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Brain-Derived Neurotrophic Factor as a Biomarker for Aging and Dementia

by

Krishna Lajwanti Bharani

A dissertation submitted to the faculty of the Medical University of South Carolina in partial fulfillment of the requirement for the degree of Doctor of Philosophy in the College of Graduate Studies.

Department of Neuroscience

2018

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To my parents, **Laju and Madu Bharani**, who instilled in me a love of learning and sacrificed extensively to make it possible for me to pursue my passions.

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LIST OF ABBREVIATIONS

%ICV = Percent Intracranial Volume
%Linearity = Percent Linearity
%Recovery = Percent Recovery
AAV = Adeno-Associated Virus
AD = Alzheimer's Disease
ANOVA = Analysis of Variance
APOE = Apolipoprotein E
Aβ = Amyloid β
Aβ40 = Amyloid β with 40 amino acid
Aβ42 = Amyloid β with 42 amino acid
BA = Brodmann Area
BBB = Blood-Brain Barrier
BCA = Bicinchoninic Acid
BDNF = Brain-Derived Neurotrophic Factor
BF = Basal Forebrain
BG = Basal Ganglia
BL = Baseline
BA46 = Brodmann Area 46
CBL = Cerebellar Cortex
CG = Cingulate Gyrus
CNPase = 2',3'-Cyclic-Nucleotide 3'-Phosphodiesterase
Con = Control
CSF = Cerebrospinal Fluid
CSF-C = Cranial CSF
CSF-L = Lumbar CSF
DAPI = 4′,6-Diamidino-2-Phenylindole
DG = Dentate Gyrus
DLPFC = Dorsolateral Prefrontal Cortex
DSM = Diagnostic and Statistical Manual of Mental Disorders
DSP4 = N-(2-Chloro Ethyl)-N-Ethyl-Bromo-Benzyl Amine
DTI = Diffusion Tensor Imaging
DV = Dependent Variable
Educ = Education
EF = Executive Function
ELISA = Enzyme-Linked Immunosorbent Assay
ENT = Entorhinal cortex
EOAD = Early-Onset Alzheimer’s Disease
EtBr = Ethidium Bromide
F344 = Fischer 344
FA = Fractional Anisotropy
FC = Frontal Cortex
FDA = Food and Drug Administration
FTD = Frontotemporal Dementia

FU = Follow-Up
GFAP = Glial Fibrillary Acidic Protein
HB = Human Brain
HD = Huntington's Disease
HIP = Hippocampus
HLA-DR = Human Leukocyte Antigen-Antigen D Related
HPC = High Pathology Control
IACUC = Institutional Animal Care and Use Committee
ICP = Internal Control Proteins
IHC = Immunohistochemistry
Inflam = Basal Inflammation
IQR = Interquartile Range
IRB = Institutional Review Board
kDa = Kilodalton
K-S = Kolmogorov-Smirnov
LBD = Dementia with Lewy Bodies
LC = Locus Coeruleus
LC-NE = Locus Coeruleus Noradrenergic
LNF = Lane Normalization Factor
LOAD = Late-Onset Alzheimer's Disease
LPS = Lipopolysaccharide
LTD = Long-Term Depression
LTP = Long-Term Potentiation
mBDNF = Mature BDNF
MC = Motor Cortex
MCI = Mild Cognitive Impairment
MD = Mean Diffusivity
MHC = Major Histocompatibility
MIND = MR Imaging of Neurodegeneration
MRI = Magnetic Resonance Imaging
mTOR = Mammalian Target of Rapamycin
MUSC = Medical University of South Carolina
M-W = Mann-Whitney
NA= Not Available
NACC-UDS = National Alzheimer's Coordinating Center Uniform Data Set
ND = Not Detectable
NE = Norepinephrine
NFTs = Neurofibrillary Tangles
NGF = Nerve Growth Factor
NHR = Not Human Research
NIA = National Institute of Aging
NIA-AA = National Institute on Aging and the Alzheimer's Association
NMDA = N-Methyl-D-Aspartate
N-PER = Neuronal Protein Extract Reagent
OC = Occipital cortex

OD = Optical Density
PC = Parietal Cortex
PET = Positron Emission Tomography
PD = Parkinson's disease
PHFs = Paired Helical Fragments
PI3K = Phosphoinositide 3-Kinase
PLC = Phospholipase C Cascade
PMI = Post Mortem Interval
PSEN1 = Presenilin 1
PSEN1 = Presenilin 2
pTau = Phosphorylated Tau
PUT = Putamen
Ras-MAPK = Ras-Mitogen-Activated Protein Kinase
RB = Rat Brain
RIPA = Radioimmunoprecipitation Assay
ROI = Region of Interest
RT = Room Temperature
SD = Standard Deviation
SE = Standard Error
Ser = Serum
Sig = Significant
tBDNF = Total BDNF
TBSS = Tract-Based Spatial Statistics Pipeline
TC = Temporal Cortex
TH = Tyrosine Hydroxylase
THL = Thalamus
TrkB = Tropomyosin-Related Kinase B
U.S. = United States
VaD = Vascular Dementia
WB = Western Blot

KRISHNA LAJWANTI BHARANI. Brain-Derived Neurotrophic Factor as a Biomarker for Aging and Dementia. (Under the direction of ANN-CHARLOTTE GRANHOLM)

ABSTRACT

The number of people with dementia due to Alzheimer's disease (AD) is increasing worldwide. Although AD pathology begins well before clinical symptoms are apparent, identifying individuals at risk of developing AD to provide early interventions is still a challenge. Brain-derived neurotrophic factor (BDNF) is produced by neurons and glial cells and has complex interactions with AD pathology. BDNF also crosses the blood-brain barrier and can be measured in serum or plasma. In this dissertation, I investigated the biomarker potential of serum BDNF for AD using *post mortem* human samples, an aged rat model of cognitive impairment, and blood samples from cognitively healthy older adults. In the first part of my dissertation, I investigated the relationship between CSF, serum, and brain tissue levels of BDNF and AD-related pathology using *post mortem* human brain tissue samples. In these studies, I demonstrated, for the first time, that serum proBDNF levels reflect brain BDNF levels and that low brain BDNF levels are associated with increased accumulation of pTau and amyloid in the hippocampus. In the next part of my dissertation, I investigated whether serum BDNF levels were altered in an aged rat model exposed to a combination of the locus coeruleus selective neurotoxin and inflammation caused by lipopolysaccharides (LPS). I found that serum BDNF levels have an inverse relationship with inflammatory markers, and BDNF levels increase at two weeks

post LPS injection, suggesting a compensatory increase. Finally, in the last part of my dissertation, I investigated whether serum BDNF levels can predict early changes in neuropsychological performance and neuroimaging measures associated with AD in cognitively healthy older adults. In this cohort of older adults, we found significant differences between women and men. Women had higher serum BDNF levels than men, the changes of neuropsychological performance over time were different in women and men, and higher baseline BDNF levels were associated with greater declines in hippocampal volume and limbic FA in men but not in women. Overall, results from this dissertation indicate that serum BDNF levels correlate with brain BDNF levels, are reduced by neurodegeneration and inflammation, and may reflect clinically-relevant changes in older adults and, therefore, should be considered for inclusion in routine blood workup in a clinical setting.

CHAPTER 1.

GENERAL INTRODUCTION

1.1 Dementia: An emerging health crisis

The older adult population, defined as those aged 65 and over, is growing at an unprecedented rate in the western world, and the United States (U.S.) Census Bureau has projected that the world older adult population will double to a total of 1.6 billion between 2025 and 2050 (He et al. 2016). As the ratio of older adults to younger adults increase, the health care system will need to adapt to address the unique health challenges the elderly population face, including dementia.

Dementia is a syndrome characterized by a significant and progressive decline in two or more modalities of cognition (including aphasia, inability to plan and initiate complex behavior, attention, executive function, language, learning and memory, perceptual-motor, or social cognition) that interferes with daily function and independence. Dementia is associated with several diseases including Alzheimer's disease (AD), Dementia with Lewy bodies (LBD), Frontotemporal dementia (FTD), Huntington's disease (HD), Parkinson's disease (PD), and Vascular dementia (VaD) (Association 2018). In low- and middle-income countries, dementia is the leading cause of disability and dependence in older adults (Prince et al. 2015). Total Medicaid cost for care related to dementia is projected to exceed $47 billion dollars in 2018 (Association 2018). Alzheimer's disease accounts for approximately 60 to 80 percent of dementia cases, and the number of older adults aged 65 years or older with AD in the United States is expected to more than double by 2050 and exceed 13.8 million people (Association 2018).

1.2 Biological Causes of Alzheimer's disease

AD was described more than a century ago by Dr. Alois Alzheimer in his accounts of a 51-year-old woman, Auguste D, who showed progressive impairment in comprehension and memory as well as aphasia, disorientation, paranoia, and psychosocial impairment already in her 50s (Alzheimer 1907, Maurer et al. 1997). After the death of Auguste D, Dr. Alzheimer noted in Auguste D's brain that "in the centre of an otherwise almost normal cell there stands out one or several fibrils due to their characteristic thickness and peculiar impregnability" and that "[n]umerous small miliary foci are found in the superior layers" (Maurer et al. 1997). The latter is now known as plaques and the former as neurofibrillary tangles (NFT). In addition to plaques and NFTs, hippocampal atrophy, cortical thinning, and ventricle enlargement were also noted in AD brains (**Figure 1-1**). The eponym Alzheimer was introduced by German psychiatrist Dr. Emil Kraepelin several years later after prominent medical scientists made similar reports on other patients. Currently, the only way to definitively diagnosis AD is through *post mortem* analysis of the brain, even though evidence indicates that the underlying pathology of AD begins decades before clinical symptoms manifest themselves (Dubois et al. 2007, Jack and Holtzman 2013, Jack et al. 2013). A probable AD diagnosis is clinically determined for patients who show impairment in at least two cognitive domains (learning and memory, reasoning and judgment, visuospatial, language, or behavior and comportment) with a decline in functioning overtime that disrupts their activities of daily living

(McKhann et al. 2011). Additionally, these clinical features must have an insidious onset, have a clear-cut history of worsening, and not be explained by vitamin B12 deficiency, delirium, medications or another major psychiatric disorder. The clinical diagnosis of probable AD is confirmed through histopathologic examination of amyloid plaques and NFT *post mortem* (Hyman et al. 2012, Montine et al. 2012). The etiology of AD is highly debated, and the following is a review of several of the prevailing biological mechanisms proposed.

1.2.1 Amyloid cascade hypothesis

The primary components of AD plaques are neurotoxic amyloid β (Aβ) peptides. These Aβ peptides are created through sequential cleavage of the amyloid precursor protein (APP) by β-secretase and γ-secretase (**Figure 1-1D**) and comprise of 40-42 amino acids (amyloidogenic pathway). The 42 amino acid form of Aβ (Aβ42) is considered to be the more toxic form of Aβ when compared with the 40 amino acid form (Aβ40) due to its higher propensity to oligomerize, form fibrils, and resist degradation (Walsh and Selkoe 2004, Baranello et al. 2015). Alternatively, APP can be cleaved by α-secretase and γ-secretase to produce a smaller fragment named P3 (non-amyloidogenic pathway) which does not oligomerize or have toxic properties (Dulin et al. 2008, Han et al. 2011). The compartmentalization of these processing events is critical in APP regulation, and, while α-secretase cleaving of APP occurs either in the secretory pathway or at the cell surface, β-secretase cleaving of APP occurs in endocytic vesicles at an optimum acid pH (Baranello et al. 2015). Aβ plaques are proposed by Thal et

al. (2002) to be deposited throughout the brain in five phases: 1st in the neocortex, 2nd in allocortical brain regions, 3rd in the diencephalic nuclei, striatum, and basal forebrain, 4th in the brainstem, and 5th in the cerebellum.

The *APP* gene resides on chromosome 21, and the risk to develop the clinical and histopathological features of AD are increased in those who have trisomy 21 (Down syndrome; Egensperger et al. 1999, Head et al. 2016). The population with DS develops AD with near complete penetrance in their 4th or 5th decade. Genetic mutations of *APP*, presenilin 1 (*PSEN1*), or presenilin 2 (*PSEN1*) genes are associated with increased production of Aβ plaques and the development of early-onset AD (EOAD), which is defined as AD where symptoms begins before the patient is 65 years old (Bekris et al. 2010). These mutations are autosomal dominant and account for fewer than 10% of all AD cases. The majority of AD cases are considered to be late-onset AD (LOAD) and occur in adults who are 65 years old or older. Although no causative gene is associated with LOAD, older adults with the ε4 allele of the apolipoprotein E (*APOE*) gene are more likely to develop sporadic LOAD (Bekris et al. 2010). In mouse models that express human apoE protein, researchers found that apoE protein isoforms do not alter APP processing or increase Aβ synthesis; instead, the *APOE* genotype differentially modulates clearance. The ε4 allele is associated with lower Aβ clearance when compared with ε3 and ε2 (Castellano et al. 2011).

These genetic associations between Aβ formation and AD largely support the amyloid cascade hypothesis which asserts that Aβ production and

accumulation is the critical step that initiates a cascade characterized by activated microglia and astrocytes, NFT formation, damaged neurons, and ultimately dementia and death (Karran et al. 2011). Accordingly, several therapeutic agents such as inhibitors targeting β- and γ-secretase or antibodies targeting Aβ were developed to either impede Aβ production or facilitate Aβ clearance (Baranello et al. 2015). Unfortunately, several Aβ-targeted Phase III clinical trials have been discontinued due to adverse outcomes or poor efficacy (Karran et al. 2011, Hung and Fu 2017). One of the challenges of testing AD drugs is identifying potential candidates for therapy early enough in the disease course to maximize therapeutic benefit (Sperling et al. 2011).

1.2.2 The tau hypothesis

Intracellular neurofibrillary tangles (NFTs) are composed of hyperphosphorylated, microtubule-associated protein tau (Grundke-Iqbal 1986). Microtubules are a critical component of the neuronal cytoskeleton that regulates transport along the axon, dendrites, and spines. The tau protein has six isoforms with varying repeats and lengths (Buee et al. 2000). The hyperphosphorylation of tau by kinases causes the tau protein to disassociate from the microtubule which destabilizes the microtubule and interferes with the neuron's ability to transport material between the cell body and dendrites (Billingsley and Kincaid 1997). These free-floating hyperphosphorylated tau aggregate to form insoluble paired helical fragments (PHFs). Paired helical fragments then self-assemble to form intracellular NFT inclusions. Both the formation of intracellular NFT inclusions

and malfunctioning microtubules ultimately cause the neuron to die and leave behind an extracellular NFT, or a so-called ghost tangle (Augustinack et al. 2001, Andorfer et al. 2003, Ballatore et al. 2007). Neurofibrillary tangle deposits in the brain have been shown to sequentially spread from the transentorhinal cortex in early stages to the isocortex in late stages (Braak and Braak 1991).

The notion that dysfunction of tau is a primary event in AD is highly debated but is supported by the finding that NFT burden better correlates with cognitive decline than amyloid burden (Nelson et al. 2012) and by the finding of NFTs in patients with mild dementia without plaques (Crary et al. 2014). In addition, abnormal tau can act as a “seed,” be transferred to healthy cells, and can promote further abnormal tau formation and aggregation (Lewis and Dickson 2016), suggesting a prion-like propagation mechanism for spreading AD pathology throughout the brain. Alternatively, AD cases with NFT and without amyloid plaque formation, so-called “tangle-prominent AD,” have been proposed to be a distinct pathological category by themselves with a potentially different etiology than AD (Crary et al. 2014, Crary 2016).

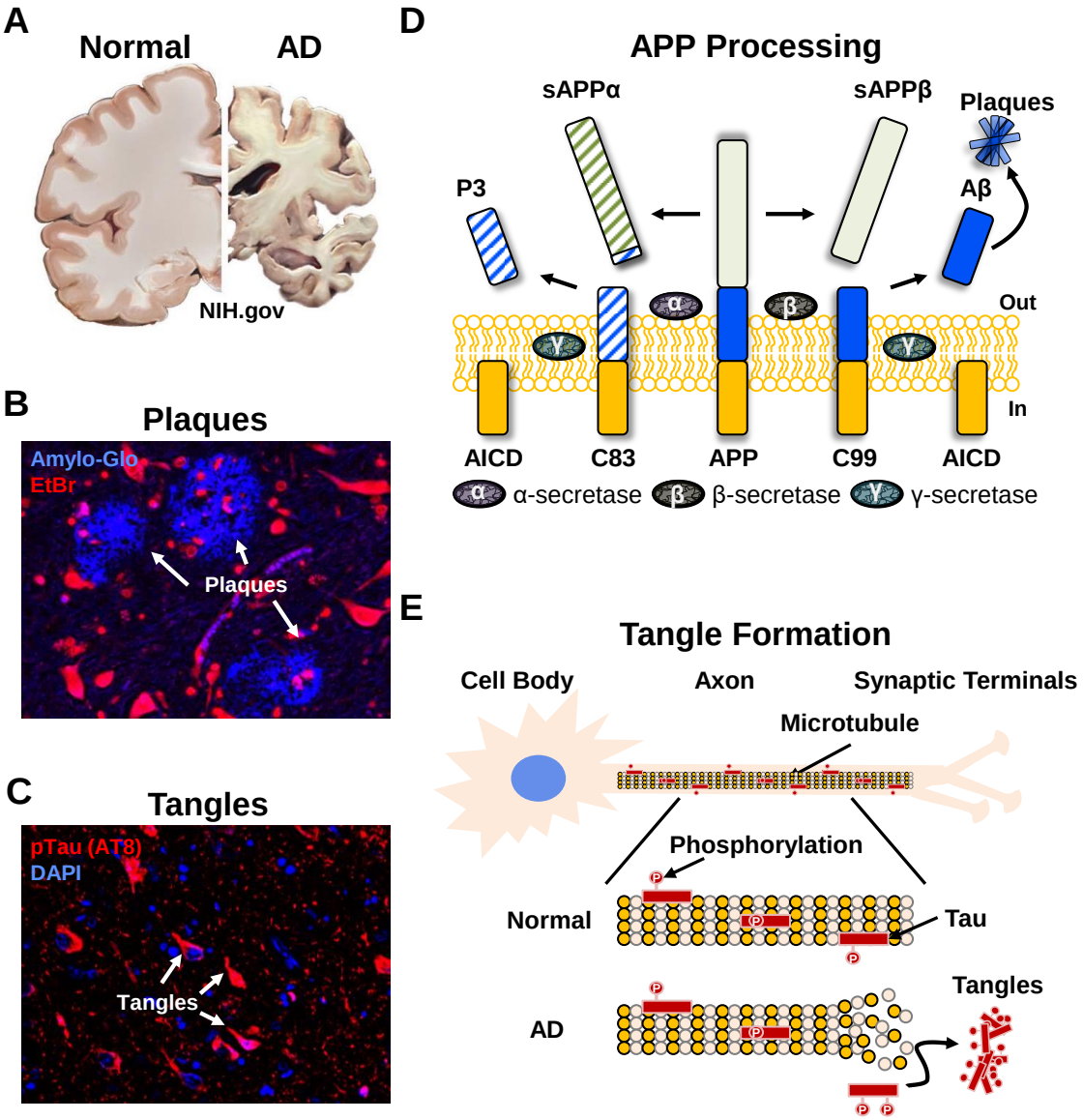


Figure 1-1. Alzheimer's disease pathology. Cortical thinning, enlarged ventricles, deeper sulci, and hippocampal atrophy are apparent in the AD brain compared to a normal brain (A). Histopathological examination of AD brains reveals numerous plaques (Amylo-Glo; B) and tangles (AT8; C) in the hippocampus. Nuclei were stained for using EtBr or DAPI. Plaques are made up of amyloid-beta (Aβ) protein which is produced by the sequential cleavage of the amyloid precursor protein (APP) by β-secretase and γ-secretase (D). Alternatively, APP can be sequentially cleaved by α-secretase and γ-secretase to form P3. Tangles are made up of tau proteins which disassociate from and destabilize microtubules when hyperphosphorylated (E).

1.2.3 Inflammation hypothesis

Alzheimer's disease has been shown to be strongly associated with chronic inflammation characterized by microglia proliferation and increased production of proinflammatory cytokines in the periphery and brain (Franceschi et al. 2006, Holmes 2013, Amor et al. 2014, Heneka et al. 2015, Heppner et al. 2015). In *in vitro* models of AD, both plaques and NFTs are shown to activate microglia which in turn release proinflammatory cytokines and can cause cell death and propagate plaque and tangle pathology (Combs et al. 2001, Sondag et al. 2009, Morales et al. 2013). In patients with dementia, *in vivo* positron emission tomography (PET) have shown increased microglial activation (Cagnin et al. 2001), and *post mortem* studies on AD brains have shown increased levels of proinflammatory cytokines compared to controls (Griffin et al. 1989, Morimoto et al. 2011). Furthermore, in mice, Chen et al. (2016) showed that NFTs stimulate microglia phagocytic activity to promote the clearance of plaques. These and other results suggest that on the one hand, microglia proliferation facilitates the clearance of plaques through phagocytosis and may instead help ameliorate AD pathology (Lai and McLaurin 2012, Heneka et al. 2015). On the other hand, the unregulated release of proinflammatory cytokines from microglia without a proper resolution can facilitate neurodegeneration and cognitive decline (Wang et al. 2015).

Neuroinflammation was traditionally viewed to only occur at the late stages of AD; however, several clinical and epidemiological studies suggested that pro-inflammatory cytokines can increase the susceptibility to cognitive

impairment in older adults (Perry 2010, Simen et al. 2011, Holmes 2013), and experimental studies in mice and rats from our group and others have suggested that immune challenges can trigger and even drive development of AD-like pathophysiology (Krstic et al. 2012, Bharani et al. 2017). Interestingly, a *post mortem* study on patients without a clinical history of dementia but with sufficient plaques and NFTs to qualify for an AD diagnosis (a.k.a. High Pathology Controls or HPC) shown that measures of inflammation (i.e., C5b-9 and LN3 immunoreactivity) were significantly reduced in HPC compared to AD cases and that HPC inflammatory status was similar to normal control cases (Lue et al. 1996). These results have fueled the hypothesis that the immune process plays an important role in AD pathogenesis and that interventions that modulate the immune system are crucial to prevent or arrest the progression of AD pathology (McGeer and McGeer 2007, Zotova et al. 2010).

1.2.4 Cholinergic hypothesis

Degeneration of the basal forebrain cholinergic neurons and the subsequent damage to cholinergic neurotransmission was one of the earliest theories of AD (Perry et al. 1977, Bartus et al. 1982, Whitehouse et al. 1982, Francis et al. 1999, Mufson et al. 2008, Ferreira-Vieira et al. 2016). This theory was supported by early *post mortem* studies which found reductions in the neurotransmitter acetylcholine (Bowen and Davison 1980), reductions in the major enzyme responsible for the synthesis of acetylcholine - choline acetyltransferase (Perry et al. 1977, Sims et al. 1983), reductions in uptake of the

acetylcholine precursor protein - choline (Rylett et al. 1983), and loss of basal forebrain cholinergic neurons in the nucleus basalis of Meynert (Davies and Maloney 1976, Whitehouse et al. 1982, Mufson et al. 2008) in AD brains compared to controls. The cholinergic hypothesis for AD suggests that degeneration of basal forebrain cholinergic neurons contributes to development and spread of AD pathology as well as the memory and attention deficits exhibited by AD patients (Whitehouse et al. 1982, Muir et al. 1992, Rogers and Kesner 2004, Ferreira-Vieira et al. 2016). Currently, three US Food and Drug Administration (FDA) approved drugs for AD are cholinesterase inhibitors (donepezil, galantamine, rivastigmine). Cholinesterase inhibitors effectively increase access to acetylcholine in the synaptic cleft by inhibiting the enzyme that degrades acetylcholine. Although these drugs do not stop the progression of AD, they do ameliorate some cognitive symptoms, enhance quality of life, diminish caregiver burden, and delay alterations in ADL (Mufson et al. 2008, Raina 2008, Sun et al. 2008, Howard et al. 2012, Hager et al. 2014, Ferreira-Vieira et al. 2016). There are still many research groups who focus on the cholinergic hypothesis, and cholinergic cell loss in AD still warrants investigation based on the close relationship between cholinergic degeneration and memory loss in AD.

1.2.5 Noradrenergic hypothesis

Degeneration of the nucleus locus coeruleus (LC) results in decreased norepinephrine (NE) levels throughout the brain (Kelly et al. 2017). With age, LC-

NE neurons degenerate, and to a greater extent, in the presence of AD pathology (Zarow et al. 2003, Marien et al. 2004, Lockrow et al. 2011). The LC-NE neurons project throughout the brain and spinal cord but densely innervate the hippocampus and neocortex where NE acts on adrenergic receptors on neurons, endothelial cells, and glia to impart potent anti-inflammatory, anti-oxidant, neurotrophic, and neuroprotective effects and to facilitate learning and memory processes (Sara 2009, O'Donnell et al. 2012, Braun et al. 2014, Uematsu et al. 2015, Kelly et al. 2017). Neurofibrillary tangle formation has been found in the LC in early stages of AD including amnestic mild cognitive impairment (MCI), preceding amyloid deposition (Grudzien et al. 2007, Braak and Del Tredici 2011) as well as NFT accumulation in other brain regions. Furthermore, lesions of the LC in mouse models of AD lead to increased inflammation, neuronal damage, and plaque burden (Kalinin et al. 2007, Lockrow et al. 2011). Converging evidence from animal models and clinical and preclinical data demonstrate the importance of the LC-NE preservation to ameliorate AD development and highlight the disease-modifying potential of pharmaceutical modification of NE (Chalermpalanupap et al. 2013, Braun et al. 2014). Several NE pharmacotherapies are FDA-approved to treat attention-deficit disorder, depression, and hypotension, and investigations of these drugs on individuals with preclinical or early clinical stages of AD are highly anticipated. **The importance of LC-NE loss for memory function and neuroinflammation will be examined in Aim 2 of this dissertation, using an aged rat model of age-**

related cognitive impairment with LC-NE degeneration combined with the injection of lipopolysaccharide (LPS).

1.3 Diagnosing Alzheimer's disease

Currently, a definitive diagnosis of AD can only be achieved through a *post mortem* histopathologic examination of the brain (Braak and Braak 1991, Beach et al. 2012, Hyman et al. 2012, Jack et al. 2018, Wolk and Dickerson 2018). Clinical criteria for an *ante mortem* probable AD dementia diagnosis have been established by the National Institute on Aging and the Alzheimer's Association (NIA-AA; McKhann et al. 2011, Wolk and Dickerson 2018). Using data from 919 subjects from the National Alzheimer's Coordinating Center Uniform Data Set (NACC-UDS), Beach et al. (2012) concluded that the sensitivity ranged from 70.9% to 87.3% and the specificity ranged from 44.3% to 70.8% for *ante mortem* AD diagnosis in relation to *post mortem* AD diagnosis. The Diagnostic and Statistical Manual of Mental Disorders (DSM) is also commonly used for the *ante mortem* diagnosis of AD, and the DSM-IV criteria have been shown to have similar accuracy to the NIA-AA criteria (Knopman et al. 2001). The accuracy for the latest DSM-V criteria for dementia, now named major neurocognitive disorder due to AD and including expanded consideration for other cognitive domains compared to DSM-IV (Association 2013), has not been tested yet. This room for improvement in clinical diagnostic accuracy in AD has fueled the search for new AD biomarkers to better identify and categorize AD.

Part of the challenge of treating AD is identifying patients early in the disease course to maximize therapeutic potential of pharmacological intervention (Sperling et al. 2011). Amnestic mild cognitive impairment (MCI) is a diagnosis given to those who exhibit progressive memory impairment, but intact cognitive and social functions to live independently without assistance (Petersen et al. 2001, Petersen et al. 2009, Albert et al. 2011). Amnestic MCI is considered to be an early stage of AD, but a meta-analysis of 41 MCI cohort studies found that the adjusted annual conversion rate for amnestic MCI convert to AD was 10 to 15 percent with some never converting to AD (Mitchell and Shiri-Feshki 2009). Accordingly, several investigations into biomarkers to better predict the conversion of MCI to AD have been undertaken. These studies have found that the presence of *APOE* ε4 genotype, increased brain atrophy on magnetic resonance imaging (MRI), and low amyloid levels and high tau levels in cerebrospinal fluid (CSF) increase the risk of conversion from MCI to AD (Petersen et al. 1995, Davatzikos et al. 2011, Gomar et al. 2011, van Maurik et al. 2017). These biomarkers do not predict the conversion from MCI to AD perfectly, but efforts to incorporate these biomarkers in a prognostic model to support clinical decisions have been promising with the overall goal to assess the individual risk of conversion and to intervene accordingly (van Maurik et al. 2017). In addition to predicting the conversion from MCI to AD, the major quest in this field is to detect the mechanistic shift from healthy brain aging to neurodegeneration (i.e., from normal control to MCI) in order to halt the disease course before any permanent damage has occurred (Jack and Holtzman 2013,

Jack et al. 2013, Fjell et al. 2014, Dubois et al. 2016). **In Aim 3, we investigated a blood-based biomarker in normal older adults in relation with both neuropsychological performance and neuroimaging measures.**

1.3.1 Modeling AD biomarkers

A hypothetical model of biomarker changes in AD suggests that the AD phenotype follows a temporal sequence with abnormal changes first occurring in Aβ regulation and then followed by tau phosphorylation (pTau), neuronal survival, memory function, and finally clinical function (Jack and Holtzman 2013, Jack et al. 2013). It is also suggested that AD pathology begins in one area of the brain and progresses to other brain regions via a prion-like process (Braak and Braak 1991, Malkki 2015). In fact, current neuropathological staging is based on progression of both NFTs and amyloid to involve progressively more brain regions (Montine et al. 2012). Although this amyloid-first hypothesis is well supported, to accommodate the alternative hypothesis that tau may precede amyloid formation but is difficult to detect based on limitations of current technologies, a detection threshold was added to the current AD disease progression model (**Figure 1-2**). The amyloid-first hypothesis model best mimics what is seen in the clinical AD population and is accurately described above this detection threshold. Below the detection threshold, the model suggests that pTau abnormalities may precede Aβ dysfunction, but Aβ levels increase and accumulate faster than pTau and are detected earlier. Neurodegeneration

ensues after Aβ and pTau pathological accumulation and subsequently leads to memory dysfunction.

