expressed in the human brain and affects the generation of amyloid beta-peptide." J Neurosci **26**(49): 12838-12846.

Wai, M. S., Y. Liang, C. Shi, E. Y. Cho, H. F. Kung and D. T. Yew (2009). "Co-localization of hyperphosphorylated tau and caspases in the brainstem of Alzheimer's disease patients." Biogerontology **10**(4): 457-469.

Wallace, W., S. T. Ahlers, J. Gotlib, V. Bragin, J. Sugar, R. Gluck, P. A. Shea, K. L. Davis and V. Haroutunian (1993). "Amyloid precursor protein in the cerebral cortex is rapidly and persistently induced by loss of subcortical innervation." Proc Natl Acad Sci U S A **90**(18): 8712-8716.

Wang, J., H. M. Wright, P. Vempati, H. Li, J. Wangsa, A. Dzhuan, K. Habbu, L. A. Knable, L. Ho and G. M. Pasinetti (2013). "Investigation of nebivolol as a novel therapeutic agent for the treatment of Alzheimer's disease." J Alzheimers Dis **33**(4): 1147-1156.

Wang, J. Z., Y. Y. Xia, I. Grundke-Iqbal and K. Iqbal (2013). "Abnormal hyperphosphorylation of tau: sites, regulation, and molecular mechanism of neurofibrillary degeneration." J Alzheimers Dis **33 Suppl 1**: S123-139.

Wang, Y., P. R. Musich, M. A. Serrano, Y. Zou, J. Zhang and M. Y. Zhu (2014). "Effects of DSP4 on the noradrenergic phenotypes and its potential molecular mechanisms in SH-SY5Y cells." Neurotox Res **25**(2): 193-207.

Ward, S. M., D. S. Himmelstein, J. K. Lancia and L. I. Binder (2012). "Tau oligomers and tau toxicity in neurodegenerative disease." Biochem Soc Trans **40**(4): 667-671.

Webster, S. J., A. D. Bachstetter, P. T. Nelson, F. A. Schmitt and L. J. Van Eldik (2014). "Using mice to model Alzheimer's dementia: an overview of the clinical disease and the preclinical behavioral changes in 10 mouse models." Front Genet **5**: 88.

Weingarten, M. D., A. H. Lockwood, S. Y. Hwo and M. W. Kirschner (1975). "A protein factor essential for microtubule assembly." Proc Natl Acad Sci U S A **72**(5): 1858-1862.

Weinshenker, D. (2008). "Functional consequences of locus coeruleus degeneration in Alzheimer's disease." Curr Alzheimer Res **5**(3): 342-345.

Weinshenker, D., N. S. Miller, K. Blizinsky, M. L. Laughlin and R. D. Palmiter (2002). "Mice with chronic norepinephrine deficiency resemble amphetamine-sensitized animals." Proceedings of the National Academy of Sciences **99**(21): 13873-13877.

Wenge, B. and H. Bonisch (2009). "Interference of the noradrenergic neurotoxin DSP4 with neuronal and nonneuronal monoamine transporters." Naunyn Schmiedebergs Arch Pharmacol **380**(6): 523-529.

Wiseman, F. K., T. Al-Janabi, J. Hardy, A. Karmiloff-Smith, D. Nizetic, V. L. Tybulewicz, E. M. Fisher and A. Strydom (2015). "A genetic cause of Alzheimer disease: mechanistic insights from Down syndrome." Nat Rev Neurosci **16**(9): 564-574.

Wolfman, C., V. Abo, D. Calvo, J. Medina, F. Dajas and R. Silveira (1994). "Recovery of central noradrenergic neurons one year after the administration of the neurotoxin DSP4." Neurochem Int **25**(4): 395-400.

Xu, F., R. R. Gainetdinov, W. C. Wetsel, S. R. Jones, L. M. Bohn, G. W. Miller, Y. M. Wang and M. G. Caron (2000). "Mice lacking the norepinephrine transporter are supersensitive to psychostimulants." Nat Neurosci **3**(5): 465-471.

Xu, H., T. W. Rosler, T. Carlsson, A. de Andrade, J. Bruch, M. Hollerhage, W. H. Oertel and G. U. Hoglinger (2014). "Memory deficits correlate with tau and spine pathology in P301S MAPT transgenic mice." Neuropathol Appl Neurobiol **40**(7): 833-843.

Xu JQ, M. S., Kochanek KD, Bastian BA (2016). Deaths: Final Data for 2013. N. v. s. reports. Hyattsville, MD. **64**.

Yang, J. H., E. O. Lee, S. E. Kim, Y. H. Suh and Y. H. Chong (2012). "Norepinephrine differentially modulates the innate inflammatory response provoked by amyloid-beta peptide via action at beta-adrenoceptors and activation of cAMP/PKA pathway in human THP-1 macrophages." Exp Neurol **236**(2): 199-206.

Yang, S., W. L. Kuan and M. G. Spillantini (2016). "Progressive tauopathy in P301S tau transgenic mice is associated with a functional deficit of the olfactory system." Eur J Neurosci **44**(6): 2396-2403.