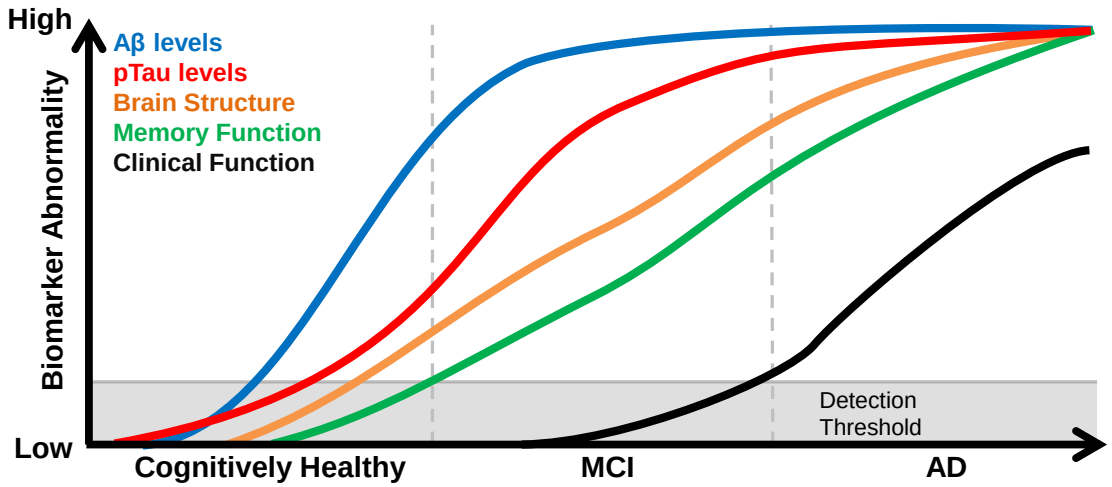


Figure 1-2. Temporal model of changes in AD phenotype. The vertical axis represents severity from low to high, and the horizontal axis represents time and progression from cognitively normal to MCI to AD. The detection threshold is signified by a horizontal gray area and represents the limit of current technology to detect pathophysiological changes. Above the detection threshold, biomarkers detect dysfunction in Aβ first and then pTau, brain structure, memory function, and finally overall health and independence (clinical function). Patients gradually deteriorate when the elevation of AD biomarkers have reached a plateau. Below the detection threshold, pTau is hypothesized to precede Aβ dysfunction but accumulate slower than Aβ pathology. Adapted from Jack et al. (2013).

1.3.2 AD Research Framework

In an attempt to better incorporate biomarkers into AD research, an initiative by the NIA-AA led to the development of a research framework to identify AD patients for use in observational and interventional research (Jack et al. 2018). This framework defines AD by its neuropathological hallmarks (i.e., accumulation of Aβ and pTau) and proposes an AT(N) biomarker system to classify research participants by biomarkers identified in fluid or through neuroimaging. The AT(N) biomarker system describes the biomarker presence

(+) or absence (-) of Aβ plaque (labeled “A”), fibrillary tau (labeled “T”), and neurodegeneration or neuronal injury (labeled “N”) in order to build eight different AT(N) biomarker profiles to better individually categorize patients. Although this framework was largely built with the modified amyloid cascade hypothesis in mind, it is flexible enough to incorporate other AD hypotheses, including the tau hypothesis and hypotheses implicating an upstream pathological process that preceeds and causes Aβ plaque and NFT formation, such as chronic inflammation. **A major focus of my dissertation (all three aims) is to assess the biomarker potential of brain-derived neurotrophic factor (BDNF) for AD in the context of this framework.**

1.4 Brain-derived neurotrophic factor

Neurotrophic factors support neuronal survival and function (Kazim and Iqbal 2016) and are being actively investigated for their therapeutic potential to mitigate AD neuropathology (Nagahara and Tuszynski 2011). The neurotrophin family of growth factors consists of nerve growth factor (NGF), neurotrophin-4/5, as well as BDNF (Nockher and Renz 2005). BDNF is expressed by both glial cells and neurons in the brain, and has significant beneficial effects for both development and maintenance of many neuronal populations (Gonzalez et al. 2016). BDNF has been shown to have a dose-dependent protective effect against amyloid toxicity in AD mouse models (Arancibia et al. 2008), and reduction in BDNF levels has been correlated with the accumulation of neuritic amyloid plaques in AD human brain tissues (Lee et al. 2005) and in rat

hippocampal cell cultures (Matrone et al. 2008). In addition, BDNF crosses the blood-brain barrier (BBB) through a high-capacity, saturable transport system (Poduslo and Curran 1996, Pan et al. 1998). In animal and human studies, both blood and brain BDNF levels have been shown to be sensitive to neurological insults (Connor et al. 1997, Murer et al. 2001, Song et al. 2015, Bharani et al. 2017), suggesting that monitoring blood BDNF levels could be useful for early detection of neuropathological changes.

1.4.1 BDNF production and function

The BDNF gene is located on chromosome 11, and a single nucleotide polymorphism Val66Met has been linked to the development of various neuropsychiatric and neurodegenerative disorders (Vepsalainen et al. 2005, Verhagen et al. 2010, Molendijk et al. 2012). Specifically, the presence of a Val66Met allele has been associated with lower activity-dependent secretion of BDNF, memory impairment, and abnormal hippocampal activity during memory processing in contrast to the BDNF Val homozygote (Egan et al. 2003, Lim et al. 2016). BDNF is synthesized as a precursor pre-proBDNF protein in cortical neurons and is anterogradely transported in dense-core vesicles to target areas (Altar et al. 1997). Pre-proBDNF is then proteolytically cleaved to produce the 32-kDa proBDNF protein. ProBDNF can subsequently be either proteolytically cleaved intracellularly by furin or pro-convertases or extracellularly by plasmin or metalloproteinases to produce the 14-kDa mature BDNF protein (Lessmann et al. 2003, Cunha et al. 2010). Both proBDNF and mature BDNF are stored in pre-

and postsynaptic compartments and can undergo retrograde and anterograde transport, and can be secreted by an activity-dependent secretory pathway (Murer et al. 2001, Kohman et al. 2007, Cunha et al. 2010).

Mature BDNF binds to the tropomyosin-related kinase B (TrkB) receptor, a member of the tyrosine kinase family, to induce receptor dimerization and autophosphorylation on multiple tyrosine residues (**Figure 1-3**). These phosphorylated tyrosine residues become binding sites for SH2 domains of various intracellular target proteins and can activate intracellular signaling cascades such as the phosphoinositide 3-kinase (PI3K) cascade, the Ras-mitogen-activated protein kinase (Ras-MAPK) cascade, and the phospholipase C cascade (PLC). Through these pathways, BDNF can regulate CREB and mammalian target of rapamycin (mTOR) to increase gene expression and protein translation, respectively, and facilitate neuronal survival and synaptic strengthening (Binder and Scharfman 2004). ProBDNF can oppose the effects of mature BDNF by acting on the p75 receptor ($p75^{NTR}$), a low-affinity pan-neurotrophin receptor, to initiate programmed cell death (apoptosis) via JNK signaling pathway (Teng et al. 2005). Alternatively, proBDNF can synergize with mature BDNF by acting on $p75^{NTR}$ to promote cell survival via NF-kB transcription factor (Mandel et al. 2009).

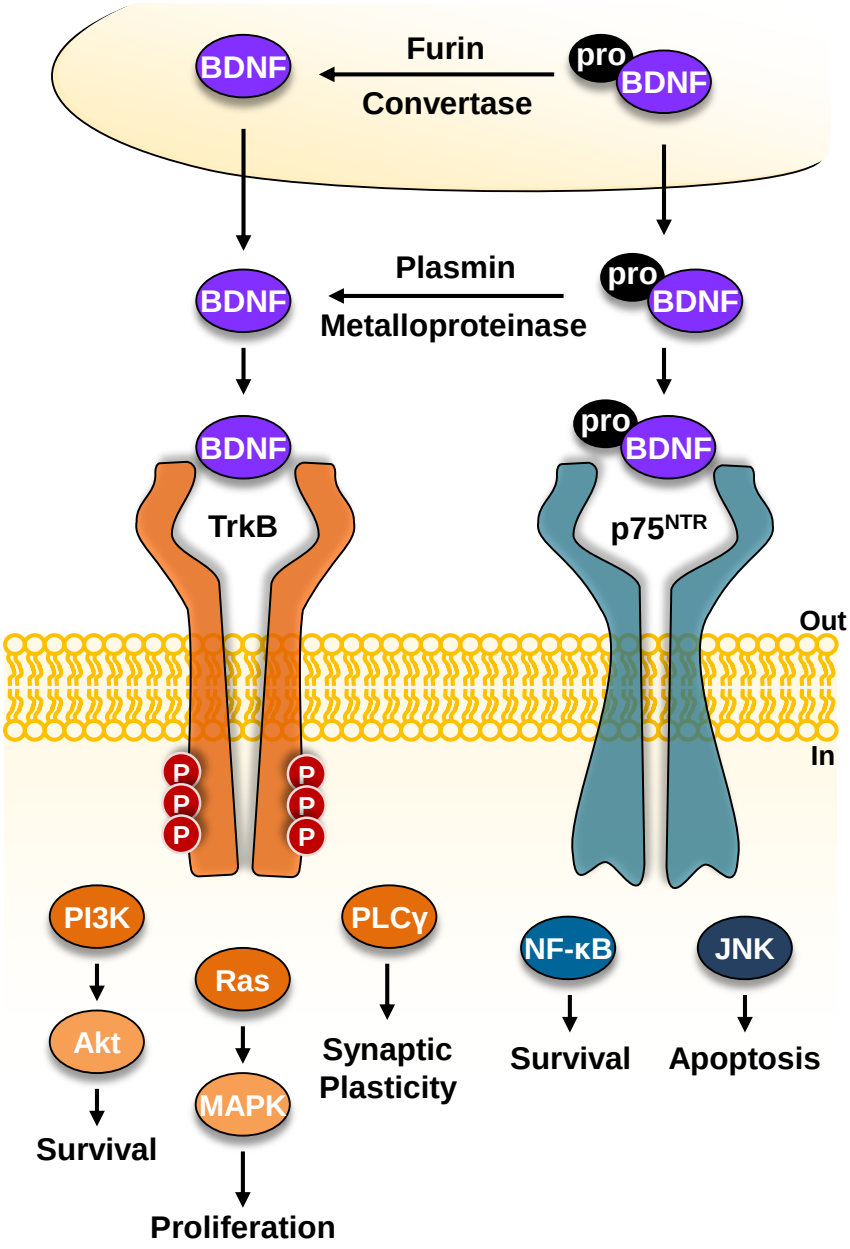


Figure 1-3. BDNF and proBDNF receptors. ProBDNF can be cleaved intracellularly by furin or convertase and extracellularly by plasmin or metalloproteinase into mature BDNF. Mature BDNF acts on the TrkB receptor which autophosphorylates and activates the intracellular signaling cascades phosphoinositide 3-kinase (PI3K), Ras-mitogen-activated protein kinase (Ras-MAPK), and phospholipase C cascade (PLC) to promote cell survival, proliferation, and synaptic plasticity. ProBDNF binds to the $p75^{NTR}$ receptor and can activate NF-κB to promote cell survival or JNK to initiate programmed cell death (apoptosis).

In addition to BDNF's role to promote proliferation and neuronal survival, BDNF is important for hippocampal learning and memory processes. Long-term depression (LTD) and long-term potentiation (LTP) are two models of synaptic plasticity underlying learning and memory. While LTD can inactivate an associative memory (i.e., a memory of a relationship between unrelated items), LTP can reactivate the same memory (Nabavi et al. 2014). BDNF can act on the TrkB receptor to induce LTP, and proBDNF can act on the $p75^{NTR}$ receptor to modify the NMDA receptor and induce LTD (Bliss and Cooke 2011). Alonso et al. (2002) showed that neutralizing endogenous BDNF in the hippocampus using an anti-BDNF antibody blocked short-term and long-term memory formation in rat and infusion of recombinant human BDNF into the hippocampus strongly facilitated short-term and long-term memory formation. These results suggest that BDNF is necessary for hippocampal-mediated learning. **In Aim 2 of this dissertation, I investigated the interplay between LC-NE degeneration and BDNF levels in an aged rat model.**

1.4.2 Detecting BDNF

Several methods are employed to detect BDNF in various sample matrixes. Enzyme-linked immunosorbent assays (ELISAs) and western blots are the most commonly used techniques to detect BDNF in biological fluids and tissue homogenates. Both of these techniques depend on using an antibody that is specific for BDNF. BDNF ELISAs are commonly used to quantify BDNF levels in serum and tissue homogenates due to their commercial availability from

several providers, their high sensitivity to BDNF levels, and their relatively straight-forward protocol that can be automated. Commercial ELISAs also have the added benefit of being validated by the manufacturing company to have low interassay variability; however, this interassay variation declared by the manufacturer has been reported to be higher in practical use by several research groups (Lim et al. 2015, Polacchini et al. 2015). In addition, an ELISA specificity is limited by the specificity of the antibody used to detect the antigen of interest, and, while ELISAs have low detection limits, commercial BDNF ELISA kits specificity to mature BDNF is questioned due to their antibody cross-reactivity to proBDNF (Fahnestock et al. 2002, Yoshida 2012, Lim et al. 2015, Polacchini et al. 2015). Western blots can distinguish mature BDNF (14 kDa) from proBDNF (36 kDa) by their molecular weight (Fahnestock et al. 2002) and have high specificity but low sensitivity compared to ELISAs. In addition, the western blot protocol is relatively labor intensive compared to the ELISA protocol, and there may be high interassay variability between blots due to variations in gel composition and transfer procedure used. Western blot interassay variability is reduced through standardizations in materials and user technique as well as internal controls. **Chapter 2 Section 2.4** will further explore the strengths and limitations of ELISAs and western blot techniques in detecting BDNF.

1.4.3 BDNF and aging

BDNF is crucial for healthy development and aging. The majority of BDNF homozygous knockout (–/–) mice die postnatally due to cardiorespiratory defects

and those that do survive only live for up to 3 weeks and show severe hypomyelination (Huang and Reichardt 2001, Binder and Scharfman 2004, Xiao et al. 2010). BDNF heterozygous knockout (+/–) mice are viable and live normal life spans, but these mice display hypomyelination, develop obesity, exhibit aggressiveness, and have impaired learning at an early age (Linnarsson et al. 1997, Lyons et al. 1999, Xiao et al. 2010). Petzold et al. (2015) further showed that the spatial learning impairment found in BDNF heterozygous knockout (+/–) mice increases with age despite having stable BDNF and TrkB protein levels over time. These results suggest that chronically low BDNF levels influence brain structure and function over time, and these accumulating changes can result in learning deficits at an older age. In rat studies, aging is associated with reduced BDNF protein levels, reduced BDNF mRNA expression, synaptic loss in the dentate gyrus, and a decline in spatial memory (Geinisman et al. 1992, Schaaf et al. 2001). In both rat and mouse studies, brain protein levels of the BDNF receptor TrkB also decline with age (Rage et al. 2007, Petzold et al. 2015). These animal studies highlight the importance of BDNF for proper brain and behavior development and suggest that early deficits in BDNF levels may have consequences at later ages. **However, it is not known whether serum BDNF levels are correlated with amyloid or pTau pathology or underlie neurodegenerative changes associated with AD. This will be explored in <u>Aim 1</u> of this dissertation**.

Normal aging in humans is generally associated with cortical and hippocampal degeneration, white matter degeneration, and cognitive decline,

including decline in executive function and memory (Falangola et al. 2008, Harada et al. 2013). Several studies, but not all, have shown that circulating BDNF levels in the blood decrease with increasing age (Lommatzsch et al. 2005, Ziegenhorn et al. 2007, Gunstad et al. 2008, Erickson et al. 2010), and a carefully performed mediation analysis on serum BDNF and hippocampal volume in adults aged 59-81 years further found that age-related decline in BDNF levels partially contributes to the age-related volumetric shrinkage of the hippocampus (Erickson et al. 2012). While this finding highlights the importance of serum BDNF in age-related hippocampal volume decay in a cross-sectional study, **the longitudinal relationship between serum BDNF and neuropsychological performance and neuroimaging measures have not been reported, and studying this relationship was one of the goals for Aim 3 in this dissertation.**

1.5 BDNF and AD

Abnormal BDNF levels in the brain and blood have been linked to the development of depression and schizophrenia (Angelucci et al. 2005, Yoshida et al. 2012), and a growing body of literature has implicated disruption of BDNF biosynthesis in the pathogenesis of AD (Diniz and Teixeira 2011, Laske et al. 2011). Although it is unknown whether the observed loss of BDNF in AD is a primary or secondary pathological event, the interaction between BDNF and AD pathology is complex, and the loss of neuroprotection normally conferred by

BDNF may contribute to the progression of AD (Arancibia et al. 2008, Tapia-Arancibia et al. 2008, Wang et al. 2011, Zhang et al. 2012).

1.5.1 BDNF and AD pathology

Interestingly, BDNF functioning and APP processing to produce amyloid are linked by γ-secretase, and γ-secretase inhibitors have been shown to disrupt BDNF trafficking and signaling while also reducing the amount of Aβ produced (Weissmiller 2015). In *post mortem* analysis of AD brains, the BDNF receptor TrkB was found to be trapped in senile plaques (Connor et al. 1996) and increasing plaque load correlated with the loss of BDNF (Lee et al. 2005), suggesting that amyloid can contribute to loss of BDNF neuroprotection. While mature BDNF has been shown to protect cells from plaque toxicity, proBDNF synergizes with Aβ toxicity and induces more cell death than Aβ alone in SH-SY5Y human neuroblastoma cell cultures (Lim et al. 2015). Overall, these results indicate that mature BDNF mitigates amyloid toxicity while proBDNF promotes amyloid production and toxicity. BDNF may also protect against tangle toxicity; neurons immunoreactive for BDNF protein did not have NFTs in *post mortem* evaluation of human AD brain samples (Murer et al. 1999). This result was supported by *in vitro* studies where BDNF stimulation of the TrkB receptor in neuronally differentiated P19 mouse embryonic carcinoma cells resulted in a rapid decrease in tau phosphorylation (Elliott et al. 2005).

1.5.2 BDNF and inflammation

Inflammation further complicates the relationship between BDNF and AD pathology. Both plaques and tangles are shown to activate microglia which in turn release proinflammatory cytokines (Combs et al. 2001, Sondag et al. 2009, Morales et al. 2013). Pro-inflammatory cytokines such as IL-1β can inhibit the synthesis and function of BDNF *in vivo* and *in vitro* (Barrientos et al. 2004, Calabrese et al. 2014, Di Benedetto et al. 2017) while anti-inflammatory cytokines such as IL-10 has been shown to stimulate BDNF production *in vitro* (Zhu et al. 2015). However, microglia also secrete BDNF, and mice depleted of BDNF produced by microglia showed deficits in multiple learning tasks and a significant reduction in motor-learning-dependent synapse formation, suggesting that BDNF secreted from microglia has an important role in learning and memory (Parkhurst et al. 2013). BDNF can also reduce inflammation, and intracerebroventricular delivery of BDNF in rats with pneumococcal meningitis resulted in downregulation of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and upregulation of anti-inflammatory cytokine (IL-10) in the hippocampus and cortex (Xu et al. 2017).

These results suggest that BDNF can mitigate AD pathology directly through interactions with plaques and tangles or indirectly through its anti-inflammatory properties. However, high levels of AD pathology and inflammation can suppress BDNF production and function and ultimately facilitate neurodegeneration by blocking the neuroprotective actions of BDNF (**Figure 1-4**). The link between deterioration of BDNF function and AD pathology is

scientifically interesting and deserves attention based on the lack of coherent data either in patients or in *post mortem* tissue or animal models to date.

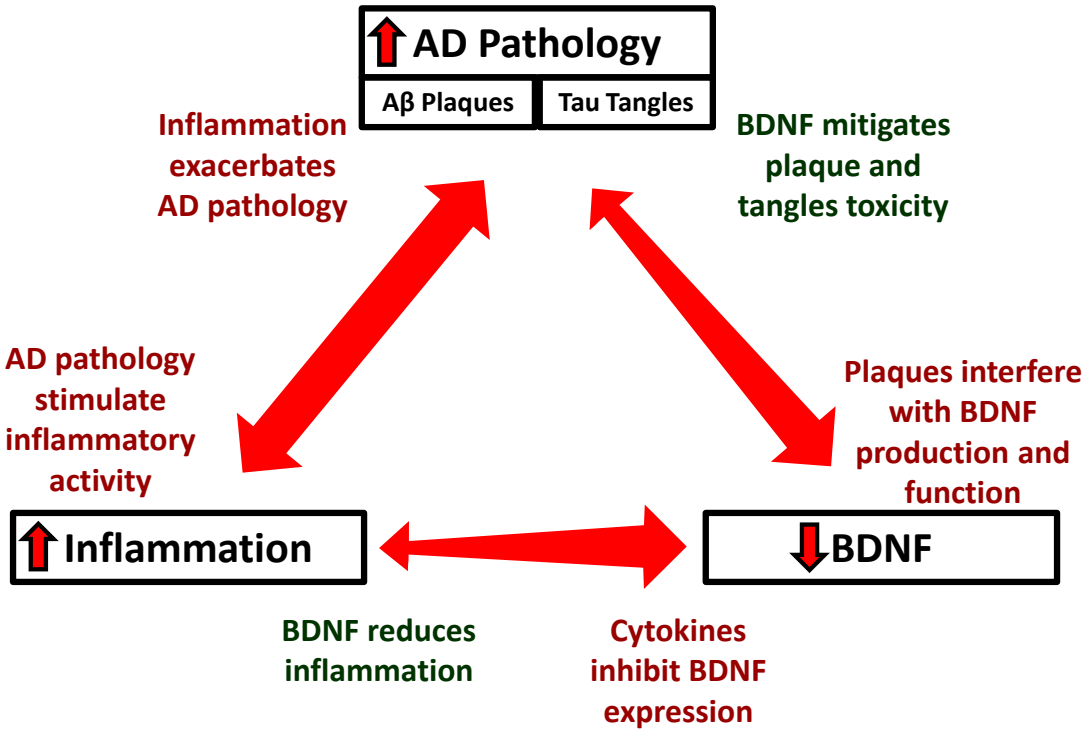


Figure 1-4. Interactions between AD pathology, BDNF, and inflammation. AD pathology including Aβ plaques and tau tangles aggravate inflammation and can ultimately interfere with BDNF production and function. The loss of neuroprotection from BDNF may further contribute to the progression of AD.

1.6 BDNF as a biomarker

BDNF and TrkB protein and mRNA are expressed predominantly in the CNS with particularly high protein expression in the hippocampus (Connor et al. 1996, Connor et al. 1997, Yan et al. 1997, Murer et al. 1999, Durany et al. 2000, Murer et al. 2001). The ability of BDNF to cross the blood-brain barrier and be detected in venous blood has led to several investigations of blood BDNF levels as a biomarker for normal and pathological aging in older adults. However, BDNF is also produced peripherally in leucocytes, macrophages, muscles, and

endothelial cells and is stored in blood platelets, and blood BDNF levels do not only reflect BDNF secreted from the brain (Fujimura et al. 2002, Phillips et al. 2014). **Although a positive relationship between brain and blood BDNF levels has been demonstrated in several animal models (Karege et al. 2002, Sartorius et al. 2009, Klein et al. 2011), the relationship between blood BDNF levels and brain BDNF levels in humans has yet to be reported and was one of the goals for Aim 1 in my dissertation.**

1.6.1 Serum BDNF levels in AD

Although BDNF can be readily detected in serum, plasma, and whole blood, this dissertation will be focused on BDNF levels in serum. This decision was made for several reasons. First, serum BDNF is considered to be a more reliable source of BDNF since plasma BDNF levels are commonly affected by choice of incubation time and anti-coagulant (Tsuchimine et al. 2014, Polacchini et al. 2015, Polyakova et al. 2017). Second, serum BDNF levels have been shown to be 100 times higher than plasma BDNF due to the release of BDNF from platelets during the clotting process (Radka et al. 1996). In plasma, platelet degranulation is difficult to control for and can occur from simple shearing force during blood collection or even due to inherent platelet instability from increasing age or pharmacological intervention (Elfving et al. 2010). Third, serum BDNF levels have comparable BDNF levels to whole blood and have been shown to be identical to BDNF levels in washed platelet lysates (Fujimura et al. 2002). Thus

serum BDNF levels represent BDNF stored in platelets and reflect total circulating BDNF produced by both the brain and peripheral organs.

Table 1-1 summarizes findings from studies on serum BDNF levels in AD and controls completed by groups around the world. Details on these studies can be found in **Appendix 1**. Most of these studies used commercially available ELISA kits from a variety of suppliers. Although the majority of studies report lower serum BDNF levels in AD compared to controls, several studies have found higher serum BDNF levels in AD compared to control or have failed to find a difference in serum BDNF levels between AD and controls. One of the possible reasons for this discrepancy is the different stages of AD that were studied. Laske et al. (2006) found that serum BDNF levels were increased during early AD (MMSE scores ≥ 21) when compared to late AD (MMSE scores < 21). Similarly, Konukoglu et al. (2012) found that although serum BDNF levels in the AD group (n=22) as a whole was not significantly different from the controls (n=20), AD patients with early dementia (n=12; MMSE scores ≥ 21) had significantly higher serum BDNF levels than both the controls and AD patients with late dementia (n=10; MMSE scores < 21). Overall these results suggest that BDNF levels may vary with disease severity and that a protective compensatory increase in BDNF may exist in early stages of AD and MCI to mitigate AD toxicity. When this compensation fails, lower BDNF levels persist, and a decline in cognition and clinical status to AD may occur**. In <u>Aim 2</u> of my dissertation, I investigated how BDNF levels fluctuate with mild cognitive impairment in a rat model of aging.**

Table 1-1. Summary of studies on brain BDNF levels in AD. AD = Alzheimer's disease, ELISA = Enzyme-linked immunosorbent assay, MCI = Mild Cognitive Impairment

Result	Origin	# of Studies	Total AD Participants	Total Control Participants	Method
Decreased BDNF levels in AD compared to Control	Brazil, China, Germany, Italy, Japan, Korea, Turkey, USA,	17	907	2890	ELISA (Abexa, Chemikine, Promega, R&D, Raybio)
Increased BDNF levels in AD compared to Control	Germany, India, Italy, Turkey	4	179	120	ELISA (Promega, R&D, Raybio)
No difference in BDNF levels between AD and Control	Germany, Spain, Turkey, USA	6	607	429	ELISA (Promega, R&D, Rule Based Medicine)

1.6.2 Brain BDNF levels in AD

Several groups have investigated changes in BDNF protein levels in AD and control brains and have reported mixed results (**Table 1-2, Appendix 2**). The discrepancy between studies may be due to *post mortem* interval (PMI); however, PMI and handling of *post mortem* samples is often limited by the management of the brain bank (Ferrer et al. 2008). Storage and handling of brain material may also influence BDNF levels in human brains, and BDNF levels have been shown to decay up to 15% in rat brains when stored at 4^{o}C for 24 hours (Ferrer et al. 1999). Alternatively, certain *ante mortem* clinical features that influence BDNF levels such as medication, depression, lifestyle (e.g., smoking, alcohol, exercise), and pathological staging are rarely reported in *ante mortem*

studies (Balietti et al. 2018), let alone in *post mortem* studies where such information may not be easily obtained.

Nevertheless, *post mortem* assessment of BDNF levels in AD and control human brain tissue have concluded that lower BDNF levels are found in certain regions of the AD brain. Generally, in AD brains compared to controls, there have been more reports of lower BDNF levels in the hippocampus, parietal cortex, and temporal cortex. BDNF levels in the basal forebrain, basal ganglia, cerebellum, frontal cortex, occipital cortex, putamen, and thalamus have generally not been found to be different between AD and control. Two studies have reported increased brain BDNF levels in AD. Durany et al. (2000) used the BDNF Emax® ImmunoAssay System from Promega to measure free BDNF and found increased BDNF levels in AD compared to control in the hippocampus and parietal cortex. Lim et al. (2015) used western blotting methods to distinguish proBDNF from mature BDNF and found increased proBDNF in AD hippocampus. Considering that the Promega BDNF ELISA used by Durany et al. (2000) does not distinguish between proBDNF and mature BDNF, it is possible that the increased BDNF reported by Durany et al. (2000) reflects increased proBDNF levels similar to results from Lim et al. (2015). Increased proBDNF levels in AD brain makes conceptual sense considering the toxic synergistic relationship found between proBDNF and Aβ plaques. Overall, BDNF levels are expected to be lower in AD brains compared to control and be related AD pathology. **In <u>Aim 1</u> of my dissertation, I measured BDNF levels in human brain samples by both ELISA and western blotting methods to better understand the**

relationship between total BDNF, mature BDNF, and proBDNF and AD histopathology.

Table 1-2. Summary of studies on brain BDNF levels in AD. AD = Alzheimer's disease, BA = Brodmann Area, BF = Basal forebrain, BG = Basal Ganglia, CBL = Cerebellar cortex, CG = Cingulate gyrus, Con = Control, DG = Dentate gyrus, ELISA = Enzyme-linked immunosorbent assay, ENT = Entorhinal cortex, FC = Frontal cortex, HIP = Hippocampus, IHC = Immunohistochemistry, MC = Motor Cortex, MCI = Mild Cognitive Impairment, OC = Occipital cortex, PC = Parietal cortex, PUT = Putamen, TC = Temporal cortex, THL = Thalamus, WB = Western Blot.

Result	Region	# of Studies	Total AD Participants	Total Control Participants	Method
Decreased BDNF levels in AD compared to Con	DG, ENT, FC, HIP, MC, PC, TC	9	178	137	ELISA (Promega, R&D, Abexa), IHC, WB
Increased BDNF levels in AD compared to Con	HIP, PC	2	21	18	ELISA (Promega), WB
No difference in BDNF levels between AD and Con	BF, BG, CBL, CG, FC, HIP, OC, PC, PUT, TC, THL	7	235	144	ELISA (Promega, R&D), WB

1.6.3 CSF BDNF levels in AD

Measurement of brain portien in CSF is thought to be a more direct measure of brain tissue levels. However, several studies have reported difficulty in measuring BDNF in CSF using commercial ELISAs (Blasko et al. 2006, Laske et al. 2006, Laske et al. 2007, Jiao et al. 2016). Laske et al. (2006) was not able to detect BDNF in CSF in any of their samples using an R&D ELISA with a limit of detection (LOD) of 15 pg/ml. Using a more sensitive fluorescent ELISA with a

LOD of 0.1 pg/ml, Laske et al. (2007) was only able to detect CSF BDNF levels in 8/27 AD and 5/10 older adult controls. Similarly, Blasko et al. (2006) was only able to detect CSF BDNF levels in 2/14 AD and 0/12 older adult controls using an R&D ELISA (LOD 15 pg/ml), and Jiao et al. (2016) was only able to detect BDNF in CSF in about 7/28 AD and 11/22 older adult controls using an Abbexa BDNF ELISA (LOD 31.2 pg/ml). Although Laske et al. (2007) and Jiao et al. (2016) were not able to detect BDNF in their entire cohort, they both report that the detection rate (i.e., the number of cases with measurable BDNF divided by the total number of cases) of BDNF in CSF was lower in AD. Laske et al. (2007) further reported that BDNF in CSF did not correlate with BDNF in serum and that there were no differences in serum BDNF levels between participants with and without detectable BDNF in CSF. In studies that did detect BDNF in CSF, CSF BDNF levels were significantly lower in AD compared to healthy controls (Zhang et al. 2008, Li et al. 2009, Forlenza et al. 2015). **BDNF investigations of CSF have thus far been limited to *ante mortem* lumbar puncture samples, and BDNF levels in *post mortem* cranial CSF are unknown to date. In addition, the relationship between BDNF in CSF and the brains has not been reported and is part of the goals for Aim 1 in this dissertation**.

1.6 Purpose of this study

In this dissertation, I investigated the biomarker potential of BDNF in AD using *post mortem* human samples, an aged rat model with LC-NE degeneration, and blood samples from cognitively healthy older adults. BDNF, AD pathology,

and inflammation are highly interconnected (see **Figure 1-4**), and the balance between all three was taken into consideration across all three of my Aims. My goal was to determine whether human serum BDNF levels were related to human brain BDNF levels and AD pathology (**Aim 1; Chapter 3**), whether serum BDNF levels reflected acute neurological insults related to age-related memory impairment (**Aim 2; Chapter 4**), and whether serum BDNF levels could predict changes in neuropsychological performance or neuroimaging measures over time in healthy older adults (**Aim 3; Chapter 5**). My overall hypothesis is that BDNF fluctuates with disease course with high levels of BDNF in early stages of the disease course and low levels of BDNF in late stages of AD. This overall hypothesis is depicted in **Figure 1-5** where the hypothetical changes in BDNF and inflammation over time are plotted together with the previously proposed AD biomarker theory shown in **Figure 1-2**. In **Chapter 3 (Aim 1)**, I assessed the relationship between serum BDNF levels, brain BDNF levels (hippocampus, entorhinal cortex, and prefrontal cortex), CSF BDNF levels, AD pathology (plaques and tangles), and inflammation (cytokines, astroglia, microglia, and oligodendrocytes) in *post mortem* samples from AD and control cases. The different forms of BDNF (mature BDNF and proBDNF) as well as the stage of AD (high or low pathology) were taken into account as we assessed whether a) serum BDNF levels were correlated with brain BDNF levels, b) serum BDNF levels were correlated with brain pathology (plaques, pTau, and inflammation) and c) brain BDNF levels were related to brain pathology. In **Chapter 4 (Aim 2)**, I assessed the relationship between serum BDNF levels, brain BDNF levels

(frontal cortex and hippocampus), and inflammation (cytokines, astroglia, and microglia) in aged rats with LC neurodegeneration and an acute systemic inflammation challenge. This aged rat model is a well-recognized model for aging-related memory loss and degeneration. Assessment of serum and brain BDNF in this model allowed us to make inferences on whether a) serum and brain BDNF decreased during an inflammatory challenge and b) a compensatory increase in serum and brain BDNF occurred after the challenge. In **Chapter 5 (Aim 3)**, I assessed serum BDNF and cytokine levels in cognitively normal adults who have completed neuropsychological testing, structural MRI scans, and blood draws in two visits approximately 15 months apart to determine whether baseline BDNF levels can predict abnormal changes in a) neuropsychological performance (executive function and memory) and b) neuroimaging measures (dorsolateral prefrontal cortex thickness, hippocampal volume, and white matter microstructure of limbic system). Overall, these studies were designed to evaluate the ability of serum BDNF levels to reflect brain BDNF levels and/or neuropathophysiological changes related to AD in cognitively healthy older adults, aged rats with memory loss, and AD.

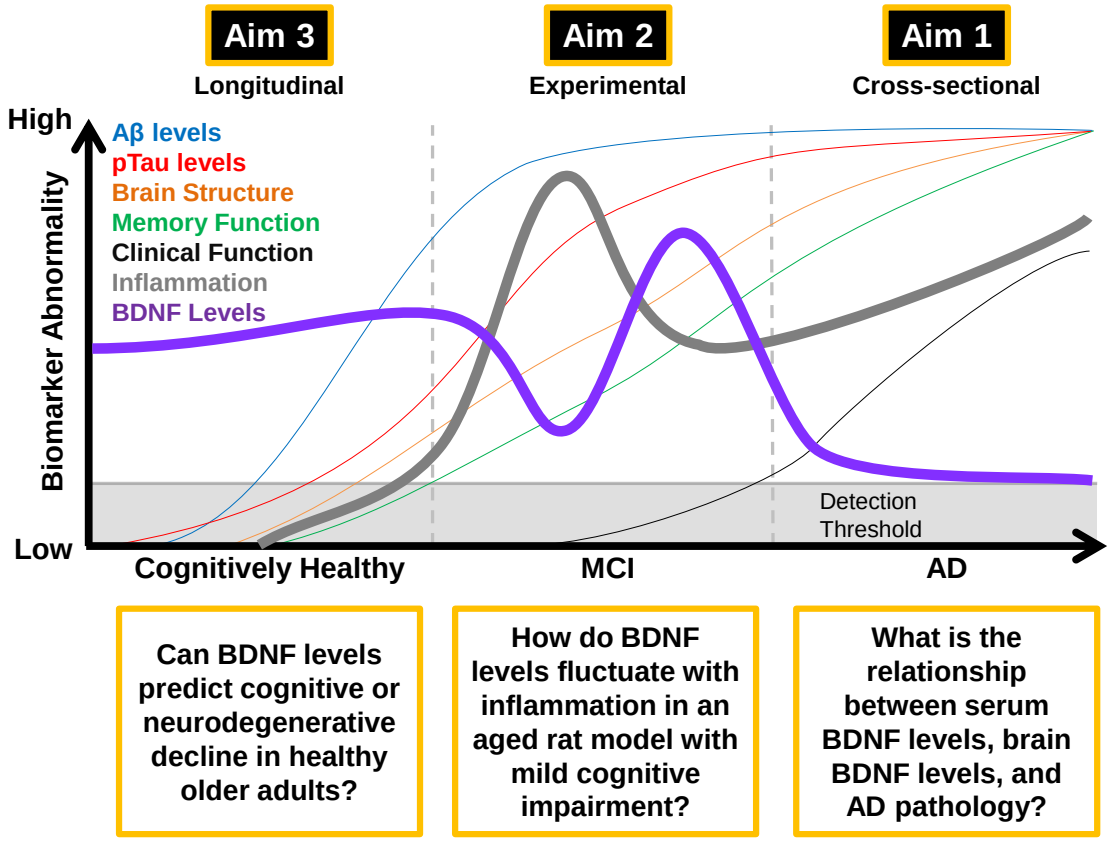


Figure 1-5. Framework of major dissertation questions. Hypothetical changes in BDNF and inflammation over time and clinical diagnosis are added to the model in Figure 1-2. The vertical axis represents biomarker severity from low to high, and the horizontal axis represents time and progression from cognitively healthy to cognitively impaired to AD. BDNF can be detected in serum at young ages and is always above the detection threshold. An inverse relationship between inflammation and BDNF is depicted with a compensatory increase in BDNF occurring after peak inflammatory activity. Failure of BDNF compensation over time is hypothesized to facilitate the progression to AD and increasing inflammation.

1.7 Manuscripts

Manuscripts in preparation:

Bharani, K. L., A. Ledreux, A. Gilmore, S. L. Carroll, and A. C. Granholm. “Serum brain-derived neurotrophic factor (BDNF) levels positively correlates with phosphorylated tau in Alzheimer’s disease.” Planned submission to Acta Neuropathologica

Bharani, K. L., A. Ledreux, A. C. Granholm, Benitez A. “Investigation of serum BDNF levels as a predictor for changes in neuropsychological performance and neuroimaging measures in healthy older adults.” Planned submission to Neurology

Peer-reviewed articles published during the course of this dissertation (chronological):

Baranello, R. J., **K. L. Bharani**, V. Padmaraju, N. Chopra, D. K. Lahiri, N. H. Greig, M. A. Pappolla and K. Sambamurti (2015). "Amyloid-beta protein clearance and degradation (ABCD) pathways and their role in Alzheimer's disease." Curr Alzheimer Res **12**(1): 32-46. PMC: 25523424. PMCID: PMC4820400.

Morrison, R. G., P. J. Reber, **K. L. Bharani** and K. A. Paller (2015). "Dissociation of category-learning systems via brain potentials." Front Hum Neurosci **9**: 389. doi: 10.3389/fnhum.2015.00389. PMC: 26217210. PMCID: PMC4493768.

Bharani, K. L., K. A. Paller, P. J. Reber, S. Weintraub, J. Yanar and R. G. Morrison (2016). "Compensatory processing during rule-based category learning in older adults." Neuropsychol Dev Cogn B Aging Neuropsychol Cogn **23**(3): 304-326. doi: 10.1080/13825585.2015.1091438. PMC: 26422522. PMCID: PMC4828326.

Ledreux, A., E. D. Hamlett, J. Lockrow, **K. L. Bharani**, and A. C. Granholm (2016). “Neurodegeneration and Memory Loss in Down Syndrome: Perspectives on Potential Therapeutics.” Research Progress in Alzheimer's Disease and Dementia Volume 6, Chapter: 7, pp.169-194

Bharani, K. L., R. Derex, A. C. Granholm and A. Ledreux (2017). "A noradrenergic lesion aggravates the effects of systemic inflammation on the hippocampus of aged rats." PLoS One **12**(12): e0189821. doi: 10.1371/journal.pone.0189821. PMC: 29261743. PMCID: PMC5736222.

CHAPTER 2.

GENERAL METHODS

2.1 Study population

All studies involving humans or animals were reviewed and approved by MUSC's Institutional Review Board (IRB) or Institutional Animal Care and Use Committee (IACUC), respectively, to ensure compliance with ethical guidelines set by the NIH. **Aim 1** was conducted using *post mortem* human brain tissue and fluids obtained from the Carroll A. Campbell, Jr. Neuropathology Laboratory at MUSC. The IRB and MUSC's Office of Research Integrity determined that this study met the Not Human Research (NHR) criteria set forth by the Code of Federal Regulations (45CFR46). All samples were obtained *post mortem* from donors whose next of kin provided consent, and donor identity was kept anonymous with an assigned random identifier. **Aim 2** was conducted using twenty-month-old male Fischer 344 (F344) rats from the aging colony at the Charles River laboratories. These rats were pair-housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited animal care facility at MUSC, were maintained on a 12-h light/dark cycle, and received food and water *ad libitum* according to the National Institute of Aging (NIA) guidelines for aged rats. **Aim 3** was conducted on cognitively healthy older adults recruited for the MR Imaging of Neurodegeneration (MIND) study. All participants gave written informed consent to participate in annual neuropsychological tests, MRI scans, and a fasting blood draw. Sample numbers and demographics for each study population are detailed in each Aim's respective chapter. Benefits and limitations of using different study designs and population for each Aim can be found in **Table 2-1** below.

Table 2-1. Benefits and limitations of different study designs and population types in Aims 1-3.

Aim	Study Type	Study Population	Benefits	Limitations
Aim 1	Cross-sectional	*Post Mortem* Human Samples	• Direct assessment and relevance to disease pathology	• Cross-sectional • Potential recruiting bias
Aim 2	Interventional Cross-sectional	Aged Rats	• Able to control for multiple confounders • Can perform more invasive procedures • Excellent model for aging-related cognitive impairment	• May not accurately model all symptoms found in a clinical population with AD
Aim 3	Longitudinal	Healthy Older Adults	• Clinically relevant • Can suggest a cause-and-effect relationship	• Risk of drop out over time • Costly • Potential recruiting bias

2.2 Serum collection

Aim 1 *post mortem* human blood and **Aim 2** rat blood from cardiac puncture was collected into BD Vacutainer Serum Separator Tubes® and allowed to clot for 60 minutes at room temperature (RT) before being centrifuged for 20 minutes at 1,500 x g. Serum was then carefully collected, aliquoted, and stored in a -80^{o}C freezer until further analysis. **Aim 3** *ante mortem* blood was collected into serum separator tubes, inverted five times to mix with clot activator, and allowed to clot for 30 minutes at room temperature (RT). After being centrifuged for 10 minutes at 3000 rpm at 4^{o}C, serum was then carefully aliquoted into 0.5 ml aliquots and stored at -80^{o}C.

2.2.1 Glass vs. plastic tubes

The collection of blood has been traditionally done in glass tubes; however, plastic tubes, which are cheaper and less likely to break, have become more ubiquitous. Most studies show that the use of glass vs plastic tubes does not affect analyte concentration in serum (Hill et al. 1992, Preissner et al. 2004). Although Smets et al. (2004) reported a small but statistically significant difference ($p<0.05$) in some hormones in blood collected in glass versus plastic test tubes, this difference was not clinically significant. The effect of glass vs plastic test tubes on BDNF concentration in serum has not been reported and may be relevant based on the proposed "stickiness" of this protein, likely due to its abundance of positively charged moieties (Waterhouse and Xu 2009). To test the effects of glass or plastic test tubes on BDNF levels in the same assay, the BDNF Emax® ImmunoAssay System (G7610, Promega, Madison, WI) was used to measure BDNF in standards and human brain (HB) tissue processed in either glass or plastic pipettes. We found no significant difference in optical density (OD) values from the BDNF assay in different concentrations of the BDNF standard diluted in manufacturer provided Block & Sample buffer between glass or plastic processing **(Figure 2-1A).** Similarly, we did not find a significant difference in OD from the BDNF assay in three human brain sample homogenates between glass- or plastic-based processing, strongly suggesting that this particular issue does not affect the outcome of experiments, either in serum or in brain tissue samples **(Figure 2-1B).**

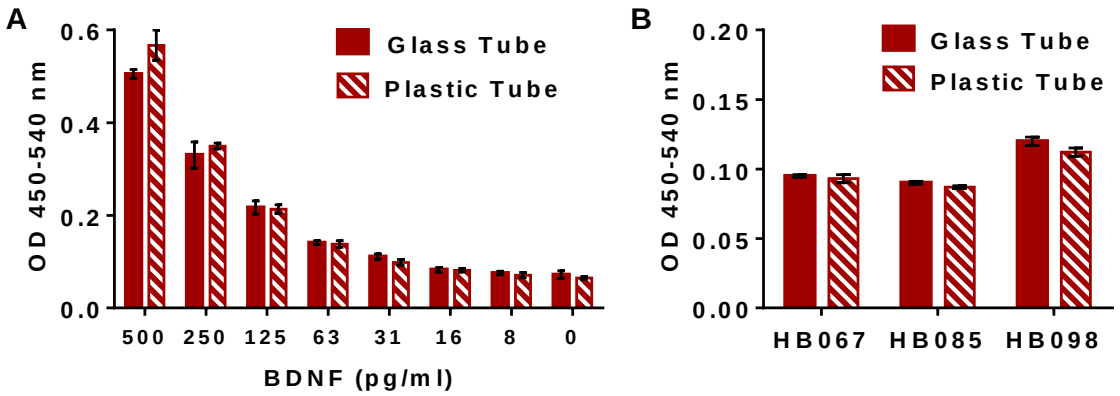


Figure 2-1. Glass vs. plastic effects on optical density (OD) from a BDNF immunoassay. Buffer with differing concentrations of BDNF (A) and three different human brain (HB) homogenates (B) were processed through a glass or plastic pipette before being run on the BDNF Emax® ImmunoAssay System (G7610, Promega, Madison, WI). OD values from this assay indicate that BDNF levels in buffer and HB homogenates were not significantly altered from processing in glass or plastic pipette.

2.3 Procurement of human brain tissue and homogenization

2.3.1 Human samples

In **Aim 1**, the left hemisphere of the brain from human donors was cut into 1 cm coronal slabs and photographed. Frozen pieces of human middle frontal gyrus (Brodmann area 46; BA46), the entorhinal cortex (ENT), and the hippocampus (HIP) were isolated from the left hemisphere and stored at -80^{o}C until homogenization.

2.3.2 Rat samples

In **Aim 2**, rats were anesthetized deeply with isoflurane before the right frontal cortex and right hippocampus were isolated, snap frozen on dry ice, and stored at -80^{o}C until homogenization.

2.3.3 Homogenization protocol

The final homogenization protocol for brain tissues used in **Aim 1** and **Aim 2** has been published previously (Bharani et al. 2017). Briefly, 200-500 mg of brain tissue were homogenized for 20 seconds using a battery-powered pestle mixer (Argos Technologies) with 1:10 w/v homogenization buffer (20 mM Tris-HCl, 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 10% glycerol, 1% NP-40) and cOmplete™ Protease Inhibitor Cocktail (Roche Diagnostics, Ltd., Mannheim, Germany). Samples were then incubated on ice for 30 minutes before the suspension was centrifuged at 10,000 x g for 20 minutes at 4˚C. The pellet was then re-homogenized with 1:5 w/v homogenization buffer for 20 seconds, incubated on ice for 30 minutes, and centrifuged at 10,000 x g for 20 minutes at 4˚C. The supernatants were combined, aliquoted, and stored in a -80˚C freezer prior to use. Protein quantification was determined using a bicinchoninic acid (BCA) protein assay (Thermo Scientific, Rockford, IL, USA).

2.3.4 Buffer optimization

Several different lysis buffers have been used for BDNF extraction from human and rat brain tissue in past literature (Ferrer et al. 1999, Lee et al. 2005, Angelucci et al. 2010, Elfving et al. 2010), but optimization of the lysis buffer for extraction of protein and BDNF from brain tissue has not been previously done in the Granholm lab. Radioimmunoprecipitation assay buffer (RIPA buffer) is commonly used to solubilize all membranes but interferes with antibody binding

in commercially-available ELISAs. Accordingly, homogenization buffers with milder, nonionic detergents, such as NP-40 or Triton-X 100 were considered and compared. Promega (Madison, WI) recommended the detergent NP-40 for use in their BDNF Emax® ImmunoAssay System (G7610, Promega, Madison, WI) while several investigators used varying concentrations of the detergent Triton-X 100 instead of NP-40 (Ferrer et al. 1999, Lee et al. 2005, Elfving et al. 2010). To ensure optimal protein extraction, protein was extracted from human brain using homogenate buffer (20 mM Tris-HCl, 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 10% glycerol) with cOmplete™ Protease Inhibitor Cocktail (Roche Diagnostics, Ltd., Mannheim, Germany) containing either 1% NP-40 or 0.1% Triton-X 100. Protein quantification from these two homogenates was assessed using a BCA protein assay (Thermo Scientific, Rockford, IL, USA). Less variability and approximately 13% more protein were observed using 1% NP-40 compared to 0.1% Triton-X 100 (**Figure 2-2A**). Therefore, 1% NP-40 was used instead of 0.1% Triton-X 100 in the extraction of BDNF from human and rat brain tissue. This buffer will be referenced to as Lab buffer from here on forward.

2.3.5 Buffer comparison

Neuronal Protein Extract Reagent (N-PER) is a proprietary cell lysis reagent from Thermo Scientific that is optimized for efficient extraction of protein from neuronal tissue. To test whether the Lab buffer was as potent in protein extraction as N-PER, frozen parietal cortices from two young rats were homogenized using the Lab buffer or N-PER, and protein was quantified using a

BCA protein assay. Protein extraction from two rat brain samples was found to be similar between the homogenization protocols using Lab buffer or N-PER (**Figure 2-2B**).

2.3.6 Protocol optimization

The resultant pellet after one round of homogenization and centrifugation may still have protein, and the effects of combining two rounds of homogenization, one on the tissue and one on the pellet, has not been tested previously. To test whether homogenization of the pellet will extract more protein, frozen parietal cortices from two young rats were first homogenized using the Lab buffer, incubated on ice for 30 minutes, and centrifuged at 10,000 x g for 20 minutes at 4˚C. The subsequent pellet was then re-homogenized with 1:5 w/v homogenization buffer for 20 seconds, incubated on ice for 30 minutes, and centrifuged at 10,000 x g for 20 minutes at 4˚C. Protein quantification of homogenates from both steps and their combination suggested that a higher amount of protein was extracted per mg of tissue (**Figure 2-2C**) when combining homogenates from both steps.

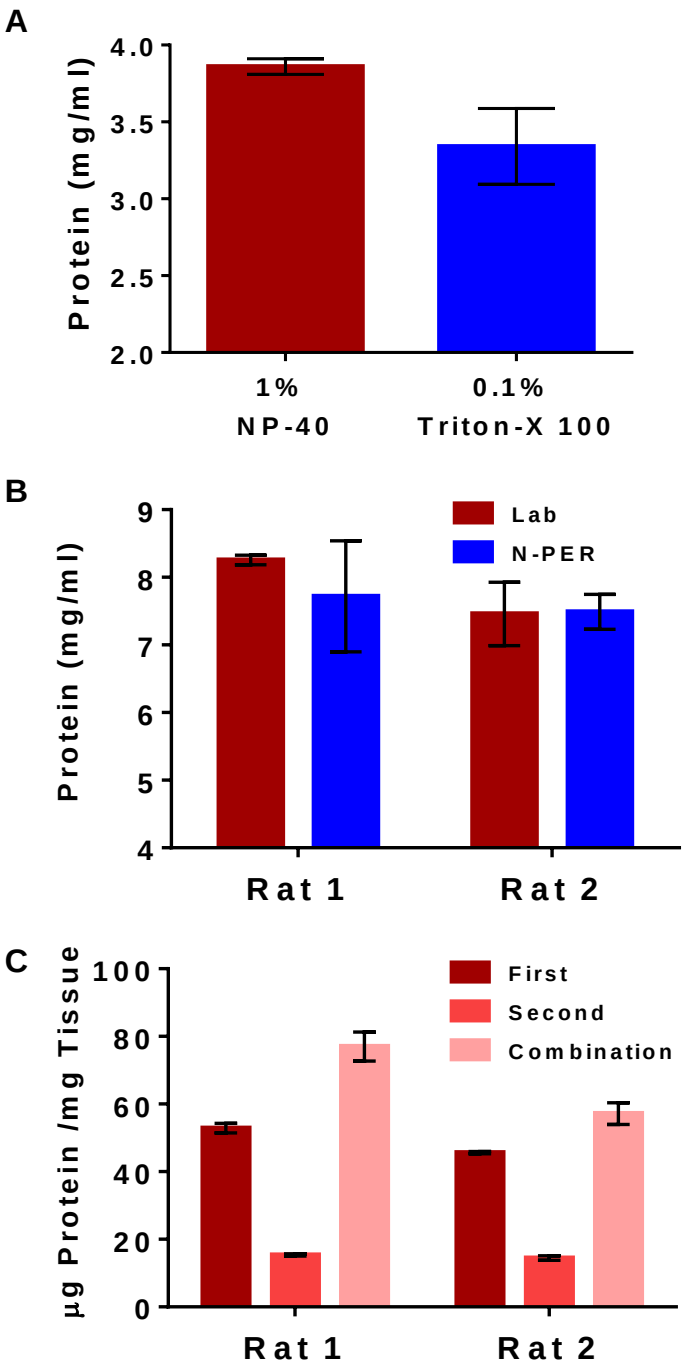


Figure 2-2. Homogenization buffer and protocol optimization. Protein levels (mean ± SD), measured by BCA assay, of human brain tissue homogenized with 1% NP-40 or 0.1% Triton-X 100 show that use of 1% NP-40 extracted more protein than 0.1% Triton-X 100 (A). Comparison between homogenization with Lab buffer vs N-PER buffer showed that the buffers extract equivalent amounts of protein from two rat brain samples (B). In two rat brain samples, we show that the first homogenization step extracts the most protein per tissue but more protein can be extracted through a second homogenization of the pellet from the first step. The combination of supernatants from both the first and second homogenization steps yields the most protein per tissue (C).

2.4 BDNF quantification

2.4.1 BDNF ELISA optimization

In general, an ELISA involves the quantitative detection of an analyte in a liquid sample using antibodies specific to the analyte and a linked enzyme that undergo an optical change which can be quantitatively measured by spectrophotometry. A sandwich ELISA is a type of ELISA in which the analyte of interest is captured by two specific antibodies, one that is bound to the plate (capture antibody) and one that is added after nonspecific binding sites are blocked (primary antibody). After the analyte is effectively 'sandwiched' between these two antibodies, an enzyme-linked secondary antibody is added to bind specifically to the primary antibody. The enzyme-substrate is then added to be converted by the enzyme to produce a colored or fluorescent product that can be detected by spectrophotometry and related to the concentration of the analyte of interest.

A large number of research groups have quantified BDNF in human whole blood, serum, and plasma using the commercially available Human Free BDNF Quantikine ELISA Kit (DBD00, R&D Systems, Minneapolis, USA). This kit (R&D BDNF ELISA kit), although validated for use on human and rat blood/serum/plasma, was not validated for use on human or rat tissue. To validate and assess the accuracy of this R&D BDNF ELISA on human and rat brain homogenates, a spike-and-recovery and linearity-of-dilution experiment was performed using the Spike, Recovery, and Linearity Protocol for Validating Untested Samples in R&D Systems ELISA provided by R&D Systems support. A

spike-and-recovery experiment involves adding a known amount of analyte (BDNF) to the sample matrix (human or rat brain homogenate) and to a standard diluent. A linearity-of-dilution experiment involves preparing dilutions of the sample matrix with the addition of a known amount of analyte (known as a 'spike'). If the quantification of the spike in the sample diluent is not identical to the spike in the sample matrix, or, if the spike does not exhibit a linear dilution, then there is something in the sample matrix that is interfering with the ELISA.