Yankner, B. A. (1989). "Amyloid and Alzheimer's disease--cause or effect?" Neurobiol Aging **10**(5): 470-471; discussion 477-478.

Yasuda, M., K. Yokoyama, T. Nakayasu, Y. Nishimura, M. Matsui, T. Yokoyama, K. Miyoshi and C. Tanaka (2000). "A Japanese patient with frontotemporal dementia and parkinsonism by a tau P301S mutation." Neurology **55**(8): 1224-1227.

Yates, C. M., J. Simpson, A. Gordon, A. F. Maloney, Y. Allison, I. M. Ritchie and A. Urquhart (1983). "Catecholamines and cholinergic enzymes in pre-senile and senile Alzheimer-type dementia and Down's syndrome." Brain Res **280**(1): 119-126.

Yiannopoulou, K. G. and S. G. Papageorgiou (2013). "Current and future treatments for Alzheimer's disease." Therapeutic Advances in Neurological Disorders **6**(1): 19-33.

Yoshiyama, Y., M. Higuchi, B. Zhang, S. M. Huang, N. Iwata, T. C. Saido, J. Maeda, T. Suhara, J. Q. Trojanowski and V. M. Lee (2007). "Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model." Neuron **53**(3): 337-351.

Yu, J. T., L. Tan, J. R. Ou, J. X. Zhu, K. Liu, J. H. Song and Y. P. Sun (2008). "Polymorphisms at the beta2-adrenergic receptor gene influence Alzheimer's disease susceptibility." Brain Res **1210**: 216-222.

Yuan, Y., Z. X. Gu and W. S. Wei (2009). "Fluorodeoxyglucose-positron-emission tomography, single-photon emission tomography, and structural MR imaging for prediction of rapid conversion to Alzheimer disease in patients with mild cognitive impairment: a meta-analysis." AJNR Am J Neuroradiol **30**(2): 404-410.

Zarow, C., S. A. Lyness, J. A. Mortimer and H. C. Chui (2003). "Neuronal loss is greater in the locus coeruleus than nucleus basalis and substantia nigra in Alzheimer and Parkinson diseases." Arch Neurol **60**(3): 337-341.

Zetterberg, H., U. Andreasson, O. Hansson, G. Wu, S. Sankaranarayanan, M. E. Andersson, P. Buchhave, E. Londos, R. M. Umek, L. Minthon, A. J. Simon and K. Blennow (2008). "Elevated cerebrospinal fluid BACE1 activity in incipient Alzheimer disease." Arch Neurol **65**(8): 1102-1107.

Zhang, B., J. Carroll, J. Q. Trojanowski, Y. Yao, M. Iba, J. S. Potuzak, A. M. Hogan, S. X. Xie, C. Ballatore, A. B. Smith, 3rd, V. M. Lee and K. R. Brunden (2012). "The microtubule-stabilizing agent, epothilone D, reduces axonal dysfunction, neurotoxicity, cognitive deficits, and Alzheimer-like pathology in an interventional study with aged tau transgenic mice." J Neurosci **32**(11): 3601-3611.

Zhang, X., D. M. Zuo and P. H. Yu (1995). "Neuroprotection by R(-)-deprenyl and N-2-hexyl-N-methylpropargylamine on DSP-4, a neurotoxin, induced degeneration of noradrenergic neurons in the rat locus coeruleus." Neurosci Lett **186**(1): 45-48.

Zhao, Q. F., L. Tan, H. F. Wang, T. Jiang, M. S. Tan, L. Tan, W. Xu, J. Q. Li, J. Wang, T. J. Lai and J. T. Yu (2016). "The prevalence of neuropsychiatric symptoms in Alzheimer's disease: Systematic review and meta-analysis." J Affect Disord **190**: 264-271.

Zhong, Z., M. Ewers, S. Teipel, K. Burger, A. Wallin, K. Blennow, P. He, C. McAllister, H. Hampel and Y. Shen (2007). "Levels of beta-secretase (BACE1) in cerebrospinal fluid as a predictor of risk in mild cognitive impairment." Arch Gen Psychiatry **64**(6): 718-726.

Zipser, B. D., C. E. Johanson, L. Gonzalez, T. M. Berzin, R. Tavares, C. M. Hulette, M. P. Vitek, V. Hovanesian and E. G. Stopa (2007). "Microvascular injury and blood-brain barrier leakage in Alzheimer's disease." Neurobiol Aging **28**(7): 977-986.

Zornetzer, S. F. (1985). "Catecholamine system involvement in age-related memory dysfunction." Ann N Y Acad Sci **444**: 242-254.

Zweig, R. M., C. A. Ross, J. C. Hedreen, C. Steele, J. E. Cardillo, P. J. Whitehouse, M. F. Folstein and D. L. Price (1989). "Neuropathology of aminergic nuclei in Alzheimer's disease." Prog Clin Biol Res **317**: 353-365.