The R&D BDNF ELISA kit comes with two different calibrator diluents: animal serum (RD6P) with preservatives for use with serum/plasma samples and buffered protein base with preservatives (RD5K) for cell culture supernatant. We ran a spike-and-recovery and a linearity-of-dilution experiment on human brain and rat brain homogenate diluted with either RD6P or RD5K. A spike was created using the standard BDNF protein provided by the R&D BDNF ELISA kit, and brain homogenates were spiked with 4000 pg/ml of BDNF. Spiked and unspiked brain homogenates were then serially diluted with either RD6P or RD5K to 1:2, 1:4, and 1:8. BDNF levels were then quantified using the R&D BDNF ELISA standard curve. Percent Recovery (%Recovery) of the spiked sample was calculated using the 1:2 dilution of the spiked and unspiked samples as follows: %Recovery = 100 x (Spiked – Unspiked)/2000. Percent Linearity (%Linearity) was calculated for the 1:4 and 1:8 dilutions as follows and averaged together: %Linearity 1:4 = 100 x [1:4 Spiked/(1:2 Spiked/2)] and %Linearity 1:8 = 100 x [1:4 Spiked/(1:2 Spiked/4)]. Percent recovery and linearity for HB and rat brain (RB) homogenates diluted in either RD6P or RD6K are plotted in **Figure 2-**

3. The acceptable range for %Recovery and %Linearity is 80-120%. Only RB diluted in RD6P had acceptable %Recovery and %Linearity for the R&D BDNF ELISA, and my results indicate that the R&D BDNF ELISA kit should not be used to measure BDNF levels in human brain homogenates without additional optimization such as protein purification.

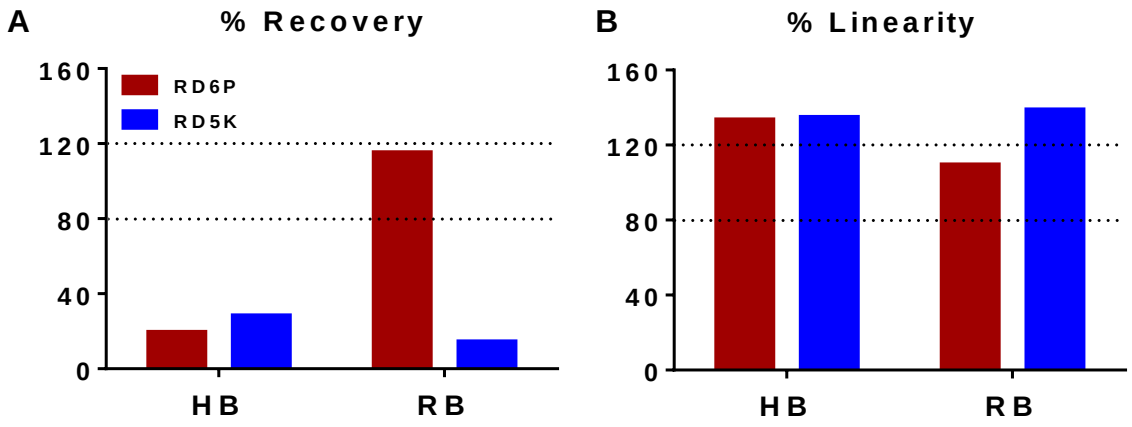


Figure 2-3. **Percent recovery and linearity for the R&D BDNF ELISA kit using human brain (HB) and rat brain (RB) homogenates.** Spike-and-recovery and linearity-of-dilution studies done on HB and RB homogenates with R&D diluents RD6P and RD5K demonstrate that the R&D Human Free BDNF Quantikine ELISA kit can accurately measure BDNF levels in RB homogenates using the diluent RD6P but not in HB homogenates regardless of diluent used. The acceptable range for %Recovery and %Linearity is 80-120% and is noted on the graphs by dotted horizontal lines.

The R&D BDNF ELISA kit is claimed by the manufacturer to be specific for mature BDNF with some cross-reactivity to proBDNF. To investigate this cross-reactivity further, we ran increasing concentrations of proBDNF protein (Alomone, B-245) with the manufacturer provided mature BDNF standard (**Figure 2-4**). Results from this experiment indicate that the R&D BDNF ELISA is sensitive to mature BDNF with a limit of detection of less than 62.5 pg/ml but

does detect proBDNF at levels greater than 500 pg/ml. This result somewhat conflicts with Polacchini et al. (2015) where the R&D BDNF ELISA antibody was found to be very reactive to proBDNF in line blots. Nonetheless, this cross-reactivity with proBDNF is important to consider when quantifying BDNF levels in biological samples with high levels of proBDNF compared to mature BDNF. For example, proBDNF levels have been reported to be more than 10-fold higher than mature BDNF levels in healthy Jewish subjects and not in in Japanese subjects (Levin et al. 2015, Hashimoto 2016, Hashimoto 2016). Measurement of mature BDNF levels in these subjects using the R&D BDNF ELISA would result in an artificially high result due to the high levels of proBDNF levels. To avoid such an erroneous conclusion, BDNF levels quantified by ELISA will be referred to as total BDNF levels.

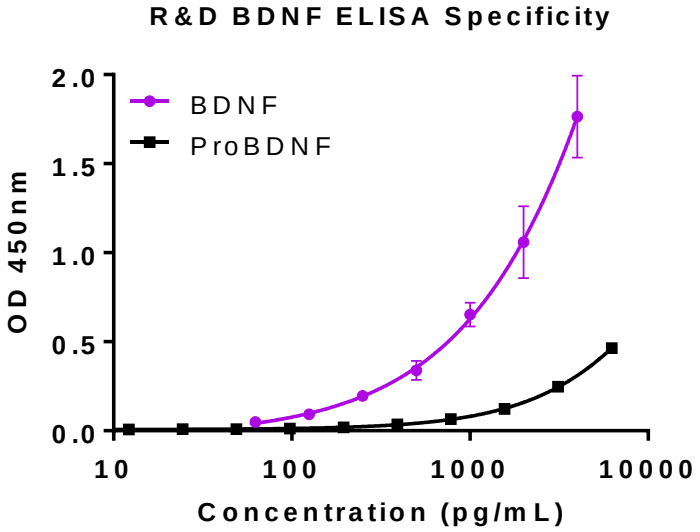


Figure 2-4. Specificity of the R&D BDNF ELISA kit to BDNF isoforms. Different concentrations of mature BDNF and proBDNF protein were measured using the R&D Human Free BDNF Quantikine ELISA kit. The primary antibody used in this kit is specific for mature BDNF but exhibits some cross-reactivity to proBDNF. Only high concentrations of proBDNF are detected by this ELISA kit.

Accordingly, total BDNF levels in undiluted cranial CSF (CSF-C), undiluted lumbar CSF (CSF-L), and serum samples (diluted 1:20 with the manufacturer's provided diluent, RD6P) and rat brain homogenates were measured in duplicates using the R&D BDNF ELISA. Total BDNF levels in brain homogenates (BA46, ENT, HIP; each diluted 1:2 with manufacturer-provided Block & Sample buffer) were measured in duplicate using the manufacture validated BDNF Emax® ImmunoAssay System (G7610, Promega, Madison, WI; Promega BDNF ELISA). Comparison of these two ELISA kits can be found in **Table 2-2**.

2.4.2 R&D BDNF ELISA protocol

The R&D BDNF ELISA was run according to the manufacturer's instructions as previously described (Bharani et al. 2017, Hakansson et al. 2017). The sensitivity of this R&D BDNF ELISA was 20 pg/mL (Polacchini et al. 2015). Briefly, the manufacturer-provided 96-well plate was pre-coated with a monoclonal antibody specific for the human free BDNF. These plates were then incubated with 1) Assay Diluent RD1S for 1 hour at RT; 2) diluted serum samples and BDNF standards for 2 hours at RT; and 3) a buffer containing monoclonal antibody specific for human free BDNF conjugated to horseradish peroxidase for 1 hour at RT. After the wells were washed with the manufacturer-provided buffer, a color reaction was produced by incubating the plate with a tetramethylbenzidine solution. This reaction was stopped with the addition of 2N sulfuric acid, and the absorbance was read by an automated microplate reader (BioTek Synergy H1) at 450 nm with wavelength corrections set to 540 nm within

30 minutes. BDNF concentration in test samples was extrapolated from a 4-parameter logistic curve fit calculated from the manufacturer-provided standards. BDNF levels in serum samples were measured twice in duplicate, and all four measurements were averaged together to one value which was used in subsequent analyses.

2.4.3 Promega BDNF ELISA protocol

The Promega BDNF ELISA was run according to the manufacturer's instructions. The Promega BDNF ELISA has been previously validated for use on human brain samples, is comparable to the R&D BDNF kit, and has a sensitivity of 15.6 pg/ml (Yoshida 2012, Polacchini et al. 2015). Briefly, Costar 96-well flat-bottom EIA plates (Costar #2240096) were coated with anti-BDNF monoclonal antibody diluted in carbonate buffer (25 mM sodium bicarbonate, 25 mM sodium carbonate, pH 9.7) overnight at 4^{o}C. The next day, plates were incubated with 1) Block & Sample buffer for 1 hour at RT; 2) diluted brain samples and BDNF standards for 2 hours at RT with agitation; 3) Block & Sample buffer containing anti-Human BDNF polyclonal antibody for 2 hours at RT with agitation; and 4) Block & Sample buffer containing anti-IgY antibody conjugated with horseradish peroxidase for 1 hour at RT with agitation. Each step was followed by washing as recommended by the manufacturer. A color reaction was produced by incubating the plate with a tetramethylbenzidine solution. This reaction was stopped with the addition of 1N hydrochloric acid, and the absorbance at 450 nm was read by an automated microplate reader within 30 minutes. BDNF concentration in test

samples was extrapolated from a 4-parameter logistic curve fit calculated from the manufacturer-provided standards.

Table 2-2. Manufacture Declared Properties of BDNF ELISA kits. Table content modified from Polacchini et al. (2015) and Yoshida (2012). NA = Not Applicable.

Company	R&D System	Promega
Kit Name (Cat #)	Quantikine ® (DBD00)	BDNF Emax® Immuno-Assay System (G7610)
Type of Assay	Sandwich ELISA	Sandwich ELISA
Sensitivity (pg/ml)	20	15.6
Range of Detection (pg/ml)	62.5 - 4000	7.8 - 5000
BDNF Standard	Human recombinant	Type not declared
Coating/Capture Antibody	anti-BDNF (mouse monoclonal)	anti-BDNF (mouse monoclonal)
Primary Antibody	anti-BDNF (mouse monoclonal) HRP conjugated	anti-BDNF (chicken polyclonal)
Secondary Antibody	NA	anti-IgY HRP conjugated
Species Cross-Reactivity	Only human	Not specified
ProBDNF Cross-Reactivity	13%†	NA‡
Processing Time	4–5 hours	23–24 hours

† The R&D BDNF ELISA declared cross-reactivity with proBDNF is 13%; however, labs have reported cross-reactivity with proBDNF up to 30% (Lim et al. 2015).

‡ Although the cross-reactivity with proBDNF has not been quantified, the Promega BDNF ELISA kit is based on an antibody that targets the carboxyl terminal of BDNF and recognizes both the proBDNF and mature BDNF (Yoshida 2012, Lim et al. 2015).

2.4.4 BDNF western blot optimization

Western blotting separates protein by molecular weight from a fluid through denaturing gel electrophoresis. Protein from the gel is then transferred or 'blotted' to a membrane. The membrane is then blocked with a buffer to prevent nonspecific binding of an antibody to the membrane. Then the membrane is incubated with a primary antibody specific for the protein of interest. A fluorophore-linked or enzyme-linked secondary antibody directed to the primary antibody is then added and detected with an appropriate spectrophotometer.

Independent studies have shown that the antibodies used in the R&D Systems-Quantikine® and the Promega-Emax® ELISA are specific for mature BDNF (mBDNF) but also cross-react with proBDNF up to 30% (Lim et al. 2015, Polacchini et al. 2015). Accordingly, BDNF levels measured from ELISAs will be referred to as total BDNF (tBDNF). Western blotting was used to differentiate further and quantify mBDNF (14-16 kDa) and proBDNF (28-32 kDa) band intensities normalized to internal control proteins (ICP) for serum (transferrin; 77 kDa) and brain homogenate (β-actin; 42 kDa). Information on antibodies used for western blotting can be found in **Table 2-3**. Strengths and limitations of ELISAs and western blotting can be found in **Table 2-4**.

Table 2-3. Antibodies used for western blotting.

Target	Host	Dilution	Source	Identifier
β-actin	Mouse	1:200,000	Sigma-Aldrich	A5441
mBDNF	Rabbit	1:1,000	Alomone	ANT-010
ProBDNF	Rabbit	1:1,000	Alomone	ANT-006
Transferrin	Rabbit	1:2,000	Abcam	ab109503
Ms IgG (H+L)	Goat	1:10,000	LI-COR	IRDye® 680RD 925-68070
Rb IgG (H+L)	Goat	1:10,000	LI-COR	IRDye® 800CW 925-32210

To assess ICP saturation in HB homogenates and human serum, dilutions of HB homogenate from 40 to 1 µg and dilutions of human serum from 125-3.9 µg were run in separate blots and probed with anti-actin and anti-transferrin, respectively. A band for actin was detected between 50 kDa and 37 kDa in HB homogenates, and a band for transferrin was detected around 75 kDa in human serum. These bands progressively became smaller and weaker with decreasing protein load (**Figure 2-4**). Based on this blot, 30 µg of HB homogenate and 50 µg of human serum were used for future analysis.

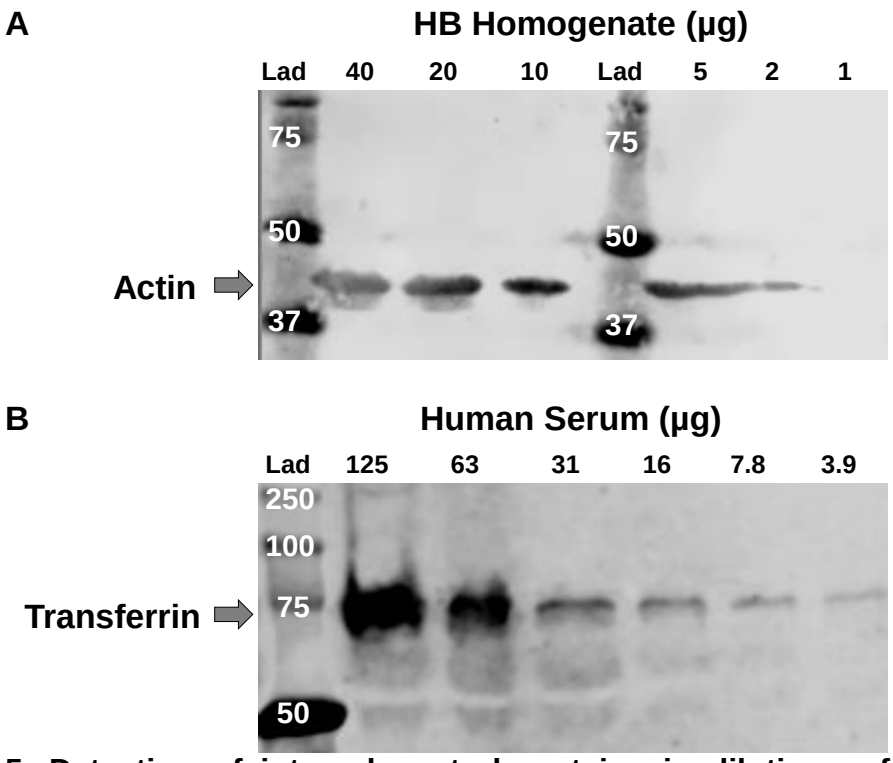
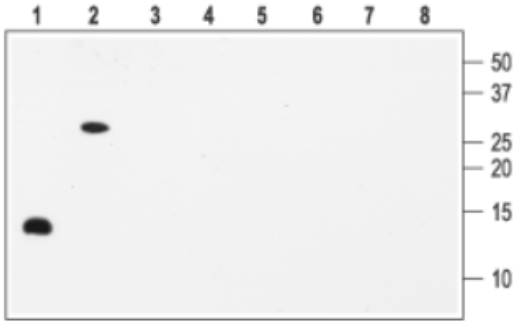


Figure 2-5. Detection of internal control proteins in dilutions of human brain (HB) homogenates and serum. 40-1 µg of HB homogenate (A) and 125-3.9 µg of human serum were run in separate blots and probed with anti-actin and anti-transferrin, respectively. A band for actin was detected between 50 kDa and 37 kDa in HB homogenates, and a band for transferrin was detected around 75 kDa in human serum. These bands progressively became weaker and smaller with decreasing protein load, suggesting that control protein saturation has not been reached.

Anti-BDNF (ANT-010) and anti-proBDNF (ANT-006) antibodies were purchased from Alomone Labs (Jerusalem, Israel). The specificity of the ANT-010 (**Figure 2-5**) and the ANT-006 (**Figure 2-6**) antibodies were tested by the company. The anti-BDNF antibody is specific for human mBDNF and proBDNF but does not detect closely related nerve growth factor (NGF) or neurotrophin-3 (NT-3). The anti-proBDNF antibody is specific for human proBDNF, and not human mature BDNF, NGF, or NT-3.

Alomone Anti-BDNF (ANT-010)



Western blot analysis with Anti-BDNF antibody:

1, 5. human BDNF (#B-250).

2, 6. proBDNF (WT-mouse) (#B-240).

3, 7. human beta-NGF (#N-245).

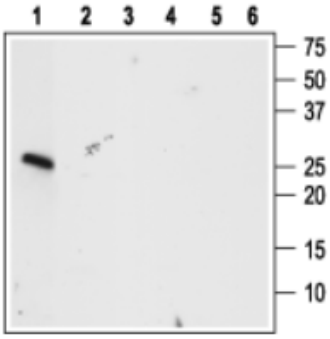
4, 8. human NT-3 (#N-260).

Lanes 1-4: **Anti-BDNF Antibody** (#ANT-010), (1:200).

Lanes 5-8: Anti-BDNF Antibody, preincubated with the control peptide antigen.

Figure 2-6. Specificity of Alomone anti-BDNF. The anti-BDNF antibody from Alomone (ANT-010) detects both mBDNF and proBDNF isoforms but does not detect nerve growth factor (NGF) or neurotrophin-3 (NT-3). Figure adapted from the Alomone website (www.alomone.com).

Alomone Anti-proBDNF (ANT-006)



Western blot analysis using Anti-proBDNF Antibody (#ANT-006), (1:400):

1. **proBDNF (WT-human)** (#B-257), (20 ng).
2. **proNGF (WT-human)** (#N-280), (200 ng).
3. recombinant proNT-3 (200 ng).
4. **human BDNF** (#B-250), (200 ng).
5. **mouse NGF 2.5S (Grade II)** (#N-100), (200 ng).
6. **human NT-3** (#N-260), (200 ng).

Figure 2-7. Specificity of Alomone anti-proBDNF. The anti-proBDNF antibody form Alomone (ANT-006) detects proBDNF but does not detect mBDNF, proNGF, or NT-3. Figure adapted from the Alomone website (www.alomone.com).

The sensitivity of the Alomone antibodies to mBDNF and proBDNF was tested by running dilutions of mBDNF protein (Alomone, B-250) and of proBDNF protein (Alomone, B-245) from 100 to 0.3 ng in a western blot. Mature BDNF was detected around 15 kDa, and proBDNF was detected around 25 kDa. The limit of detection for mBDNF was 10 ng and for proBDNF was 0.3 ng using the Alomone antibodies (**Figure 2-8**).

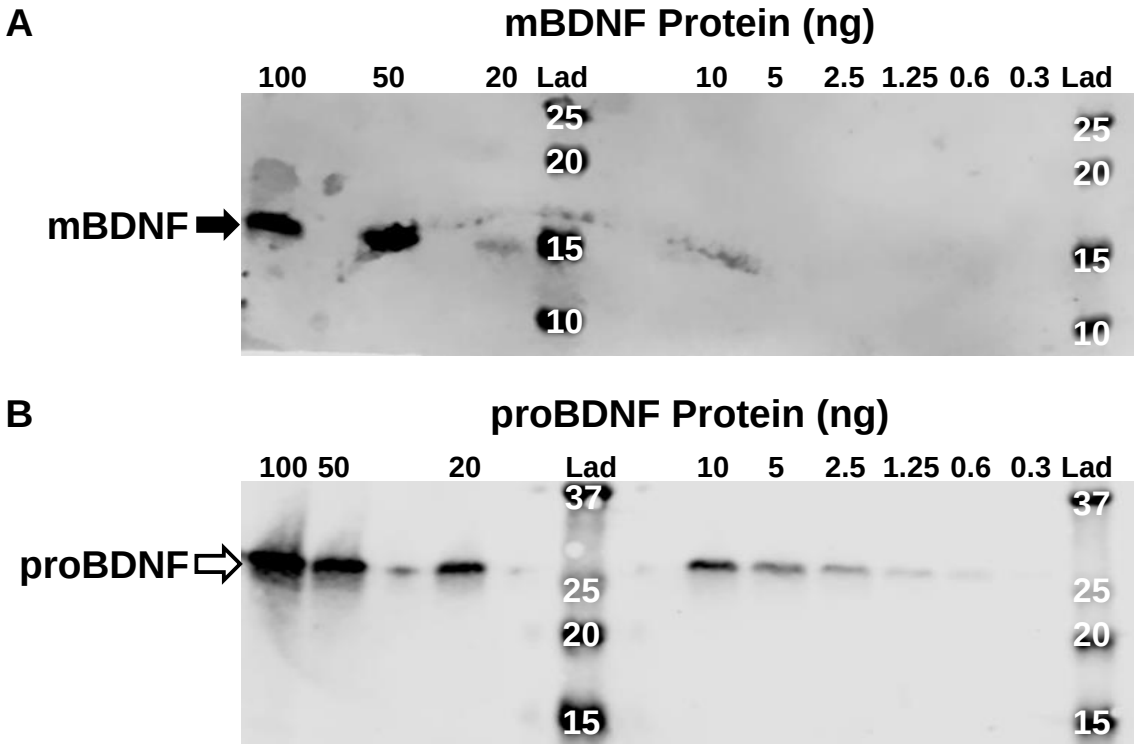


Figure 2-8. Sensitivity of Alomone antibodies to different dilutions of mBDNF and proBDNF proteins. 100-0.3 ng of mBDNF and proBDNF were run in separate blots and probed with the anti-BDNF (ANT-010) or the anti-proBDNF (ANT-006) antibodies from Alomone. Greater than 10 ng of mBDNF protein was reliably detected around 15 kDa (A), and greater than 0.3 ng of proBDNF protein was reliably detected around 25 kDa (B).

Peptide competition was used to test the specificity of the Alomone antibodies to mBDNF and proBDNF in HB homogenate and human serum (**Figure 2-9**). Specific binding of anti-BDNF and anti-proBDNF antibodies was tested through peptide competition on 30 µg human brain (HB) homogenate and 50 µg of human serum (Ser). For peptide competition, equal amounts of the peptide (BDNF or proBDNF) was mixed with the antibody (anti-BDNF or anti-proBDNF) in blocking buffer and incubated for 30 minutes at RT with agitation before use in the western blot protocol. A mBDNF band at 15 kDa could be

detected in HB homogenate but not in human serum. A small proBDNF band around 25 kDa was detected in HB homogenate while a strong, large proBDNF band was detected in human serum. The mBDNF and proBDNF bands were not detectable when the membranes were probed with peptide-blocked antibodies.

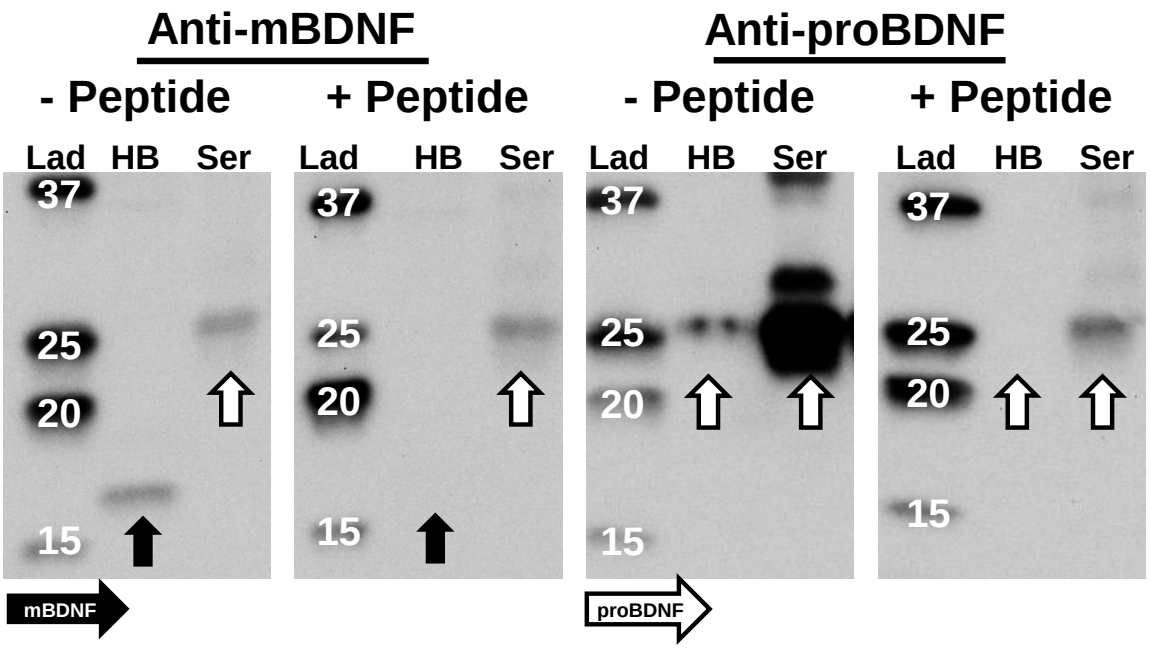


Figure 2-9. Specificity of Alomone anti-BDNF and anti-proBDNF in human brain homogenate and serum. A mBDNF band detected around 15 kDa (black arrows) and a proBDNF band detected around 25 kDa (white arrows) did not appear in membranes probed with peptide-blocked antibodies.

2.4.5 Western Blot Protocol

All cases of each sample type (serum, BA46, ENT, HIP) were run at the same time on 4 gels using the mini-PROTEAN® Tetra Cell with Precision Plus Protein Dual Color ladder (Bio-Rad #161-0374), 120 ng of recombinant human mature BDNF protein (Alomone Labs #B-250), and 2 ng of recombinant human proBDNF protein (Alomone Labs #B-257) included in every gel. Briefly, Any kD™ Mini-PROTEAN® TGX™ Precast Protein gels (Bio-Rad #456-9036) were used to

separate 0.5 µL of serum or 30 µg of human brain homogenate at 150 V for 45 minutes. Separated gel proteins were then transferred onto a 0.2 µm nitrocellulose membrane (Bio-Rad #162-0112) in transfer buffer (25 mM BisTris, 25 mM Bicine, 0.1mM EDTA, 10% methanol) using a Thermo Scientific Pierce G2 Fast Blotter with constant voltage (25 V) for 30 minutes. Blots were then incubated in blocking buffer (0.1% casein in phosphate-buffered saline) at RT for 1 hour with agitation. Next, serum blots were cut at the 20 kDa and 75 kDa bands, and the top blot was incubated in anti-transferrin antibody, the middle blot was incubated in anti-proBDNF antibody, and the bottom blot was incubated in anti-mature BDNF antibody. Brain homogenate blots were cut at the 20 kDa band, and the top blot was incubated with anti-β-actin and anti-proBDNF antibodies, and the bottom blot was incubated with anti-mature BDNF antibody. Blots were incubated with primary antibodies (**Table 2-3**) diluted in blocking buffer with 0.1% Tween 20 overnight at 4^{o}C with agitation. The next day, after washing in TBS-T buffer (2 mM Tris base, 150 mM NaCl, 0.1% Tween 20, pH 7.6), the membrane was incubated in blocking buffer containing IRDye® secondary antibody targeting the host of the primary antibody (**Table 2-3**) for 1 hour at RT with agitation. After washing in TBS-T buffer, protein expression in blots was assessed using the 700 nm and 800 nm channels in Odyssey® FC Imaging Systems (LI-COR) and Image Studio 5.x (LI-COR). Internal controls, mBDNF, and proBDNF were quantified on the same blots using the same exposure at the same time for each sample type (i.e., serum, BA46, ENT, and HIP).

Similar to previous studies between AD and Control *post mortem* samples (Connor et al. 1997, Michalski and Fahnestock 2003, Peng et al. 2005), β-actin and transferrin levels were not significantly different between AD and Control ($p>0.05$) and therefore served as ICP in western blots quantification. Quantification of the target protein (i.e., mBDNF and proBDNF) and the ICP (i.e., β-actin for brain tissue and transferrin for serum) was completed using Image Studio™ Software (licor.com/islite) according to LI-COR's Housekeeping Protein Normalization Protocol. Briefly, the Lane Normalization Factor (LNF) for each lane was calculated by the ratio of (ICP signal for each lane)/(the highest ICP signal). Then the normalized target protein value was calculated by the ratio of (target band signal)/(LNF), and this value was used in subsequent analysis.

Table 2.4. Advantages and limitations of ELISA, westerns, and immunohistochemistry. IHC = Immunohistochemistry; mBDNF = Mature BDNF; tBDNF = Total BDNF.

Method	Primary Purpose	Sample Type	Advantages	Limitations
ELISA	Quantitative measurement of protein levels	Fluid or tissue homogenate	• High sensitivity for tBDNF (pg range) • Requires low sample volume	• Contested specificity for mBDNF
Western Blots	Qualitative measurement of protein levels	Fluid or tissue homogenate	• High specificity for mBDNF and proBDNF • Semi-quantitative measurement of protein levels	• Low sensitivity (µg range)
IHC	Localization of proteins	Frozen or Paraffin-embedded tissue	• High-resolution localization of proteins in histological context	• Sensitive to poor handling and/or fixation of tissue

2.6 Cytokine Quantification

Serum levels of cytokines and chemokines (GM-CSF, IFNy, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, IL-23, TNF-α) were determined using the 14-plex Human High Sensitivity T-Cell Discovery Array (HDHSTC14) by Eve Technologies (Canada) in **Aims 1-3**. This assay, run by Eve Technologies, measures all fourteen biomarkers simultaneously in 75 uL of serum using a kit from Millipore MILLIPLEX on a BioPlex 200 system (Mineyko et al. 2012, Kim et al. 2018). In **Aims 1 and 2**, levels of the pro-inflammatory cytokine IL-1β in the hippocampus were quantified using a commercial ELISA kit (R&D Systems, Minneapolis, USA) following manufacturer's instructions. IL-1β levels in tissue

homogenates are expressed per mg of protein as determined by a BCA protein assay (Thermo Scientific, Rockford, IL, USA).

In **Chapters 3 and 5**, a composite serum cytokine score was calculated as follows. Standard z-scores were calculated for IL-1β and IL-10 based on the mean and standard deviation of each measurement in all the samples. IL-10 has anti-inflammatory properties and was reversed coded so that a high z-score represented low IL-10 levels. IL-1β z-score and IL-10 reversed z-score were averaged into a composite score to estimate a serum cytokine score where a positive score represents a proinflammatory status in serum.

There are many different methods reported in the literature on how to make a composite inflammation score. Many assign scores based on cutoffs (e.g., percentile) and then add the scores together for a composite (Boelen 1995, Wakelkamp et al. 2003, Xu et al. 2013). The serum cytokine score used in this dissertation is based on a previously published composite cytokine score (Osuchowski et al. 2010, Osuchowski et al. 2012, Stevens et al. 2017). However, there are some important differences between the composite cytokine score and the serum cytokine score. For the composite cytokine score, cytokine levels are normalized to the median, and composite scores for proinflammatory cytokines and anti-inflammatory cytokines are either kept as distinct, separate scores or a ratio of proinflammatory cytokines scores and anti-inflammatory cytokines scores are calculated. In contrast, the serum cytokine score used in this dissertation uses the mean to normalize cytokine levels and averages the scores for proinflammatory cytokines and the reverse-coded score for anti-inflammatory

cytokines to make one value to reflect inflammation. The serum cytokine score is similar to the composite cytokine score and is calculated to make better conceptual sense in relation to serum BDNF levels.

CHAPTER 3.

SERUM BDNF LEVELS POSITIVELY CORRELATE WITH PHOSPHORYLATED TAU IN ALZHEIMER'S DISEASE

This chapter is being prepared for publication:

Bharani, K. L., A. Ledreux, A. Gilmore, S. L. Carroll, and A. C. Granholm. "Serum brain-derived neurotrophic factor (BDNF) levels positively correlate with phosphorylated tau in Alzheimer's disease." Planned submission to Acta Neuropathologica

3.1 Introduction

Previous literature on BDNF levels in serum, brain, and CSF is discussed in **Chapter 1 Section 1.6**. The relationship between BDNF levels in serum, CSF, and brain tissue has not been fully investigated and represents an important step to validate the use of blood levels of BDNF as a brain health biomarker.

Measures of BDNF levels in brain tissue or serum have led to mixed results (see **Appendix 1 and 2**). Measurements of BDNF in CSF have also led to mixed results with the added difficulty of limited detection levels (Laske et al. 2006, Soderquist et al. 2009). Furthermore, investigations of BDNF levels in the CSF have thus far been limited to *ante mortem* lumbar CSF. BDNF levels in *post mortem* cranial and lumbar CSF was unknown when this project was initiated, and the relationship between BDNF levels in the CSF and brain tissue had not been reported either.

Methodological issues or the disease stage of AD are likely reasons for this discrepancy (Balietti et al. 2018). BDNF levels in serum have been proposed to vary with disease severity, where higher BDNF levels are found in those with MCI or mild AD compared to controls, and lower BDNF levels are found in those with severe AD (Laske et al. 2006, Angelucci et al. 2010, Laske et al. 2011, Forlenza et al. 2015, Kim et al. 2017). Another yet to be resolved issue is the relationship between serum BDNF levels and brain BDNF levels. Although significant correlations have been found between serum and brain BDNF levels in animal models (Karege et al. 2002, Sartorius et al. 2009, Elfving et al. 2010,

Klein et al. 2011), this relationship has not been directly investigated in humans, and was therefore one of the aims in the current study.

In addition to the complexity of brain/blood BDNF levels, the interaction between BDNF and biomarkers of AD (Aβ and pTau) is also complex (Diniz and Teixeira 2011, Zhang et al. 2012, Rosa and Fahnestock 2014, Budni et al. 2015). Lee et al. (2005) reported a weak correlation between the loss of BDNF and the accumulation of neuritic amyloid plaques in AD patients. BDNF has been shown to have direct dose-dependent protective effects against the toxicity induced by Aβ peptide in animal models (Arancibia et al. 2008), and reduced high-affinity BDNF receptor tropomyosin-related kinase B (TrkB) exacerbates hippocampal signaling dysfunction in AD transgenic mice (Devi and Ohno 2015). TrkB receptors have also been found to be trapped in senile plaques (Connor et al. 1996) which could contribute to loss of neurotrophic support from BDNF due to reduced BDNF binding to its high-affinity receptor. Lower TrkB receptor expression in AD hippocampi has been consistently reported (Connor et al. 1996, Allen et al. 1999, Ferrer et al. 1999, Ginsberg et al. 2006). However, $p75^{NTR}$ receptor expression in AD hippocampi has been reported as higher (Hu et al. 2002, Chakravarthy et al. 2012) or not different (Ginsberg et al. 2006) compared to control, suggesting that a shift between the high-affinity receptor and the $p75^{NTR}$ receptor may contribute to or be causative in AD neuropathology. Aβ peptides have been shown to bind to the $p75^{NTR}$ receptor (Sotthibundhu et al. 2008) and positively correlate with $p75^{NTR}$ receptor expression (Chakravarthy et

al. 2012), providing indirect evidence for a role of BDNF in protection against AD pathology.

Despite these previous findings, no cohesive examination of BDNF, TrkB, and AD pathology had been undertaken prior to our study. The aims of the current study were to examine total BDNF, proBDNF, and mBDNF levels in *post mortem* CSF, serum, and brain tissue samples. We aimed to assess the relationship between BDNF levels in CSF and serum and BDNF levels in brain tissue (BA46, ENT, HIP), as well as the relationship between BDNF levels and the distribution of BDNF receptors, Aβ, pTau, and neuroinflammation in the hippocampus to characterize the role of BDNF in the pathology of AD.

3.2 Methods and Materials

3.2.1 Human Cases

Post mortem human brain tissue and fluids were obtained from the Carroll A. Campbell, Jr. Neuropathology Laboratory at the Medical University of South Carolina (MUSC). Thirty-one *post mortem* cases (20 AD, 11 non-cognitively impaired controls) were selected for this study. The neuropathological diagnoses of AD and control cases were assessed by a board-certified neuropathologist at MUSC. Bielschowsky silver staining, Amylo-Glo (Schmued et al. 2012) and pTau immunostaining were used to confirm staging of AD pathology in the hippocampus.

3.2.2 Human Brain and Fluids Collection

Post mortem blood was collected into BD Vacutainer Serum Separator Tubes® and allowed to clot for 60 minutes at room temperature (RT) before being centrifuged for 20 minutes at 1,500 x g. Serum was then carefully collected, aliquoted, and stored in a -80^{o}C freezer until analysis. Cranial (CSF-C) and lumbar (CSF-L) cerebrospinal fluid were collected at autopsy and centrifuged for 20 minutes at 1,500 x g before being aliquoted and stored in a -80^{o}C freezer. The left hemisphere was cut into 1 cm coronal slabs and placed into clear plastic bags for storage in a -80^{o}C freezer. Right hemisphere 1 cm slabs were fixed free-floating in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 48-72 hours at 4^{o}C. The paraformaldehyde-fixed slabs, were then transferred to a 0.1 M phosphate buffer solution (pH 7.4) containing 30% sucrose for at least 24 hours at 4^{o}C. Subsequently, samples were transferred into cyroprotectant and stored at -20^{o}C. Frozen pieces of the prefrontal cortex (Brodmann area 46; BA46), the entorhinal cortex (ENT), and the hippocampus (HIP) were isolated from the left hemisphere and stored at -80^{o}C before homogenization and use in further assays. Fixed pieces of the 16 areas identified by Montine et al. (2012) were embedded in paraffin, and 5 µm thick sections were used for immunohistochemistry and for hematoxylin and eosin (H&E) stain, and Bielschowsky silver stains. Paraffin sections (5 µm thick) of BA46, ENT, and HIP were used for analysis of AD-related and inflammation-related markers with IHC. Brain homogenates were prepared as described in **Chapter 2 Section 2.3.3**.

3.2.3 Cytokine and Chemokine Quantification

Serum cytokines were measured, and a serum cytokine score was calculated as described in **Chapter 2 Section 2.6**. The serum cytokine score was not different between AD and Control (M-W *U*=69, p-0.561), did not correlate with any of the measurements in this analysis, and consequently was not considered a confounder.

3.2.4 Total BDNF Quantification

Total BDNF levels in undiluted CSF-C, undiluted CSF-L, and serum samples (diluted 1:20 with manufacturer's provided diluent, RD6P) were measured in duplicates using the Human Free BDNF Quantikine ELISA Kit (DBD00, R&D Systems, Minneapolis, USA), as described in **Chapter 2 Section 2.4.2**. Total BDNF levels in brain homogenates (BA46, ENT, HIP; each diluted 1:2 with manufacturer-provided Block & Sample buffer) were measured in duplicate using the BDNF Emax® ImmunoAssay System (G7610, Promega, Madison, WI) as described in **Chapter 2 Section 2.4.3**.

3.2.5 Mature BDNF and proBDNF Quantification

Western blotting was used to differentiate further and quantify mBDNF (14-16 kDa) and proBDNF (28-32 kDa) as described in **Chapter 2 Section 2.4.4**. Internal controls, mBDNF, and proBDNF were quantified on the same blots using the same exposure at the same time for each sample type (i.e., serum, BA46, ENT, and HIP). Representative blots for serum and brain homogenates are

presented in **Figure 3-1**. Serum blots had strong proBDNF bands but undetectable mBDNF bands. This is consistent with Carlino et al. (2011) where western blot analysis of BDNF isoforms in *ante mortem* human serum samples resulted in strong proBDNF bands and weak mBDNF bands using an anti-BDNF antibody from Santa Cruz Biotechnology (N-20/sc-546). Conversely, brain homogenate blots had strong mBDNF bands and weak proBDNF bands.

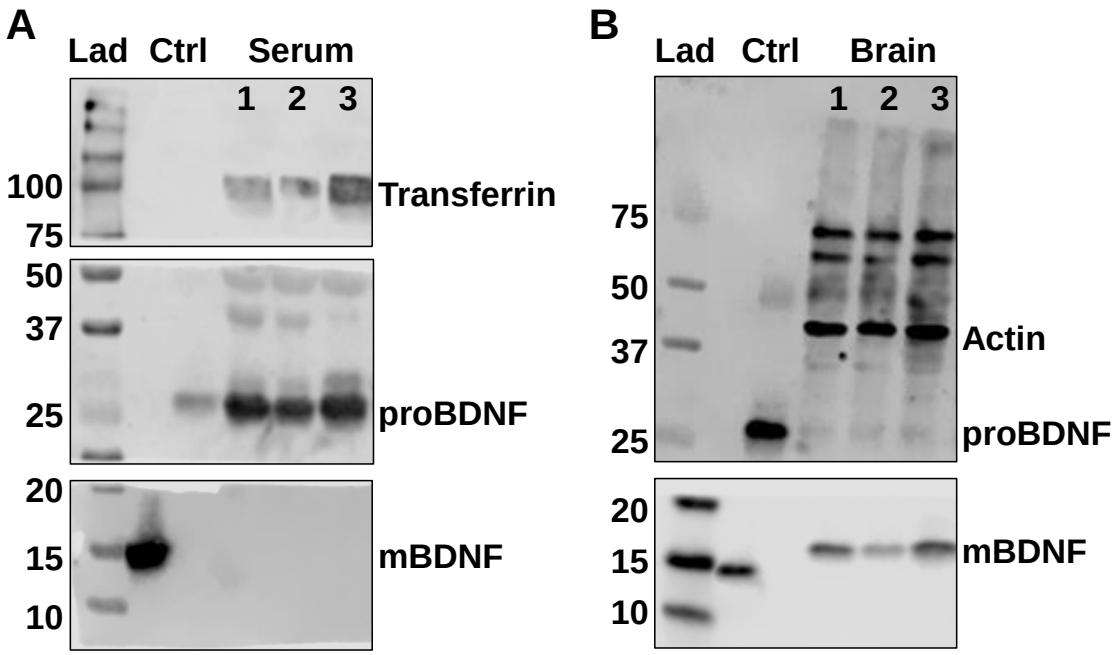


Figure 3-1. ProBDNF and mBDNF representative western blots of serum (A) and brain homogenate (B) samples. The first three lanes in both blots contain Precision Plus Protein Dual Color ladder, 120 ng of recombinant human mature BDNF protein (mBDNF), and 2 ng of recombinant human proBDNF protein. The last three lanes contain 0.5 µL of serum (A) or 30 µg of human brain homogenate (B) from three different cases. Strong proBDNF bands can be seen in serum samples (A). Weak proBDNF and strong mBDNF bands can be seen in brain samples (B).

3.2.6 Immunohistochemistry

Five-micron paraffin-embedded sections were baked at 60^{o}C for 1 hour before deparaffinization in xylene twice for 5 minutes each followed by serial rehydration in graded ethanol [100%, 95%, 70%, and phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 43 mM Na_2HPO_4, 14.7 mM KH_2PO_4)] for 5 minutes at each step. Sections were then submerged in Tris-EGTA buffer (10mM Tris, 0.5 mM EGTA) and heated in a microwave for 9 minutes at 80% power and 15 minutes at 40% power. After cooling on the bench for 15 minutes, sections were blocked in 2% normal goat serum (NGS) in PBS for 30 minutes and then incubated in 2% NGS-PBS with primary antibody (see **Table 3-1**) overnight at 4^{o}C with agitation. The next day, sections were washed with PBS and incubated with 1% bovine serum albumin solution made in PBS containing Alexa Fluor® 568 secondary antibodies targeting the host of the primary antibody (see **Table 3-1**) for 1 hour at RT with agitation. Sections were incubated in 0.04 ug/mL bisbenzamide for 10 minutes and then incubated in 0.3% Sudan Black B (ab146284) in 70% ethanol for 10 minutes before being washed and coverslipped with ProLong® Diamond Antifade (Thermo Fisher Scientific P36961).

Table 3-1. Antibodies used for immunohistochemistry. CNPase = 2',3'-Cyclic-nucleotide 3'-phosphodiesterase; GFAP = Glial fibrillary acidic protein; HLA-DR = Human Leukocyte Antigen – antigen D Related; pTau (AT8) = Phosphorylated tau (Ser202, Thr205); TrkB = Tropomyosin receptor kinase B.

Target	Host	Dilution	Source	Identifier
Astroglia (GFAP)	Rabbit	1:1,000	Agilent	DAKO 20334
Microglia (HLA-DR)	Mouse	1:100	Thermo Fisher Scientific	MA5-11966
Neurons (NeuN)	Mouse	1:500	Millipore Sigma	MAB377
Oligodendrocytes (CNPase)	Mouse	1:200	Abcam	ab6319
p75NTR	Mouse	1:100	R&D Systems	MAB367
pTau (AT8)	Mouse	1:750	Thermo Fisher Scientific	MN1020
TrkB	Rabbit	1:100	Alomone	ANT-019
Ms IgG (H+L)	Goat	1:1,500	Thermo Fisher Scientific	Alexa Fluor® 568 A11004
Rb IgG (H+L)	Donkey	1:1,500	Thermo Fisher Scientific	Alexa Fluor® 568 A10042

Amyloid staining was completed using Amylo-Glo® RTD™ “Ready to Dilute” Staining reagent (Biosensis #TR-300-AG) following the manufacturer provided protocol. Briefly, rehydrated sections were placed in 70% ethanol for 5 minutes at RT, distilled water for 2 minutes, manufacturer provided Amylo-Glo® RTD™ solution for 10 minutes, 0.9% saline solution for 5 minutes, manufacturer-provided ethidium bromide (EtBr RTD™) solution for 3 minutes, 0.9% saline solution for 5 minutes, and then rinsed briefly in distilled water before being coverslipped with ProLong® Diamond Antifade.

Images of the Cornu Ammonis (CA1) area of the hippocampus were captured using a 40x objective on an Olympus BX53 microscope attached to an Olympus DP80 camera. Images were analyzed using Fiji ImageJ (version 1.50i, Schindelin et al. 2012). The background was subtracted using a rolling ball radius of 50.0 pixels, and thresholding was used to isolate positive staining from background staining by an experimenter blinded to the neuropathological status of each case. The percent area of positive staining (% area) was quantified using the Particle Analyzer feature in Fiji ImageJ with the size inclusion set to 200-Infinite pixels to exclude smaller punctate staining with no apparent anatomical pattern. This procedure was adapted from previous studies (Ubhi et al. 2009, Ihara et al. 2010, Holleran et al. 2017, Woerman et al. 2018), and this technique was further validated by a recent study (Holleran et al. 2017) where the investigators reported a high correlation between stereology count of pTau sites per area volume and the automated % area of pTau staining obtained using the above described ImageJ-based workflow. Quantification of positive staining was reported as an average % area from 3 non-overlapping images by an investigator blinded to the groups.

3.2.7 Statistics

Outliers were identified using the Robust regression and Outlier removal (ROUT) method in GraphPad Prism for Windows, version 6 (GraphPrism Software, La Jolla, California, USA, www.graphpad.com). All other statistics were run in IBM SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, N.Y.,

USA). BDNF has been widely reported to have a non-normal distribution (Ziegenhorn et al. 2007, Balietti et al. 2018). In this study, a non-normal distribution was confirmed by inspecting the histogram plot of the data for skewness and by using the Shapiro-Wilk test. Equality of variance across groups was assessed using Levene's F-test. Accordingly, non-parametric pairwise comparison between two groups was assessed using a Mann-Whitney (M-W) U-test for measures with equal variance between groups or a Kolmogorov-Smirnov (K-S) Z-test for measure with unequal variance between groups. Relationships between two continuous variables were assessed using nonparametric Spearman's rank-order correlation, and relationships between categorical variables were assessed using the two-sided Fisher's exact test.

3.3 Results

3.3.1 Demographics and characteristics of AD and Control groups

Summary data of age, gender, brain weight, *APOE* genotype, and *post mortem* interval (PMI) is presented in **Table 3-2**. The mean age of the AD group (76.5 ± 2.3 years) was statistically higher (t_{29}=2.117, p=0.043) than the Control group (66.2 ± 5.1 years). Previous studies have indicated that BDNF levels decrease with increasing age (Laske et al. 2007, Ziegenhorn et al. 2007, Erickson et al. 2010, Forlenza et al. 2015, Hakansson et al. 2017); however, in this cohort, age did not significantly correlate with any of our measurements due to our narrow age range (see **Table 3-3** for correlations). No significant differences were found in the ratio of females to males between the AD group

(13/20 females, 65.0%) and the Control group (5/11 females, 45.5%; p=0.449, two-sided Fisher's exact test). However, brain weight in males (1316.7 ± 38.5 grams) were found to be significantly higher (t_{28}=5.113, p<0.001) than in females (1111.2 ± 20.6 grams), but not significantly different (t_{28}=1.002, p=0.325) between the AD group and the Control group. The AD group (15/18, 83.3% *APOE* ε4 carriers) had significantly more (p<0.001, two-sided Fisher's exact test) *APOE* ε4 alleles than the Control group (0/10, 0.0% *APOE* ε4 *carrier*). PMI was statistically higher (t_{28}=2.560, p=0.016) in the Control group (15.4 ± 1.9 hours) than the AD group (10.1 ± 1.1 hours), but the PMI for all samples were under 24 hours. PMI also did not significantly correlate (p>0.05) with any measurements used in this analysis, suggesting that PMI was not a confounder in this study.

Table 3-2. Demographics of human brain samples. Mean ± SEM. NA= Not Available; PMI = *Post Mortem* Interval.

	Total	AD	Control
Age (years)	72.8 ± 2.5	76.5 ± 2.3	66.1 ± 5.2
Sex (female / male)	18 / 13	13 / 7	5 / 6
Brain weight (g)	1193±26.9	1174±36.5	1232±33.4
PMI (hours)	11.8 ± 1.1	10.1 ± 1.1	15.4 ± 1.9
***APOE* status:**			
ε2/ε3	2	0	2
ε3/ε3	11	3	8
ε3/ε4	9	9	0
ε4/ε4	6	6	0
NA	3	1	2

Table 3-3. Summary statistics of BDNF measurements from AD and Control samples. Summary statistics are reported as median (Q3-Q1). Normality was assessed using Shapiro-Wilk test. Equality of variances was assessed using Levene's F-test. Pairwise comparison was assessed using Mann-Whitney (M-W) U-test or Kolmogorov-Smirnov (K-S) Z-test. Significance is indicated with * when p<0.05. ND = Not Detectable (i.e., measurements were under detectable threshold).

Measure	AD	Control	Equality of Variance	Pair-Wise Test	AD vs Control	Correlation with Age
BDNF (ELISA)						
Serum (pg/mL)	524.8 (591-463)	496.7 (580-442)	p=0.396	M-W	p=0.547	r_S=-0.004 p=0.987
BA46 (pg/mg)	10.6 (16.9-5.8)	12.3 (16.2-7.7)	p=0.724	M-W	p=0.966	r_S=0.197 p=0.296
ENT (pg/mg)	5.9 (10.9-2.8)	6.6 (10.4-5.5)	p=0.126	M-W	p=0.660	r_S=-0.208 p=0.307
HIP (pg/mg)	18.6 (44-10)	15.7 (33.2-11.6)	p=0.457	M-W	p=0.941	r_S=-0.238 p=0.231
mBDNF (Western Blot)						
Serum	ND	ND				
BA46	1.3 (2.4-0.7)	1.9 (2.4-1.3)	p=0.629	M-W	p=0.307	r_S=0.241 p=0.199
ENT	0.8 (1.1-0.4)	1.2 (1.7-0.7)	p=0.725	M-W	p=0.104	r_S=0.205 p=0.286
HIP	1.1 (1.5-0.6)	1.5 (2.2-0.1)	p=0.235	M-W	p=0.769	r_S=0.166 p=0.389
proBDNF (Western Blot)						
Serum	22.7 (33.9-12.4)	15.6 (21.4-12.8)	p=0.024*	K-S	p=0.161	r_S=0.297 p=0.149
BA46	0.06 (0.09-0.04)	0.1 (0.14-0.07)	p=0.090	M-W	p=0.024*	r_S=-0.046 p=0.809
ENT	0.02 (0.03-0.01)	0.03 (0.05-0.02)	p=0.903	M-W	p=0.045*	r_S=0.266 p=0.163
HIP	0.04 (0.04-0.02)	0.05 (0.08-0.02)	p=0.042*	K-S	p=0.106	r_S=-0.033 p=0.868

3.3.2 Detection rate of BDNF in CSF-C was lower in the AD group

To test whether BDNF was detected at a lower rate in AD CSF (CSF-C n=13, CSF-L n=6) than in Control CSF (CSF-C n=8, CSF-L n=8), we compared the detection rate (number of samples with BDNF detected/total samples tested) between the two groups. The detection rate of BDNF in undiluted CSF-C was lower (p=0.007, two-sided Fisher's exact test) in the AD group (5/13, 38.5%, median of detected=11.2 pg/mL, Interquartile Range - IQR= 22.0-8.6 pg/mL) than the Control group (8/8, 100%, median of detected=11.3 pg/mL, IQR= 12.7-9.8 pg/mL). In undiluted CSF-L, there was no difference (p=0.627, two-sided Fisher's exact test) in the ratio of BDNF detected in the AD group (4/8, 50.0%, median of detected =27.9 pg/mL, IQR= 310.0-11.5 pg/mL) compared to the Control group (4/6, 66.7%, median of detected=14.6 pg/mL, IQR= 106.2-9.3 pg/mL). Detectable BDNF levels were not significantly different between the AD and Control groups in both CSF-C (M-W U=19.0, p=0.943) and CSF-L (M-W U=6.0, p=0.686) and did not significantly correlate (p>0.05) with any serum or brain BDNF measurements, potentially due to the low number of samples with detectable BDNF in CSF in the two cohorts.

3.3.3 Serum proBDNF levels were inversely related to HIP tBDNF and proBDNF levels

To test the relationship between serum and brain tissue levels of BDNF, a Spearman correlation table was generated to compare tBDNF and proBDNF from serum with tBDNF, proBDNF, and mBDNF from BA46, ENT, and HIP frozen

tissue samples. Serum proBDNF levels negatively correlated with HIP tBDNF (r_s=-0.435, p=0.043) and HIP proBDNF levels (r_s=-0.432, p=0.040; **Figure 3-2**), while serum tBDNF levels measured by ELISA did not correlate with any brain measurement of BDNF, suggesting either that protein levels of mBDNF or proBDNF may deteriorate more rapidly in serum than in brain tissue *post mortem*, or that levels observed in the blood are not entirely mirroring the levels observed in the CNS at the same time.

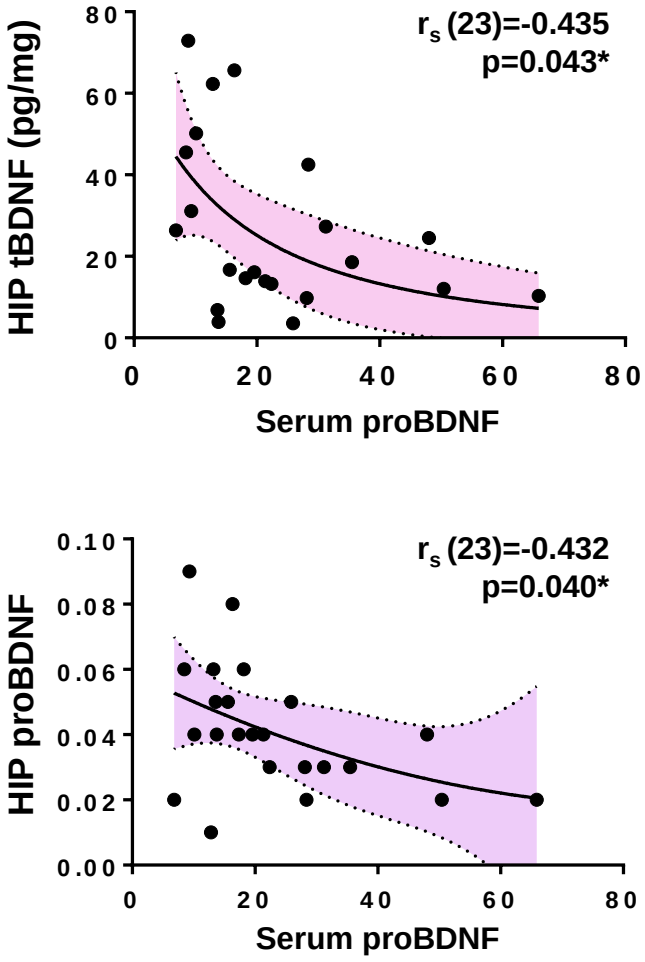


Figure 3-2. Correlations between serum proBDNF measurements and HIP tBDNF and proBDNF measurements. Non-parametric Spearman correlations were used to compare peripheral serum measurements of BDNF to central brain measurements of BDNF. Total BDNF (tBDNF) was assessed through commercial ELISA while proBDNF was quantified through western blotting after normalizing to internal control protein transferrin for serum and β-actin for HIP brain homogenates.

3.3.4 BA46 and ENT proBDNF levels were lower in AD

In line with previous findings in the parietal cortex (Michalski and Fahnestock 2003, Peng et al. 2005), proBDNF in BA46 (M-W *U*=49.0, p=0.024) and in ENT (M-W *U* = 51.0 p=0.045) was found to be statistically lower in the AD group compared to the Control group. ProBDNF in the HIP was not significantly different between the AD group and the Control group (K-S *Z*=1.266, p=0.106); however, HIP proBDNF was negatively correlated with HLA-DR (r_s=-0.498, p=0.011) and positively correlated with $p75^{NTR}$ in the hippocampus (r_s=0.480, p=0.018) across groups, suggesting that higher neuroinflammation was associated with lower proBDNF and that lower proBDNF was associated with lower $p75^{NTR}$ receptor density.

Considering that the ENT has direct and indirect inputs into CA1 of the HIP and the majority of BDNF synthesized in the ENT is transported to the HIP (Scharfman and Chao 2013), we tested if BDNF levels in the ENT correlated with IHC measures of AD pathology, BDNF receptors, neurons, and glia in the CA1. ENT tBDNF levels were found to be positively correlated with NeuN staining in CA1 (r_s=0.419, p=0.041) and negatively correlated with amyloid staining in CA1 (r_s=-0.418, p=0.047), suggesting that BDNF from the ENT provide trophic support for mature neurons and perhaps ameliorates plaque burden in the CA1 region of the HIP. Western blot measurement of mBDNF and ELISA measurement of tBDNF levels in any of the brain regions did not differ between AD and Control (M-W, p>0.05), likely due to high variability.

3.3.5 TrkB, but not p75 staining, is inversely correlated with amyloid and pTau accumulation

Frequent diffuse and neuritic plaques were apparent in all AD hippocampal sections with increased number and size in the CA1 region. Similarly, numerous NFTs and dystrophic neurites were found in all AD hippocampal sections with increased number of intracellular and ghost tangles found in the CA1 region. Control hippocampal sections presented with few to no positive amyloid plaques or tangles. These observations were supported by statistical analysis of % area measurements of Amylo-Glo® and pTau (AT8) staining where the AD group had statistically higher % area staining for amyloid (K-S Z=1.976, p=0.001) and pTau (K-S Z=1.824, p=0.003) compared to the Control group (**Figure 3-3**).

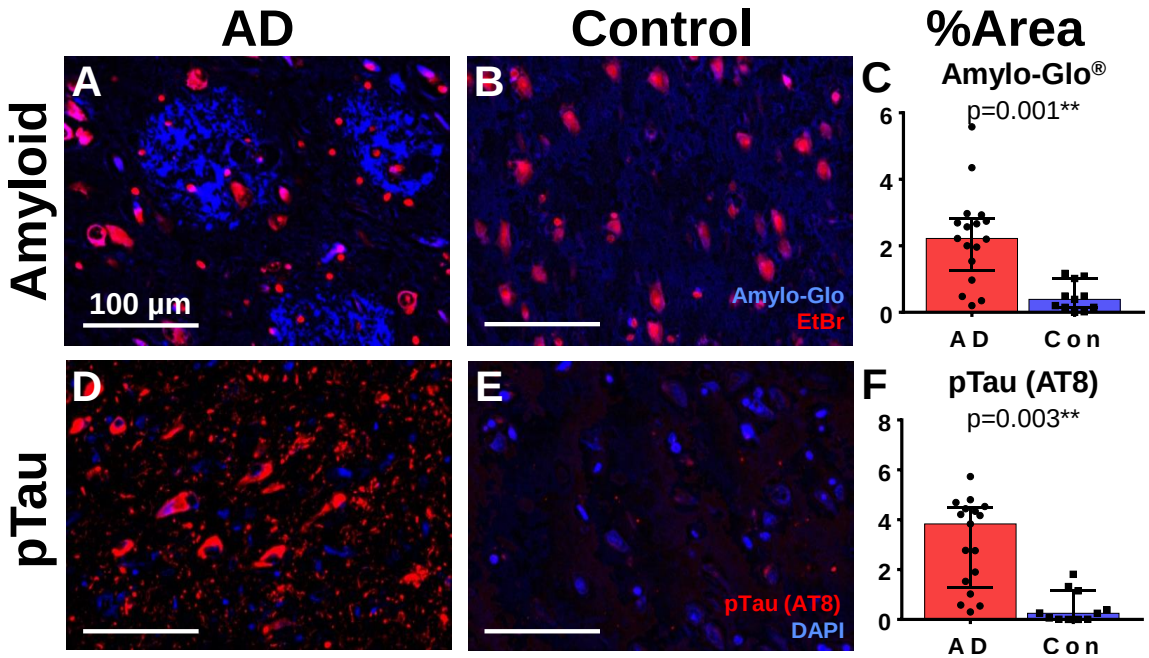


Figure 3-3. Representative images of amyloid plaques (A, B) and phosphorylated tau (D, E) in the CA1 region of the hippocampus. Bar graphs (C, F) depict median and interquartile range of the % area stained by Amylo-Glo® and pTau antibody in the AD and Control groups. The AD group had statistically higher % area staining for amyloid (K-S *Z*=1.976, p=0.001) and pTau (K-S *Z*=1.824, p=0.003) compared to the Control group. (AT8) = Phosphorylated tau (Ser202, Thr205). **p<0.001.

Microscopic evaluation of the high-affinity BDNF receptor TrkB and low-affinity BDNF receptor $p75^{NTR}$ revealed diffuse staining across the hippocampal regions with increased immunoreactivity in cell bodies compared to processes. Additionally, TrkB staining was not found in the nucleus, unlike $p75^{NTR}$, and was found in fewer neurons in the CA1 of the AD cases compared to Control cases. Statistical analysis of % area measurements of these stains confirmed that the AD group had lower % area staining of the BDNF receptor TrkB (M-W *U*=39.0, p=0.009) than the Control group, but the AD group did not differ from the Control group in % area staining of $p75^{NTR}$ (M-W *U*=58.0, p=0.148, **Figure 3-4**). TrkB %

area staining negatively correlated with amyloid (r_s=-0.386, p=0.042) and pTau (r_s=-0.383, p=0.044) immunoreactivity, while p75^{NTR} % area staining did not correlate with either amyloid (r_s=0.251, p=0.207) or pTau (r_s=0.215, p=0.282), suggesting that amyloid and pTau pathology may downregulate or interfere with TrkB but not p75^{NTR} density.

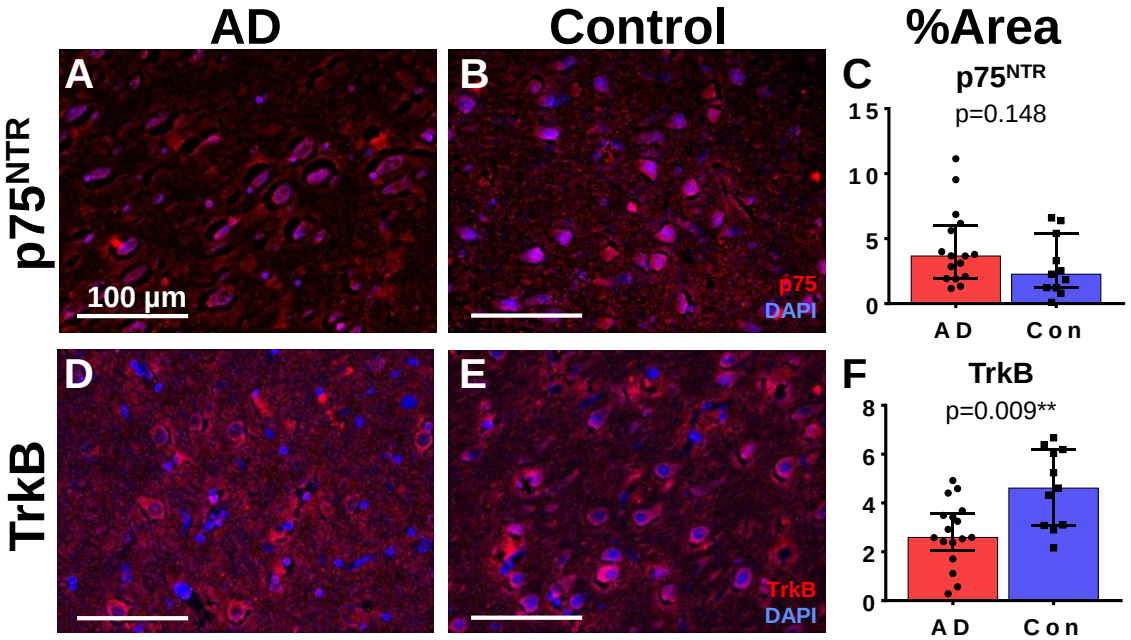


Figure 3-4. Representative images of neurotrophin receptors p75^{NTR} (A, B) and TrkB (D, E) in the CA1 region of the hippocampus. Bar graphs (C, F) depict median and interquartile range of the % area stained by TrkB and p75^{NTR} antibodies in the AD and Control groups. The AD group had lower % area staining of the BDNF receptor TrkB (M-W *U*=39.0, p=0.009) than the Control group, but the AD group did not differ from the Control group in the % area staining of p75^{NTR} (M-W *U*=58.0, p=0.148). TrkB = Tropomyosin receptor kinase B; p75^{NTR} = p75 neurotrophin receptor. **p<0.001.

3.3.6 HLA-DR staining positively correlated with amyloid and pTau staining

Microscopic evaluation of astrocytes, microglia, oligodendrocytes, and mature neurons in the hippocampus was highly variable across cases in both the AD and the Control groups. AD cases tended to have an increase in the density

of GFAP-stained astrocytes with overlapping processes and enlarged cell bodies compared to the Control cases, indicating increased astrogliosis in AD hippocampi. Similarly, ramified microglia with slender extrusions and reactive microglia with rounded cell bodies and fewer extrusions were observed more often in the AD hippocampi than in the Controls. However, % area measurements of GFAP (K-S *Z*=1.161, p=0.135**, Figure 3-5A-C**) and HLA-DR (K-S *Z*=0.912, p=0.376, Figure **3-5D-F**) in the CA1 region of the hippocampus was not significantly different between the AD and the Control groups, presumably due to the high variability observed between the different cases.

HLA-DR staining was positively correlated with amyloid staining (r_S=0.371, p=0.057) and pTau staining (r_S=0.383, p=0.049), although the former did not reach statistical significance at p<0.05. This result suggests that increased HLA-DR positive microglia is associated with increasing AD pathology. Mature, small, round oligodendrocytes and their long thin processes were observed equivalently between AD and Control hippocampi which was supported by statistical analysis of % area measurements of CNPase staining (M-W *U*=92.0, p=0.135, Figure 5G-I). Consistent with previous findings that hippocampal atrophy and not necessarily neuronal loss distinguishes AD from controls (Simic et al. 1997, Jin et al. 2004), the density of mature neurons was not decreased in the CA1 region of AD hippocampi compared to Control hippocampi. This observation was further supported by statistical analysis of % area measurements of NeuN staining (M-W *U*=83.0, p=0.492, **Figure 3-5J-L**).

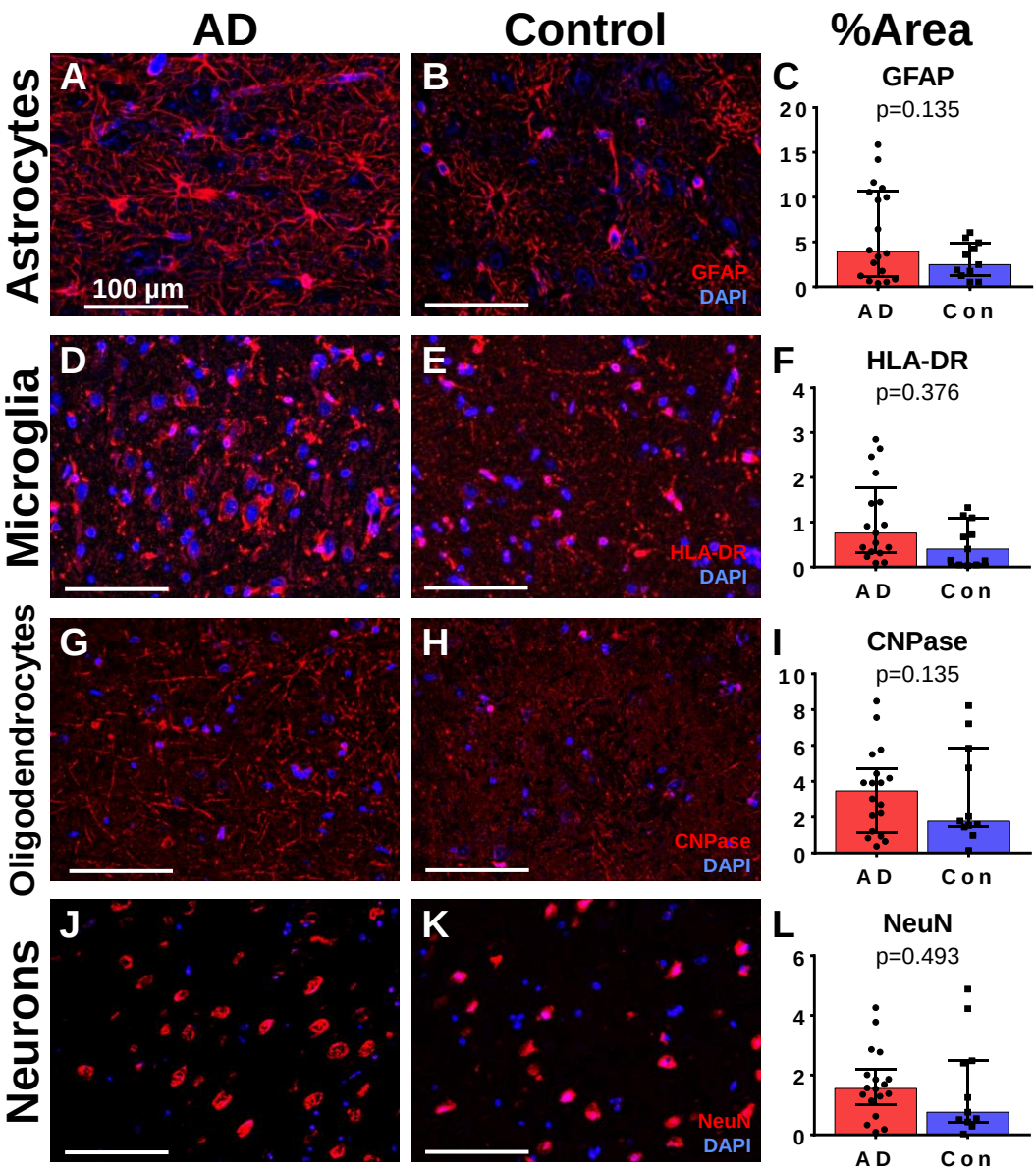


Figure 3-5. Representative images of astrocytes (GFAP; A, B), activated microglia (HLA-DR; D, E), oligodendrocytes (CNPase; G, H), and mature neurons (NeuN; J, K) in the CA1 region of the hippocampus. Bar graphs (C, F, I, L) depict median and interquartile range of the percent area stained of each marker in the AD and Control groups. The AD and Control groups did not significantly differ in % area measurements of GFAP (K-S Z=1.161, p=0.135), HLA-DR (K-S Z=0.912, p=0.376), CNPase staining (M-W U=92.0, p=0.135), or NeuN staining (M-W U=83.0, p=0.492). CNPase = 2',3'-Cyclic-nucleotide 3'-phosphodiesterase; GFAP = Glial fibrillary acidic protein; HLA-DR = Human Leukocyte Antigen – antigen D Related; NeuN = Neuronal nuclei.

3.3.7 Serum proBDNF positively correlated with pTau staining

As an exploratory analysis to see if serum tBDNF or serum proBDNF levels were correlated with hippocampal pathology, a Spearman correlation table between serum BDNF levels (tBDNF and proBDNF) and IHC density measures of amyloid, pTau, BDNF receptors, and glia in the CA1 was analyzed. Serum tBDNF levels were positively correlated with HLA-DR staining density (r_s=0.593, p=0.007), and serum proBDNF levels were positively correlated with pTau staining density (r_s=0.435, p=0.043), suggesting that serum BDNF may be upregulated with increasing neuroinflammation and AD pathology. No correlations reached significance (p>0.05).

3.3.8 High and Low AD subgroups differed in amyloid, pTau, and GFAP staining

To investigate the effects of severity, z-scores were calculated for each stain using the mean and standard deviation of the control group. A positive z-score is interpreted as higher % area staining compared to controls, and a negative z-score is interpreted as lower % area staining compared to controls. Two AD subgroups could be distinguished by density of GFAP staining. One AD subgroup (High AD) had intense GFAP staining, and the other subgroup (Low AD) was more similar to the Control group with low GFAP staining. Accordingly, AD subgroups were defined based on GFAP % area z-scores where cases with z-scores greater than 2 was classified as High AD (n=7), and cases with z-scores less than 1 were classified as Low AD (n=10). Visualization of these stains by

subgroups (**Figure 3-6A**) suggests that the High AD subgroup had higher amyloid, higher pTau, and higher GFAP staining than the Low AD subgroup. Statistical analysis between High AD and Low AD confirmed this observation in in terms of GFAP staining (M-W U=0.0, p<0.001) while only a marginally significant difference was found between High AD and Low AD in amyloid (M-W U=15.0, p=0.055) and pTau (M-W U=16.0, p=0.070). The High AD and Low AD subgroups did not differ in staining for $p75^{NTR}$ (M-W U=23.0, p=0.475), TrkB (M-W U=26.0, p=0.417), HLA-DR (M-W U=29.0, p=0.958), CNPase (M-W U=28.0, p=0.536), or NeuN (M-W U=27.0, p=0.475). Visualization of brain tBDNF z-scores by subgroup (**Figure 3-6B**) suggests that the Low AD subgroup had higher tBDNF in the ENT and HIP than the High AD subgroup and the Control group, suggesting a possible compensatory increase in BDNF in AD compared to Control when AD pathology is low. In addition, proBDNF levels seem to be higher in both the AD subgroups compared to the Control group only in serum (**Figure 3-6C**), and mBDNF was lower in all three brain regions in both the AD subgroups (**Figure 3-6D**). Although mechanistically interesting, these observations were not found to be statistically significant (p>0.05). Interestingly, the AD case which had the highest HLA-DR staining area for CA1 also exhibited very high % staining for GFAP, pTau, and among the lowest levels of BDNF, suggesting a correlation between inflammatory activation, AD pathology, and BDNF expression at the individual level. This will have to be further examined using early and late Braak stage cases in the future.

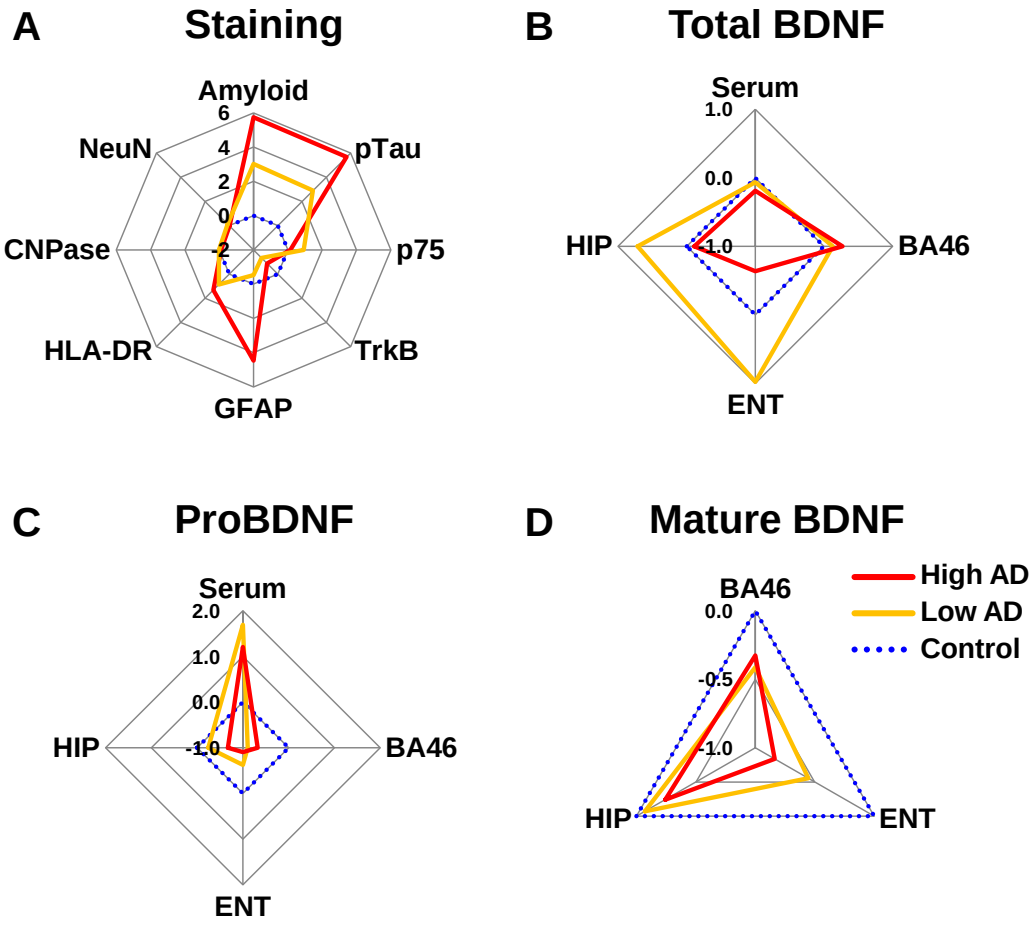


Figure 3-6. Profiles of staining (A), total BDNF (B), proBDNF (C), and mature BDNF (D) in Control and AD subgroups. Mean and standard error of Z-scores for % area of each stain and total BDNF levels for the Control group, High AD subgroup, and Low AD subgroup are plotted. Z-scores were calculated using the mean and standard deviation of the Control group. The AD subgroup was defined by GFAP % area staining where a case with a z-score greater than 2 was classified as High AD and a case with a z-score less than 1 was classified as Low AD. A significant difference between the High AD and Low AD subgroups was found in z-scores of GFAP (M-W U=0.0, $p<0.001$) while a marginally significant difference was found in z-scores of amyloid (M-W U=15.0, $p=0.055$) and pTau (M-W U=16.0, $p=0.070$). Total BDNF levels in the dorsolateral prefrontal cortex (BA46), entorhinal cortex (ENT), hippocampus (HIP) and serum and proBDNF in serum were not significantly ($p>0.05$) different between the High AD and Low AD subgroups. However, trends can be observed in BDNF levels in brain regions where the Low AD subgroup seems to have had higher total BDNF levels than the High AD subgroup in the ENT and HIP. Similarly, trends can be observed in proBDNF and mature BDNF between groups; however, these relationships were not significantly different ($p>0.05$).

3.4 Discussion

To our knowledge, this is the first investigation of BDNF in serum, CSF, and brain tissue from the same AD and Control cases. We measured the levels of tBDNF, proBDNF, and mBDNF in *post mortem* CSF, serum, and frozen brain tissue regions and correlated these findings with staining densities of amyloid, pTau, BDNF receptors (TrkB and $p75^{NTR}$), and glia (astrocytes, activated microglia, oligodendrocytes) in the CA1 region of the HIP to investigate the relationship between peripheral BDNF levels, central BDNF levels and pathology. We found that 1) BDNF in CSF was detected at a lower ratio in the AD group than in the Control group, 2) BDNF in serum was inversely related to BDNF in HIP, and 3) HIP AD pathology was correlated with decreased TrkB staining, increased neuroinflammation, and decreased HIP proBDNF levels, as measured by western blots. Further exploratory data analysis showed that serum BDNF levels were correlated with the % area of staining of HIP CA1 microglia and pTau and suggested that a low pathology AD subgroup expressed lower levels of astrogliosis and higher BDNF levels than the high pathology AD subgroup, although further in-depth analysis is warranted in future studies.

We showed that tBDNF was not reliably detected in *post mortem* CSF samples, similar to studies using *ante mortem* lumbar CSF (Blasko et al. 2006, Laske et al. 2006, Laske et al. 2007, Jiao et al. 2016). However, tBDNF was above the detection limit in cranial CSF more often in Control cases than in AD cases, suggesting reduced tBDNF levels in AD samples. This is consistent with

previous reports of lower BDNF levels in *ante mortem* lumbar CSF from AD patients compared to age-matched Controls (Laske et al. 2007, Zhang et al. 2008, Li et al. 2009, Forlenza et al. 2015). In the samples where BDNF was above the detection limit, we found that CSF BDNF levels did not correlate with serum BDNF levels (tBDNF or proBDNF), confirming findings by Laske et al. (2007) who reported a similar finding in *ante mortem* lumbar CSF and serum. These findings are unique in that neither CSF-C nor CSF-L BDNF levels correlated with BDNF levels (tBDNF, proBDNF, or mBDNF) in BA46, the ENT, or the HIP. These results underscore the difficulty in measuring and interpreting the significance of BDNF levels in CSF, which has also been pointed out previously by others (Blasko et al. 2006, Laske et al. 2006, Laske et al. 2007, Jiao et al. 2016).

Previous investigations have reported lower proBDNF levels in AD compared to Control only in the parietal cortex and not in the frontal cortex (BA9) or hippocampus (Michalski and Fahnestock 2003, Peng et al. 2005, Michalski et al. 2015). In the current study, we investigated proBDNF levels in the dorsolateral prefrontal cortex (BA46), the ENT, and the HIP, and we found lower levels of proBDNF in BA46 and ENT but not in the HIP of the AD group compared to the Control group. Similar to Michalski and Fahnestock (2003), we found no differences in mBDNF levels between the AD and Control groups in any of the three brain regions investigated (BA46, ENT, HIP). We also found no difference between tBDNF levels measured by ELISA between the AD and Control groups in any of the three brain regions investigated (BA46, ENT, HIP). This

preservation of hippocampal BDNF levels in AD compared to Control has been reported before (Connor et al. 1997, Murer et al. 1999), and may be due to the highly interconnected nature of the hippocampus which may facilitate compensatory anterograde transport of BDNF from other brain regions to the hippocampus (Scharfman and Chao 2013). Indeed, in this study, we found that tBDNF levels in the ENT positively correlated with NeuN staining density and negatively with Amylo-Glo staining in the hippocampal CA1 area, suggesting that ENT input in to CA1 through the perforant pathway may provide trophic support and mitigate amyloidosis, as suggested in tissue culture and animal models of AD (Arancibia et al. 2008). We also found that although tBDNF, proBDNF, and mBDNF levels were not significantly different between the AD and Control groups in the HIP, proBDNF levels in the HIP were inversely related to HLA-DR staining. HLA-DR staining, in turn, was positively correlated with amyloid and pTau staining. HLA-DR is an major histocompatibility (MHC)-Class II marker for microglial activation and indiactes increased inflammatory processes in the brain, which have been shown to induce and increase AD neuropathology (Busse et al. 2015, Hendrickx et al. 2017). This result highlights the antagonistic relationship between BDNF and inflammation and the potential benefit of inc/reasing BDNF levels to reduce inflammation or to compensate for downregulated BDNF expression due to inflammation as reported in previous studies (Patterson 2015, Di Benedetto et al. 2017).

We further found that high-affinity BDNF TrkB receptor staining was downregulated in AD CA1 compared to staining observed in the control cases.

Previous studies have shown that TrkB receptors may be trapped in senile plaques, leading to reduced BDNF signaling in patients with AD (Connor et al. 1996) and our finding of a negative correlation between TrkB expression and amyloid and pTau expression further supports this finding. Overall, our finding of equivalent BDNF levels but decreased TrkB receptor expression in the HIP suggests that stimulation of TrkB expression should be considered along with BDNF supplementation therapy. A recent manuscript by Zhang et al. (2018) has shown that Tau phosphorylation negatively controls BDNF-TrkB receptor activation in AD mouse brains, demonstrating a strong connection between the development of AD pathology and down-regulation of BDNF expression or receptor activation. Targeting both BDNF and TrkB expression will be interesting for future pharmaceutical investigations and will complement current trials focused on AAV delivery of BDNF to aged non-human primates and humans with AD (Nagahara et al. 2018).

Moreover, $p75^{NTR}$ receptor staining densities in the hippocampus were not different between the AD and Control groups, which is consistent with the Ginsberg et al. (2006) study where they found a downregulation in TrkB but not $p75^{NTR}$ expression in cholinergic basal forebrain neurons in patients with AD. Although we find a preservation of $p75^{NTR}$ receptor expression in the AD hippocampus compared to controls, it has been proposed that the increase of $p75^{NTR}$ expression in relation to TrkB may reflect cell fate towards apoptosis and death (Meeker and Williams 2014). Amyloid has also been shown to interact with $p75^{NTR}$ receptors to induce neuronal damage (Perini et al. 2002, Zeng et al.

2011), and a positive correlation between $p75^{NTR}$ and amyloid (Chakravarthy et al. 2012) and pTau (Hu et al. 2002) staining in the hippocampus has been previously reported. Although we found a positive correlation between $p75^{NTR}$ staining densities in CA1 of the hippocampus and proBDNF levels in the hippocampus, this result was difficult to interpret because $p75^{NTR}$ is a promiscuous receptor with several different ligands and functions (Zeng et al. 2011), and because reports of $p75^{NTR}$ expression in AD brains compared to control has been mixed with some reports of increased expression in AD (Hu et al. 2002, Chakravarthy et al. 2012) or not different between AD and control (Ginsberg et al. 2006). There is thus a complicated relationship between the expression of this pan-neurotrophin receptor and the progression of AD neuropathology.

Two subgroup within our AD cases were identified by separating AD subjects by degree of GFAP staining. The Low AD subgroup, which is characterized by low GFAP immunoreactivity, also presented with lower amyloid and pTau staining than did the High AD subgroup despite having equivalent HLA-DR staining. AD pathology has been shown to upregulate GFAP and trigger reactive astrogliosis (Heneka et al. 2015, Hol and Pekny 2015, Osborn et al. 2016). Reactive astrocytes often accumulate around senile plaques (Medeiros and LaFerla 2013) and are thought to aid with neuroprotection and degradation of amyloid (Wyss-Coray et al. 2003). However, an interesting study in the APP/PS1 mouse model of AD showed that AAV suppression of astrogliosis led to improved cognition and decreased amyloid aggregation (Furman et al. 2012). In

the current study, the Low AD subgroup also had higher tBDNF levels in the ENT and HIP compared to both the High AD subgroup and the Control group, although this was not statistically significant. Our observation of low AD pathology and higher BDNF levels in the Low AD subgroup supports the notion of a compensatory increase in serum, brain, and CSF BDNF levels in MCI or early AD compared to controls (Durany et al. 2000, Laske et al. 2006, Konukoglu et al. 2012, Kim et al. 2017). Once a compensatory increase in BDNF fails to ameliorate the pathology, the patient is thought to be at a higher risk for cognitive and clinical decline, suggesting that failure to compensate with higher BDNF expression may lead to a conversion from MCI to AD.

Unique to this study is the measurements of BDNF levels in serum and brain tissue from the same human donors to investigate the correlation between serum BDNF levels and brain BDNF levels. A positive correlation between blood and brain BDNF levels has been consistently reported in rats, mice, and pigs (Karege et al. 2002, Sartorius et al. 2009, Klein et al. 2011, Bharani et al. 2017), but the relationship between blood and brain BDNF is unknown in humans to date. In a rat model of aging and cognitive impairment, we recently demonstrated a significant correlation between serum and brain tissue levels of BDNF and also found that lower BDNF levels in aged rats were associated with increased inflammatory markers, at least following an acute neurodegenerative lesion (Bharani et al. 2017). Both in our previous work and in the current study, we used commercially available ELISA kits that are frequently used in the assessment of serum BDNF in various clinical populations as well as semi-quantitative western

blotting techniques to measure tBDNF, proBDNF, and mBDNF in serum and in three brain regions from the same donor (BA46, ENT, HIP). We hypothesized that a positive correlation between blood and brain BDNF levels would validate the use of blood BDNF levels to infer the expression of BDNF in brain tissue. However, we found a negative correlation between serum proBDNF levels and hippocampal total BDNF levels as well as hippocampal proBDNF levels. This result parallels the findings reported by Elfving et al. (2010) where a negative correlation was found between serum and hippocampal BDNF in a genetic rat model of depression and may suggest that peripheral sources of BDNF could interfere with interpretations of BDNF levels in serum as a biomarker for neurological conditions affecting the brain.

One interpretation of a negative correlation between blood and brain BDNF is that increasing pathology disrupts the blood-brain barrier such that BDNF is trapped in one compartment. However, this is highly unlikely considering that blood-brain barrier integrity has been shown to decrease with increasing AD pathology (Yamazaki and Kanekiyo 2017, Zenaro et al. 2017). Alternatively, BDNF is produced in peripheral tissues (e.g., endothelial cells and smooth muscles) (Donovan et al. 1995, Fujimura et al. 2002, Michalski and Fahnestock 2003), and peripheral BDNF levels might be upregulated to compensate for pathology-associated downregulation of BDNF levels in the brain. Further interventional studies in animal models need to be undertaken to understand the role of peripheral BDNF levels and its relationship with brain BDNF levels and function in the context of pathologic aging.

When studying *post mortem* tissues, one has to consider many different factors that can influence the outcomes and interpretation. Clinical factors known to modulate BDNF levels such as *ante mortem* medication (Leyhe et al. 2008, Leyhe et al. 2009) and comorbidities (Bus et al. 2011, Bus et al. 2012) should also be carefully considered for any analysis. In addition, as a *post mortem* cross-sectional investigation, inherent limitations exist in regards to inferring causation and so longitudinal studies need to be undertaken to investigate whether BDNF dysfunction in AD is a primary pathological event. However, the strengths of our study include using conservative statistical testing to avoid false positives, measuring different forms of BDNF (tBDNF, mBDNF, and proBDNF) with both commercial ELISA kits and western blot analyses to increase reproducibility, and uniquely relating levels of peripheral BDNF (serum and CSF) to central BDNF in three different regions of the brain (BA46, ENT, HIP). Our results thus define the relationship between serum BDNF levels, brain BDNF levels and hippocampal pathology. Our results indicate that proBDNF may be significantly associated with both amyloid load and pTau-positive NFTs in the hippocampus, and that future therapeutic strategies for AD should consider targeting both BDNF and TrkB expression.

3.5 Acknowledgements

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CHAPTER 4.

A NORADRENERGIC LESION AGGRAVATES THE EFFECTS OF SYSTEMIC INFLAMMATION ON THE HIPPOCAMPUS OF AGED RATS

This chapter is published in PLOS One:

Bharani KL, Derex R, Granholm, AC, Ledreux A (2017). A noradrenergic lesion aggravates the effects of systemic inflammation on the hippocampus of aged rats. *PloS one*, 12(12), p.e0189821.

4.1 Introduction

Aging and aging-related disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD) are characterized by a subclinical chronic inflammatory status (Tansey and Goldberg 2010, Holmes 2013, Amor et al. 2014, Heneka et al. 2015) and by increased pro-inflammatory markers in the brain (Franceschi et al. 2000). Additionally, existing neuropathology can be exacerbated by systemic inflammation via a spread of pro-inflammatory cytokines across the blood-brain barrier (BBB) and subsequent activation of microglial cells in the brain (Perry et al. 2007, Perry 2010, Bettcher and Kramer 2014, Heneka et al. 2015). Systemic inflammation induced by the bacterial endotoxin lipopolysaccharide (LPS) is well-documented to cause neuroinflammation in animal models (Qin et al. 2007, Jeong et al. 2010) despite the fact that the endotoxin does not readily cross the BBB (Banks and Erickson 2010, Banks and Robinson 2010). Instead, circulating pro-inflammatory cytokines induced by systemic LPS cross the BBB, activate microglia, and directly inhibit crucial transcription factors in hippocampal neurons to induce neurodegeneration secondary to neuroinflammation (Laflamme and Rivest 1999, Perry et al. 2007, Perry 2010, Bettcher and Kramer 2014, Heneka et al. 2015, Patterson 2015). Several clinical and epidemiological studies suggest that pro-inflammatory cytokines can increase the susceptibility to cognitive impairment in older adults (Perry et al. 2007, Cunningham et al. 2009, Simen et al. 2011, Holmes 2013, Franceschi and Campisi 2014, Cunningham and Hennessy 2015). Accordingly, factors that further damage the BBB and increase its permeability, such as aging and noradrenergic degeneration (Wolburg and

Lippoldt 2002, Kalinin et al. 2006), can exacerbate the detrimental neuronal effect of LPS-induced systemic inflammation.

Noradrenergic degeneration has been shown to occur early in AD progression and is found in amnestic mild cognitive impairment patients (Kelly et al. 2017). Understanding effects of systemic inflammation in an aged model with locus coeruleus noradrenergic (LC-NE) dysfunction will be essential for developing therapies to mitigate cognitive decline in older adults, but a combined examination of LPS and LC-NE degeneration has not been undertaken to date.

LC-NE neurons have extensive innervations of cortical and subcortical brain regions and modulate cognitive functions including memory and attention (Uematsu et al. 2015). In particular, the LC-NE influences cognitive flexibility, working memory, and attentional processes (Lockrow et al. 2011). LC-NE neurons have also been shown to degenerate in normal aging, and, to a greater extent, in both AD and PD early in the disease process (Tomlinson et al. 1981, Feinstein et al. 2016, Vermeiren and De Deyn 2017). In addition to its role as a neurotransmitter, NE also has potent anti-inflammatory effects via adrenergic receptors on astrocytes and microglia to suppress the expression of pro-inflammatory cytokines and chemokines (Heneka et al. 2002, Heneka et al. 2010, Lockrow et al. 2011). Accordingly, LC-NE degeneration leads to the loss of the anti-inflammatory state in the brain (Marien et al. 2004, Grudzien et al. 2007, Lockrow et al. 2011), and elevated neuroinflammation due to LC-NE pathway degeneration has been linked to early neuronal dysfunction and aggravated AD pathophysiology including increased amyloid accumulation in AD mouse models

(Heneka et al. 2010). Because of adrenoreceptors located on endothelial cells in the brain, the degeneration of LC-NE innervation is implicated in the disruption of tight junction assembly (Kalinin et al. 2006) and increased BBB permeability (Wolburg and Lippoldt 2002), which is likely to make the central nervous system more susceptible to systemic insults. Although these findings individually implicate LC-NE pathway degeneration and systemic inflammation in pathologies of aging, the interaction between LC-NE degeneration and systemic inflammation in the aged brain remains to be explored.

Systemic inflammation induced by LPS may also affect neurotrophic support. For example, brain-derived neurotrophic factor (BDNF) is broadly important for regulating neuronal growth, differentiation, and survival and is necessary for neuronal plasticity along with counteracting amyloid toxicity in cell culture (Counts and Mufson 2010, Liu et al. 2015, Begni et al. 2017). Systemic inflammation was shown to reduce BDNF gene expression in certain areas of the rodent brain (Guan and Fang 2006), and pro-inflammatory cytokines interfere with BDNF's neuroprotective effects in rat brain tissue cultures (Tong et al. 2008, Tong et al. 2012). Inflammatory disruption of BDNF synthesis and function can lead to dysfunctions in rat hippocampal-dependent memory (Barrientos et al. 2004, Patterson 2015). LC-NE activity and BDNF seem to have an intricate relationship. Indeed, exogenous BDNF infusion into the frontal cortex protects against age-related LC-NE degeneration in rats (Nakai et al. 2006). Because LC-NE activity regulates the expression of BDNF (Willis et al. 2005, Mello-Carpes et al. 2016), we wanted to explore whether a combined systemic inflammation and

LC-NE degeneration would affect BDNF levels in serum or in brain tissue in aged rats.

The neurotoxin DSP4 (N-(2-chloro ethyl)-N-ehtyl-bromo-benzyl amine) readily crosses the BBB to cause selective degeneration of the rat LC-NE system by first inhibiting the noradrenaline transporter, depleting intracellular NE, and finally inducing degeneration of noradrenergic terminals. Although peripheral administration of DSP4 decreases NE levels in the peripheral sympathetic system, this effect is temporary and is resolved within one week (for review, see Ross and Stenfors (2015)). Our lab has previously shown that LC-NE degeneration induced by DSP4 significantly promotes neuroinflammation and behavioral deficits in the Ts65Dn mouse model of Down syndrome, but has no neuroinflammatory and minimal behavioral effects on normosomic mice (Lockrow et al. 2011). Similarly, DSP4 treatment on rats has had minimal to no effect on behavioral performance in the open field test (Srinivasan and Schmidt 2004), elevated plus maze (Lapiz et al. 2001), water maze (Abe et al. 1997), and Cogitat holeboard paradigm (Hauser et al. 2012). Thus, the overall purpose of the current study is to examine whether a DSP4-induced LC-NE lesion would potentiate neuroinflammation and behavioral impairment specifically in aged rats subjected to LPS-induced systemic inflammation. We hypothesized that aged rats with pronounced LC-NE degeneration would develop an exacerbated response to LPS-induced systemic inflammation in terms of inflammatory markers, glial activation, and neuronal deterioration and that this would be

related to a reduction in BDNF expression and reduced performance in a novel object recognition task.

4.2 Methods

4.2.1 Animals

Twenty-month-old male Fischer 344 (F344) rats (weighing 434 ± 34 g) from the aging colony of National Institute on Aging (NIA) at Charles River were pair-housed in an Association for Assessment and Accreditation of Laboratory Animal Care accredited animal care facility at the Medical University of South Carolina (MUSC). All animals were maintained on a 12-h light/dark cycle according to NIA guidelines for aged rats and received food and water *ad libitum*. All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at MUSC and complied with NIH guidelines.

4.2.2 Treatment

Treatment groups consisted of 16 double-saline-injected rats (Ctrl), 18 saline- and lipopolysaccharide- (LPS, from *Escherichia coli*, serotype O55:B5, Sigma-Aldrich) injected rats (LPS), and 19 DSP4- [N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine, Sigma-Aldrich] and LPS-injected rats (DSP4+LPS). Rats first received one dose of DSP4 (25 mg/kg, dissolved in sterile saline 0.9%, i.p.), known to be capable of crossing the BBB and toxic to norepinephrine (NE) neurons (Kalinin et al. 2006, Lockrow et al. 2011, Ross and Stenfors 2015) or saline (i.p.). Two weeks following the DSP4 or saline injection, rats were injected

with LPS (0.75 mg/kg, dissolved in sterile saline 0.9%) or saline (i.p.). After four hours, a subgroup of rats was sacrificed by an overdose of isoflurane (short-term subgroup). The remaining subgroup of rats underwent behavioral assessment seven days after LPS injection and was then sacrificed (long-term subgroup).

4.2.3 Novel object recognition task (NORT)

Seven days following the LPS injection, the long-term subgroup of rats completed a three-day novel object recognition task. On Day 1, rats were habituated to an empty circular testing arena (80 cm wide) for 5 minutes. On Day 2, rats were first exposed to two identical objects (A, A) for a 5-minute period (Trial 1). After a 90-minute break, rats were exposed to one familiar and one novel object (A, B) and rats were free to explore for another 5 minutes (Trial 2). On Day 3, one of the objects was replaced with a new object (A, C), and rats explored for 5 minutes (Trial 3). After a 90-minute break, the novel object (C) was moved 90^{o} clockwise, and rats were placed in the testing arena for another 5 minutes (Trial 4). Each trial was recorded and analyzed through an automated tracking system (Panlab SMART v3.0, Harvard Apparatus, USA).

4.2.4 Blood and brain collection

Rats were anesthetized deeply with isoflurane, and blood was collected by cardiac puncture into BD Vacutainer® SST tubes. Blood was allowed to clot for 1 hour at room temperature before being centrifuged for 20 minutes at 1,500 x g. Serum was aliquoted and stored in -80°C freezer until further analysis. The right

frontal cortex and right hippocampus were snap frozen on dry ice and stored at -80^{o}C until homogenization.

4.2.5 Preparation of brain homogenates

Brain homogenates were prepared in the Lab buffer as described in **Chapter 2 Section 2.3.2.**

4.2.6 Quantification of cytokines and chemokines

Quantification of serum levels of cytokines (IL-1β, IL-6, IL-10, IL-17A, TNFα, and IFNγ), serum chemokine (IP-10), and hippocampal IL-1β are described in **Chapter 2 Section 2.6**.

4.2.7 Quantification of BDNF

BDNF levels in serum samples and brain homogenates were measured in duplicates by ELISA using the human BDNF Quantikine kit (R&D Systems, Minneapolis, USA), as described in **Chapter 2 Section 2.4.**

4.2.8 Immunohistochemistry

Rats were anesthetized deeply with isoflurane gas (Novaplus) and the brain was rapidly removed and dissected. The left hemisphere was fixed in 4% paraformaldehyde for 48 hours and transferred to 30% sucrose in phosphate-buffered saline (PBS) at 4°C. Sections of hippocampus (40 µm) were sectioned using a cryostat (Microm) and processed for immunohistochemistry as previously

published (Granholm et al. 1992, Ledreux et al. 2016) using the following antibodies: TH (tyrosine hydroxylase, Abcam, 1:250), GFAP (Glial fibrillary acidic protein, Dako, 1:100) and Iba1 (ionized calcium-binding adapter molecule 1, Wako, 1:500). Briefly, free-floating sections were washed 4 times in TBS (Tris-buffered saline 0.01M, pH = 7.4) and then blocked for 1 hour at room temperature with 10% normal donkey serum in TBS-T (TBS with 0.2 % Triton-X 100). Sections were incubated with primary antibodies for 24 hours at 4°C, washed with TBS, and then incubated with secondary antibodies (Alexa 594 or Alexa 488, Life Technologies, 1:250) directed against the appropriate species for 1 hour at room temperature. Sections were washed with TBS, mounted on glass slides, air-dried, and cover-slipped with ProLong Gold antifade reagent (Molecular Probes). Photomicrographs were generated using a Nikon Eclipse 80i microscope (Nikon Instruments, Inc., Melville, NY) equipped with a QImaging Fast 1394 digital camera (QImaging, Surrey, Canada).

4.2.9 Densitometry

Semiquantitative densitometric measurements were performed in Fiji (version 1.51h, http://imagej.nih.gov/ij) using the region of interest (ROI) feature (Schindelin et al. 2012). Measurements were performed blind by one experimenter and are reported as an average of 3-4 sections per brain. Immunofluorescence staining density was obtained when background staining was subtracted from ROI staining intensities for each section. For TH and GFAP densitometry measurements, the ROI included the tip of the dentate gyrus of the

hippocampus, as shown in the insert at the top of Fig. 1. For Iba1 densitometry measurements, the ROI included a portion of the stratum radiatum, just below the stratum pyramidale in the CA1 of hippocampus.

4.2.10 Statistical analysis

Data were reported as a mean ± standard error of the mean (SEM). Grubb's method was used to check for outliers. Two-way ANOVA (treatment x time) were used to detect significant interactions between treatment and time after injection on relevant measurements. Tukey's *post hoc* multiple comparison tests were used to explore, within each exposure time, which groups were significantly different. Behavior results were analyzed using one-way ANOVA with Tukey's *post hoc* multiple comparison tests to detect changes between the three treatment groups. Pearson's correlations were used when assessing relations between two variables. Statistical significance was set at $p < 0.05$. GraphPad Prism version 6.0 (GraphPad Software, Inc., La Jolla, CA) was used for all statistical analyses.

4.3 Results

4.3.1 DSP4 treatment caused degeneration of NE fibers in the hippocampus

As expected based on previous work (Lockrow et al. 2011), DSP4 lesions gave rise to a loss of tyrosine hydroxylase (TH) immunoreactivity in the hippocampus (**Figure 4-1A**). A two-way ANOVA analysis confirmed a significant

effect of the treatment on TH immunoreactive fibers in the dentate gyrus ($F_{2,15}$ = 18.52, $p < 0.0001$). Subsequent Tukey's *post hoc* tests showed that DSP4+LPS-treated rats had significantly reduced TH immunoreactivity compared to the Control group (short-term: $p = 0.004$, long-term: $p = 0.005$) and LPS-treated rats (short-term: $p = 0.003$, long-term: $p = 0.014$; **Figure 41B**), thus demonstrating the loss of NE fibers resulting specifically from the DSP4 treatment with no effect observed from LPS alone.

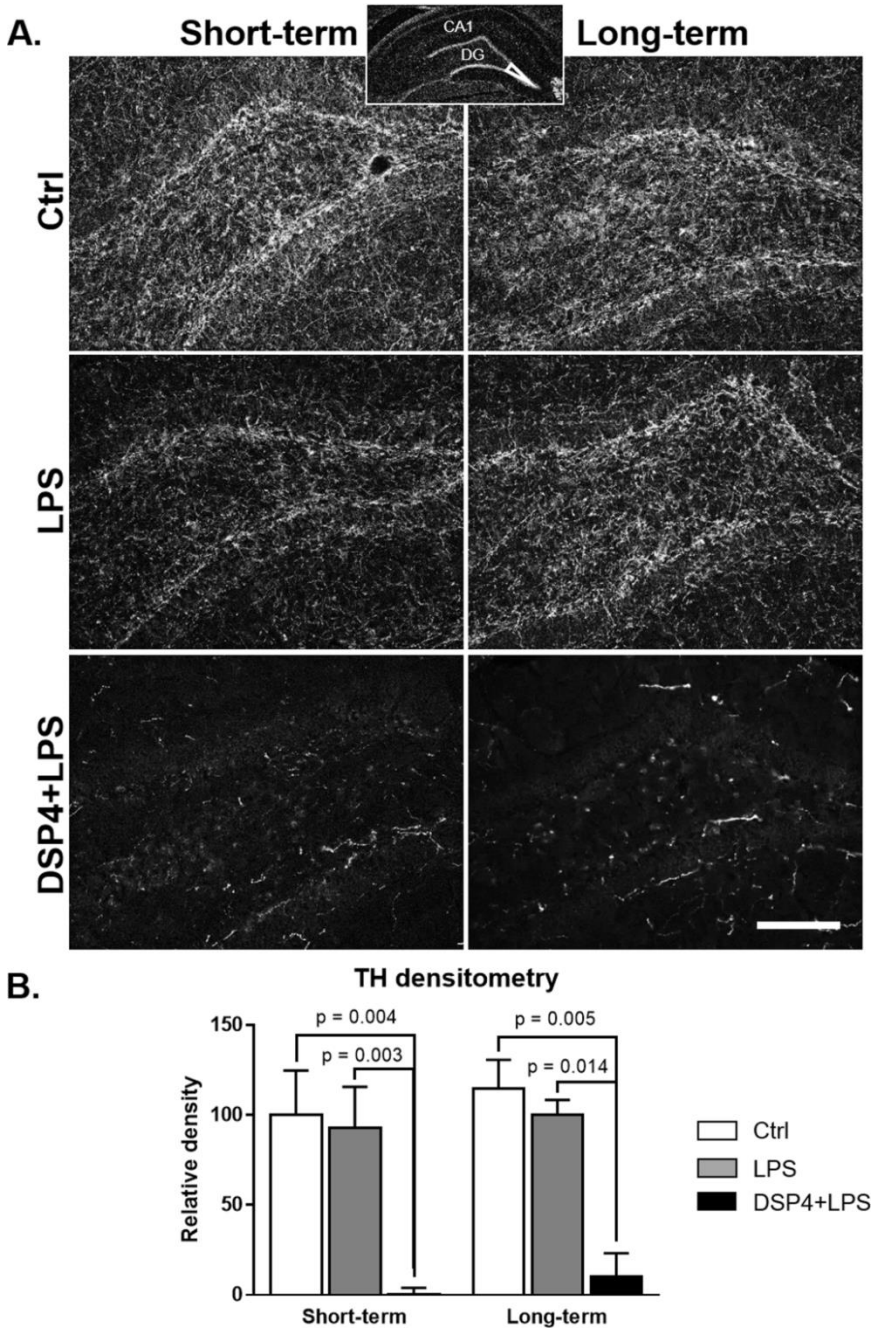

Figure 4-1. Hippocampal TH immunofluorescence. TH immunofluorescence staining shown in the hippocampal dentate gyrus (A) and densitometry (B; expressed as percent of the Ctrl group) in the short-term and long-term subgroups. The histograms demonstrate a significant reduction in TH-positive neurites in the DSP4-lesioned group, both short- and long-term, compared to the other two groups demonstrating a successful administration of the NE toxin DSP4. Densitometry confirmed observed changes, with highly significant reductions in TH density in both DSP4 groups and no changes observed in either Ctrl or LPS groups. Scale bar represents 250 μm.

4.3.2 Astroglial and microglial activity was elevated in the hippocampus after DSP4+LPS treatment

Effects of DSP4 and LPS treatments on astrocytic activation in the hippocampus were assessed through glial fibrillary acidic protein (GFAP) immunoreactivity (**Figure 4-2**). Both DSP4+LPS and LPS treatments gave rise to significantly increased staining with GFAP antibody in this brain region, with the most intense increase observed in the dentate gyrus of the DSP4+LPS-treated rats in the short-term subgroup (**Figure 4-2A**). A two-way ANOVA on GFAP densitometry measurements (expressed as percent of controls) showed significant effects of the treatment ($F_{2,33}$ = 8.92, p = 0.001) and time after LPS administration ($F_{1,33}$ = 11.36, p = 0.002) as well as a significant interaction between treatment and time ($F_{2,33}$ = 3.80, p = 0.033). Tukey's *post hoc* test revealed that in the short-term subgroup, the DSP4+LPS-treated rats had significantly higher GFAP immunoreactivity than the Control (p < 0.0001) and LPS (p = 0.016, **Figure 4-2B**) groups. GFAP immunoreactivity in the LPS group was also elevated compared to the Control group (p = 0.045; see Fig 2B). Seven days following the LPS treatment, no significant difference was observed in reactive astrogliosis in the dentate gyrus between the three groups, suggesting that the astrocytic activation following LPS and DSP4+LPS treatment had subsided at this point in the long-term group.

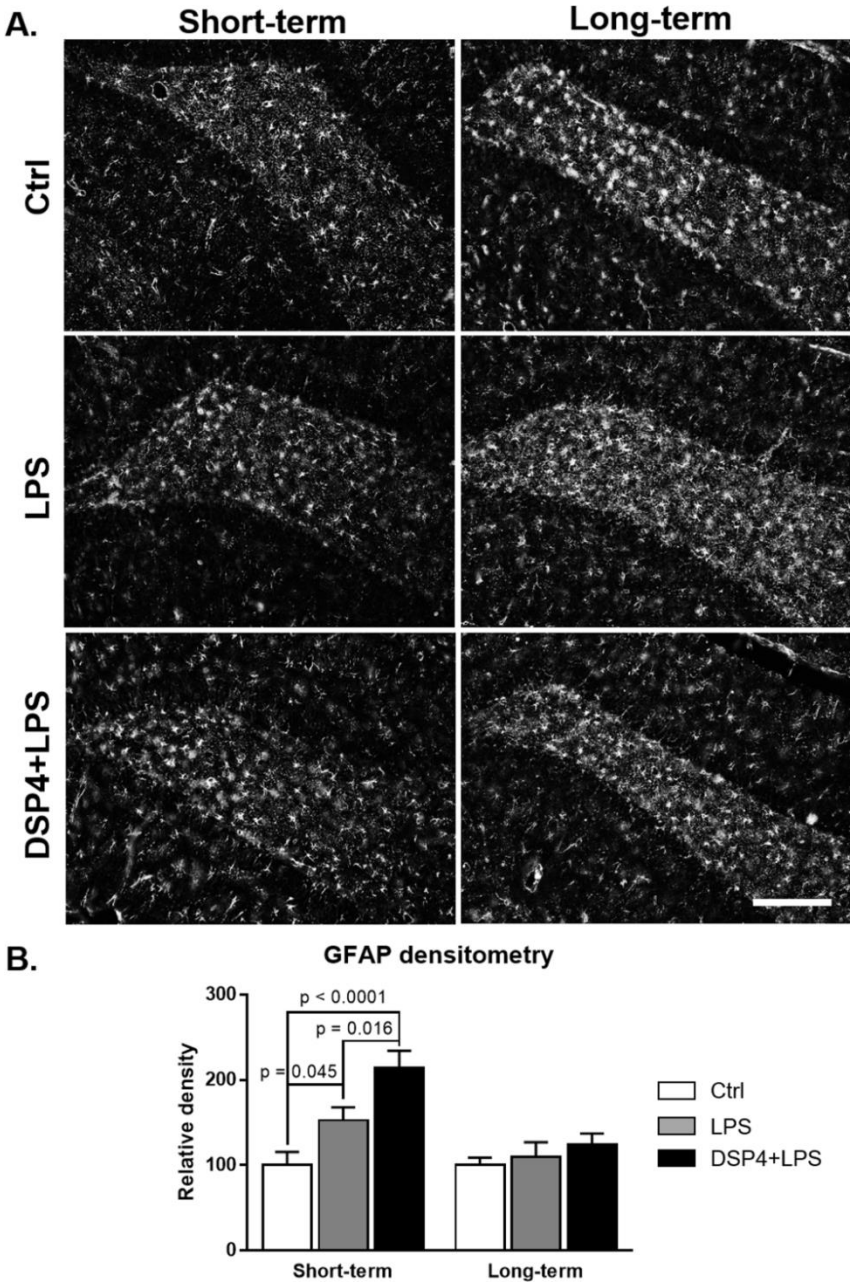

Figure 4-2. Hippocampal GFAP immunofluorescence. GFAP-immunofluorescent staining in the hippocampal dentate gyrus (A) and densitometry (B; expressed as percent of the Ctrl group) in the short-term and long-term subgroups. The histograms demonstrate a significant increase in GFAP-positive astroglia in the DSP4+LPS-treated rats in the short-term subgroup but not in the long-term subgroup. Densitometry confirmed observed changes with a highly significant increase in GFAP density in the DSP4+LPS group compared to Ctrl and LPS groups in the short-term subgroup. No significant differences were found between treatment groups in the long-term subgroup. Scale bar represents 250 µm.

Microglial activation was assessed with Iba1 immunoreactivity in the hippocampus (**Figure 4-3**). Both DSP4+LPS and LPS treatments resulted in increased Iba1 immunoreactivity in the CA1 of the hippocampus in the short-term group. A two-way ANOVA on Iba1 densitometry measurements (expressed as percent of controls) showed significant effects of the treatment ($F_{2,32} = 14.76$, $p < 0.0001$) and time after LPS administration ($F_{1,32} = 15.24$, $p = 0.0005$), with Tukey's *post hoc* tests revealing that the DSP4+LPS-treated rats had significantly higher Iba1 immunoreactivity compared to the Control ($p < 0.0001$) and LPS ($p = 0.012$, Fig 3B) groups. No significant difference was found in the long-term group.

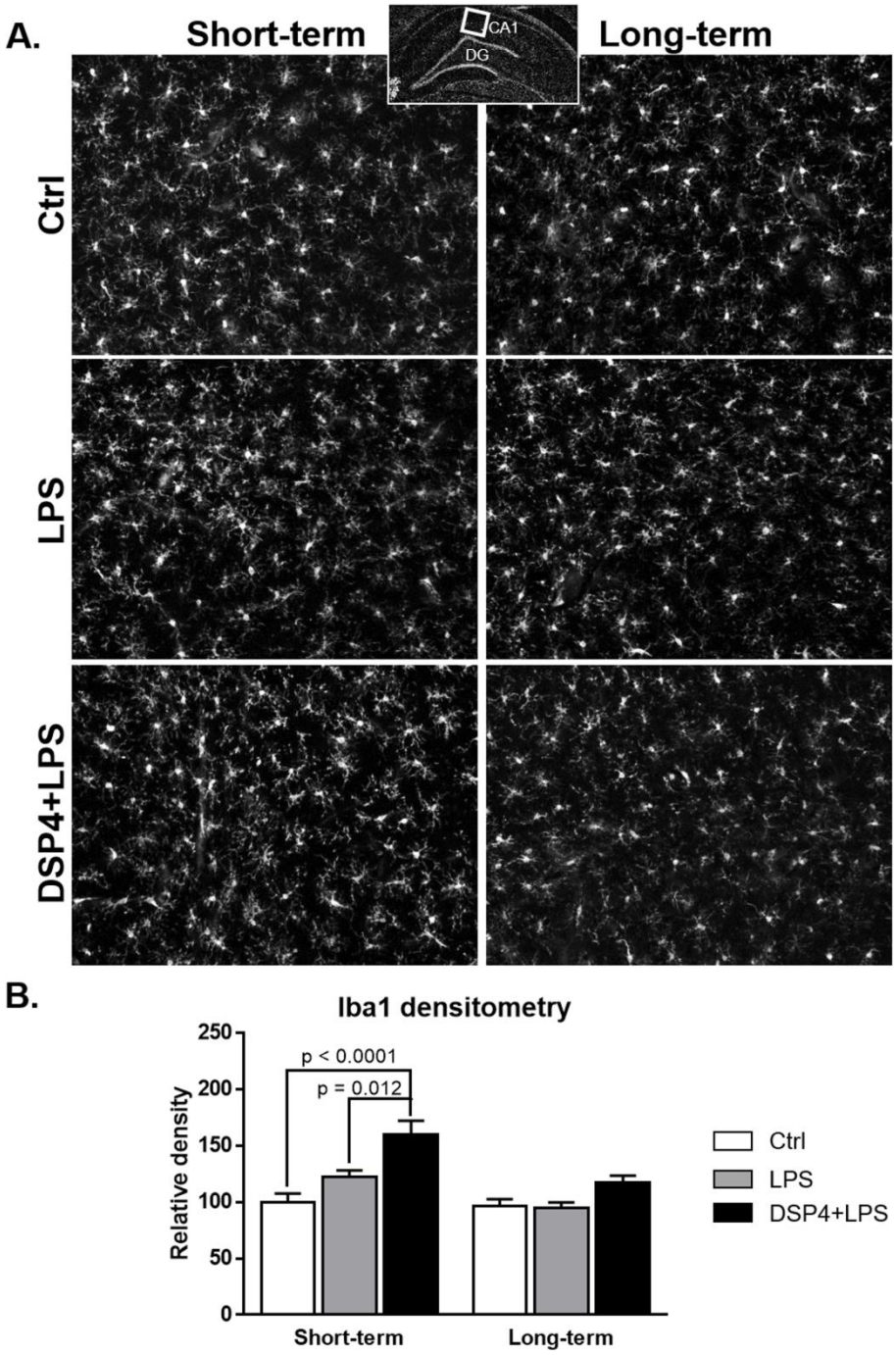

Figure 4-3. Hippocampal Iba1 immunofluorescence. Iba1-immunofluorescent staining in the CA1 (A) and densitometry (B; expressed as percent of the Ctrl group) in the short-term and long-term subgroups. Densitometry measurements demonstrate a significant increase in Iba1-microglia in the DSP4+LPS-treated rats compared to Ctrl and LPS groups in the short-term subgroup but not in the long-term subgroup. Scale bar represents 250 μm.

4.3.3 Serum cytokines and chemokines levels were increased in response to DSP4+LPS treatment

In order to determine whether treatment or time after the LPS injection affected peripheral inflammatory markers, two-way ANOVA followed by Tukey's *post hoc* multiple comparison tests were conducted on serum IL-1β, IL-6, IL-10, IL-17A, IFNγ, TNFα, and IP-10 levels. A significant statistical interaction was found between the effect of treatment and time after LPS injection, as well as a main effect of treatment and a main effect of time, as shown in **Table 4-1**.

Table 4-1. Two-way ANOVA results for serum cytokines and chemokine levels

Serum Cytokine/ Chemokine	Treatment	Time	Interaction
IL-1β	$F_{2,43} = 3.84$, $p = 0.029$	$F_{1,43} = 9.80$, $p = 0.003$	$F_{2,43} = 4.34$, $p = 0.019$
IL-6	$F_{2,46} = 3.94$, $p = 0.026$	$F_{1,46} = 6.23$, $p = 0.016$	$F_{2,46} = 3.93$, $p = 0.027$
IL-10	$F_{2,43} = 3.71$, $p = 0.033$	$F_{1,43} = 12.89$, $p = 0.001$	$F_{2,43} = 3.89$, $p = 0.028$
IL-17A	$F_{2,47} = 6.63$, $p = 0.003$	$F_{1,47} = 6.64$, $p = 0.013$	$F_{2,47} = 4.96$, $p = 0.011$
IFNγ	$F_{2,47} = 6.39$, $p = 0.004$	$F_{1,47} = 24.0$, $p < 0.0001$	$F_{2,47} = 7.54$, $p = 0.001$
TNFα	$F_{2,46} = 12.3$, $p < 0.0001$	$F_{1,46} = 43.7$, $p < 0.0001$	$F_{2,46} = 11.6$, $p < 0.0001$
IP-10	$F_{2,46} = 11.8$, $p < 0.0001$	$F_{1,46} = 29.8$, $p < 0.0001$	$F_{2,46} = 11.9$, $p < 0.0001$

In the short-term subgroup, Tukey's *post hoc* multiple comparisons tests showed consistently, significant differences between the Control and DSP4+LPS groups for all the aforementioned cytokines, revealing that NE lesions due to DSP4 treatment and peripheral inflammation caused by LPS resulted in elevated serum cytokines and chemokine levels when compared to the Control group (see **Figure 4-4** for p values). The NE lesion caused by the DSP4 toxin exacerbated

the immune response caused by the LPS challenge, which increased cytokine levels in the DSP4+LPS group compared to the LPS group. However, further statistical analysis (Tukey's *post hoc* test) revealed significant differences between these two groups only for IL-6 and IL-17A ($p = 0.011$ and $p = 0.002$, respectively) while a difference trending towards significance was found for IFNγ ($p = 0.067$), most likely because of the high variability observed within the 3 short-term treatment groups for this cytokine. Similarly, the LPS treatment resulted in elevated serum cytokines relative to the Control group, and Tukey's *post hoc* multiple comparison tests showed significant differences for the cytokines TNFα, IL-10, IFNγ ($p < 0.0001$, $p = 0.045$, and $p = 0.011$, respectively) and chemokine IP-10 ($p < 0.0001$; **see Figure 4-4**).

In the long-term subgroup, it is noteworthy that serum cytokine levels for LPS and DSP4+LPS groups were returned to levels similar to those of the Control group and were approximately one order magnitude lower when compared to the short-term subgroup (**Figure 4-4**). Overall, the absence of differences in serum cytokine levels between the 3 treatment groups 7 days after LPS treatment suggests that the cytokine and chemokine response to the LPS-induced systemic inflammation was resolved after 7 days.

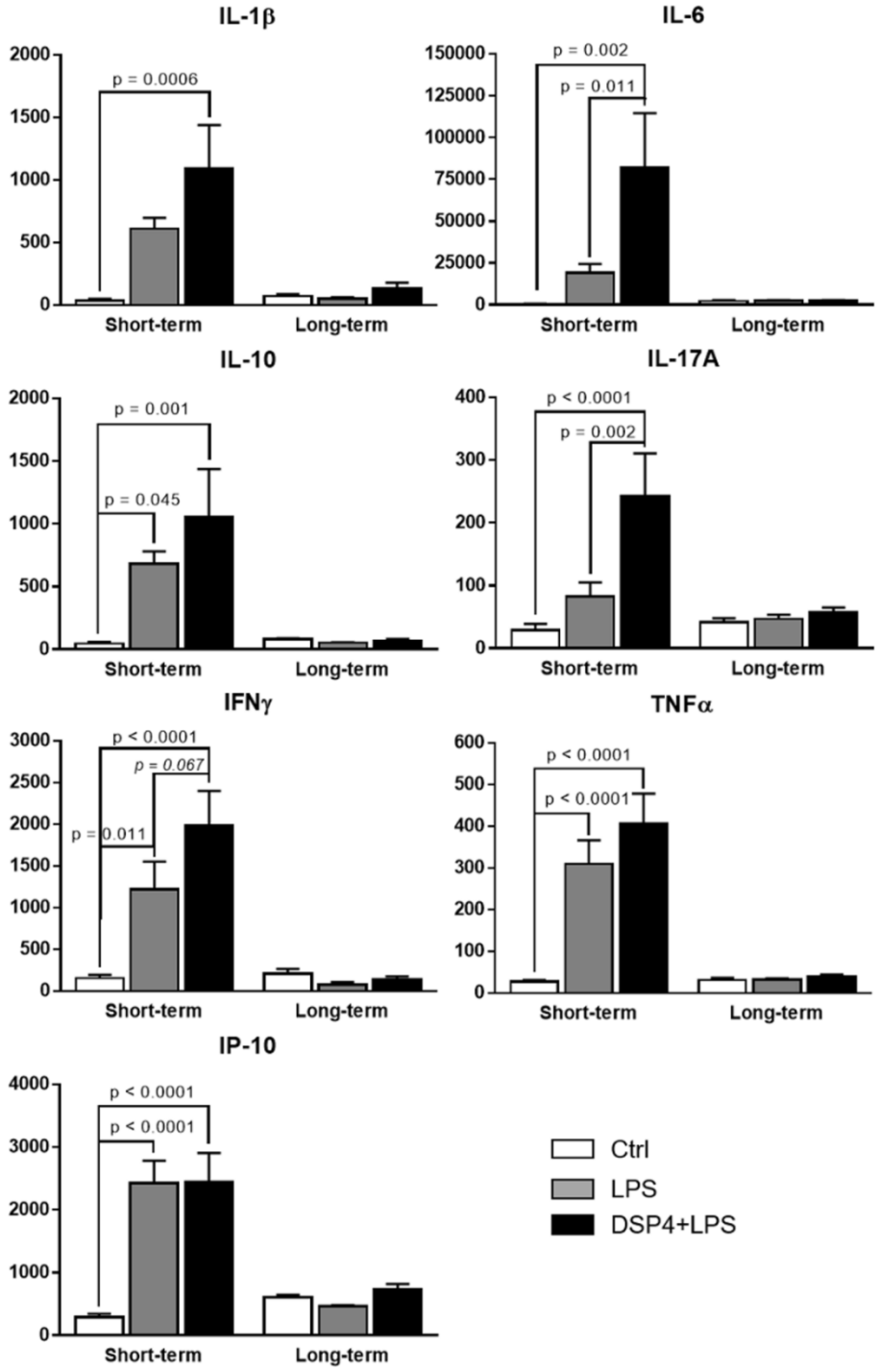

Figure 4-4. Serum cytokines/chemokine levels in the short-term and long-term subgroups for the Control, LPS-, and DSP4+LPS-treated rats. All levels are expressed in pg/mL ± SEM. In the short-term subgroup, serum cytokine levels including IL-1β, IL-6, IL-10, IL-17A, TNFα, IFNγ, and the chemokine IP-10 were significantly different between the three groups. Tukey's *post hoc* test

suggest that the NE lesion caused by the DSP4 toxin exacerbated the immune response caused by the LPS challenge in the short-term, with increased cytokine levels in the DSP4+LPS group compared to the LPS group. The absence of differences in serum cytokine levels between the 3 treatment groups in the long-term suggests that the acute cytokine response to the LPS-induced systemic inflammation was resolved after 7 days.

4.3.4 Hippocampal levels of IL-1β were transiently elevated after LPS challenge in DSP4-treated rats

Elevated levels of IL-1β in the hippocampus have been repeatedly shown to impair hippocampal-dependent memory by affecting the long-term potentiation (Hauss-Wegrzyniak et al. 1998, Murray and Lynch 1998, Pugh et al. 1998, Hauss-Wegrzyniak et al. 1999, Pugh et al. 1999, Vereker et al. 2000, Hauss-Wegrzyniak et al. 2002, Barrientos et al. 2004, Barrientos et al. 2009). In order to determine if the LPS and DSP4+LPS treatments resulted in exacerbated neuroinflammation, the levels of IL-1β were quantified by ELISA in the hippocampus of Control, LPS-treated, and DSP4+LPS-treated rats in the short-term and long-term subgroups. Our data showed that there was a significant interaction between treatment and time after LPS injection (two-way ANOVA: $F_{2,44} = 3.38$, $p = 0.043$), as wells as a significant main effect of time ($F_{1,44} = 10.30$, $p = 0.003$). In the short-term subgroup, Tukey's multiple comparison tests showed that the DSP4+LPS-treated rats exhibited higher hippocampal IL-1β levels relative to the Control group ($p = 0.019$; **Figure 4-5**). In the long-term subgroup, hippocampal IL-1β levels were not affected by the treatment and were similar to levels measured in the Control group (**Figure 4-5**).

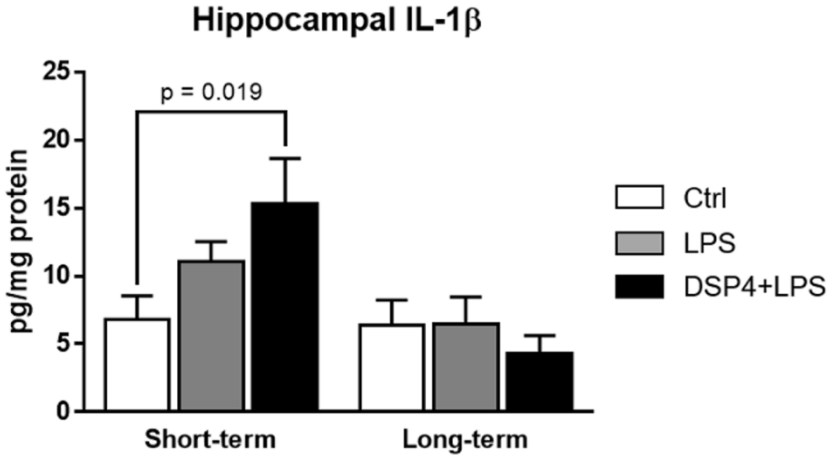

Figure 4-5. **IL-1β levels in the hippocampus as determined by ELISA in short-term and long-term subgroups**. Levels are expressed in pg/mg protein ± SEM. A significant increase in hippocampal IL-1β levels in DSP4+LPS-treated rats compared to the Control rats suggests an exacerbated neuroinflammatory response caused by the LPS challenge in the short-term subgroup, an effect that is not seen in the long-term subgroup.

4.3.5 Serum and hippocampal BDNF levels were modulated by LPS and DSP4+LPS treatments

In order to examine whether the NE lesion caused by the DSP4 toxin had further effects on BDNF levels compared to LPS effects alone, BDNF levels were assessed in serum, hippocampus, and frontal cortex by ELISA. Two-way ANOVAs were conducted to examine the effect of treatment and time after LPS injection on BDNF levels.

We found a significant interaction between the effect of treatment and time after LPS injection for serum BDNF levels ($F_{2,45}$ = 5.40, p = 0.008). In the short-term group, Tukey's *post hoc* test showed that serum BDNF levels were significantly lower in the DSP4+LPS group relative to the Control group (p = 0.012) and were decreased relative to the LPS-treated group although it did not reach significance (p = 0.069, **Figure 4-6A**). In the long-term group, serum BDNF

levels appeared increased in the DSP4+LPS group compared to the LPS, although not significantly (Tukey's *post hoc* test: p = 0.144; see **Figure 4-6A**).

A significant interaction between treatment and time after LPS injection was found in hippocampal BDNF levels (two-way ANOVA: $F_{2,45}$ = 4.395, p = 0.018, Fig 6B) but not in frontal cortex BDNF levels (data not shown). In the short-term group, hippocampal BDNF levels in the DSP4+LPS group were significantly lower relative to the Control group (Tukey's *post hoc* test: p = 0.032) and trended towards significance when compared to the LPS-treated group (p = 0.066, Fig 6B). A Pearson correlation revealed a statistically significant positive correlation between BDNF levels in the hippocampus and serum (r = 0.492, p = 0.015), possibly indicating that the elevated BDNF levels in serum observed in the short-term subgroup could reflect adaptive changes in the brain. Interestingly, in the short-term group, GFAP immunoreactivity in the dentate gyrus correlated negatively with the hippocampal BDNF levels (r = -0.762, p = 0.001) and positively with hippocampal IL-1β levels (r = 0.549, p = 0.042) and Iba1 immunoreactivity (r = 0.499, p = 0.058), suggesting that lower BDNF levels and higher IL-1β levels, as well as stronger microglia activation, were related to increased activation of astrocytes in the hippocampus. No significant treatment effect was observed in the long-term group (**Figure 4-6B**).

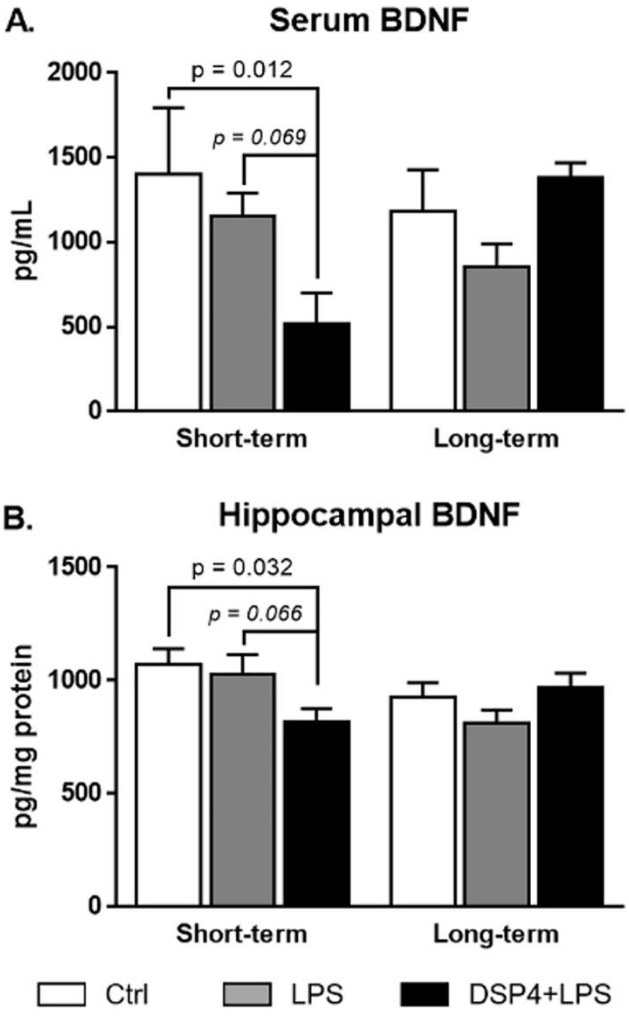

Figure 4-6. BDNF levels in serum (A) and hippocampus (B) as determined by ELISA after short-term or long-term exposure to LPS. Serum levels are expressed in pg/mL ± SEM and hippocampal levels are expressed in pg/mg protein ± SEM. BDNF levels in serum and hippocampus were significantly reduced in DSP4-lesioned animals after LPS challenge in the short-term subgroup compared to Ctrl.

4.3.6 Behavior

Reduced spontaneous locomotion after DSP4+LPS treatment

DSP4+LPS-treated rats exhibited a significant deficit in total distance traveled (**Figure 4-7A**). A one-way ANOVA showed significant effects of LPS and DSP4+LPS treatment ($F_{2,22}$ = 5.998, p = 0.008) on spontaneous locomotion. Tukey's *post hoc* analysis revealed that DSP4+LPS-treated rats performed significantly worse than LPS-treated rats (p = 0.009) and Control rats (p = 0.047),

suggesting that NE lesions gave rise to reduced spontaneous movement in the aged rats.

Novel object recognition task

The novel object recognition task (NORT) was administered 7 days after the LPS injection in DSP4-lesioned and non-lesioned rats. NORT is indicative of exploratory behavior as well as memory function (Antunes and Biala 2012). The discrimination index (DI) was calculated as the amount of time spent exploring the novel object relative to the familiar object, divided by the total amount of time exploring both objects (Lockrow et al. 2011, Antunes and Biala 2012). In order to get a more robust estimate of the discrimination index, we collapsed the data across the 3 testing phases. A one-way ANOVA showed an overall significant difference between the treatment groups ($F_{2,21}$ = 4.48, p = 0.024, **Figure 4-7B**). Tukey's *post hoc* comparison revealed that the DSP4+LPS-treated group performed worse on this task compared to the Control group (p = 0.049) and the LPS group (p = 0.033), suggesting that these rats were not able to discriminate between a familiar object and a novel object. We found that the discrimination index results were negatively correlated to Iba1 immunoreactivity (r = -0.520, p = 0.027) and to GFAP immunoreactivity (r = -0.625, p = 0.017), suggesting that rats with poorer performance had increased microglial and astroglial activation.

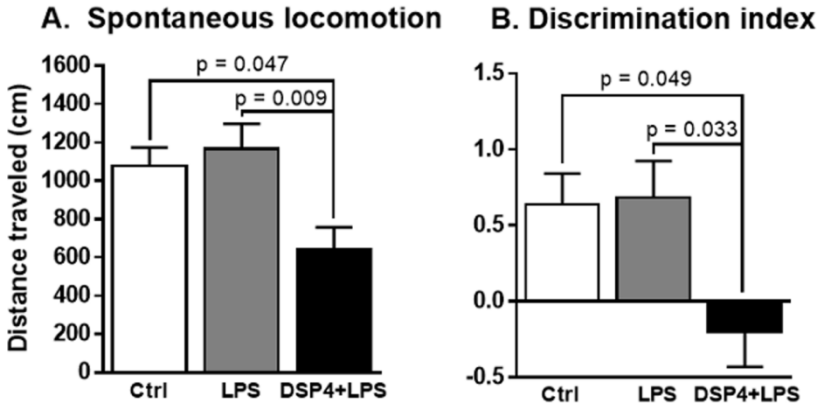

Figure 4-7. **Spontaneous locomotion and discrimination index.** The novel object recognition task (NORT) was administered 7 days after the LPS injection in control and DSP4-lesioned and non-lesioned rats. Spontaneous locomotion (A) indicates a reduction in movement in the DSP4-lesioned rats compared to both Ctrl and LPS groups. The discrimination index (B) was calculated as the amount of time spent exploring the novel object relative to the familiar object, divided by the total amount of time exploring both objects, and collapsed across all three testing phases. DSP4+LPS-treated rats performed worse on this task compared to the Ctrl group and the LPS group, suggesting that these rats were not able to discriminate between a familiar object and a novel object.

4.4 Discussion

Neuroinflammation induced by systemic inflammation has been implicated in the onset and progression of neurodegenerative diseases including AD, PD, and Down syndrome (Jeong et al. 2010, Tansey and Goldberg 2010, Holmes 2013, Trager and Tabrizi 2013, Heneka et al. 2015). Previous studies have shown that a peripheral immune challenge in animal models of neurodegeneration exacerbates pathology and cognitive deficits and implicates the immune system's disease modifying role (Qin et al. 2007, Cunningham et al. 2009, Schreuder et al. 2017). The results of the present study add to this literature and show that neuroinflammation induced by systemic inflammation is exacerbated by LC-NE lesion. Specifically, in aged rats, we found that a loss of

LC-NE neurons induced by the neurotoxin DSP4 combined with peripheral inflammation caused by LPS led to transiently increased astrogliosis and microglial activation in the hippocampus, increased accumulation of pro-inflammatory cytokines both in the hippocampus and in serum, and reduced exploratory behavior. BDNF levels were initially reduced in both serum and hippocampus following the LPS challenge in NE-lesioned rats. Similar to previous reports on LPS-treated rats, we did not find significant differences in hippocampal BDNF levels in the LPS group alone compared to the control (Shaw et al. 2001, Zhu et al. 2014). However, in the short-term subgroup, BDNF levels in the DSP4+LPS-treated rats were significantly reduced compared to Control and recovered to control levels after the long-term exposure. These results suggest that neuronal damage due to DSP4 in addition to LPS inflammation may disrupt BDNF synthesis. Thus, our results show that degeneration of the LC-NE pathway results in an exacerbated but transient systemic and neural response to a peripheral immune challenge including increased cytokine production, astrogliosis, microglial activation, and modulated BDNF levels.

We found that systemic LPS injection stimulates the release of pro-inflammatory cytokines IL-1β, IL-6, IL-17A, and TNFα in the peripheral circulation. These effects of systemic LPS administration in aged rats have also been reported by others (Nolan et al. 2005, Kohman et al. 2007, Donoso et al. 2008, Culley et al. 2014, Fu et al. 2014, Kawano et al. 2014, Sun et al. 2015). *In vitro*, LPS has also been shown to induce the anti-inflammatory cytokine IL-10 in a negative feedback manner to mitigate excessive inflammation (Iyer et al. 2010).

In our study, although an elevation of the aforementioned cytokines and chemokines was found in the short-term LPS-treated group relative to the Control group, this elevation was only statistically significant from control for TNFα, IL-10, IFNγ, and IP-10. However, we found a robust significant elevation of these cytokines and chemokine in the short-term DSP4+LPS group, suggesting that LC-NE degeneration by DSP4 potentiates the inflammatory response to LPS. This potentiation may be due to a transient neurotoxic effect of DSP4 (approximately seven days) on the peripheral sympathetic system in addition to the central nervous system (Bortel 2014, Ross et al. 2015). In the long-term group, cytokine and chemokine levels in LPS-treated and DSP4+LPS-treated rats were returned to control levels, suggesting a resolution of inflammation after seven days. While previous studies showed increased hippocampal IL-1β levels after LPS exposure in rats (Fu et al. 2014, Kawano et al. 2014), this was not the case under our experimental conditions, possibly due to differences in animal models or incubation period between LPS administration and sample collection. It is also noteworthy that the LPS dose used in our study (0.75 mg/kg) was lower than the ones used in the Fu et al. (2014) (2 mg/kg) and Kawano et al. (2014) (5 mg/kg) studies. However, we found that the NE lesion caused by the DSP4 toxin resulted in significantly elevated hippocampal IL-1β levels 4 hours after the LPS administration. Similar to previous reports showing that systemic inflammation by itself does not induce neurodegeneration (Jeong et al. 2010), our results show that a peripheral immune challenge in aged rats resolves within seven days and does not induce long-term cognitive deficits despite inducing neuroinflammation.

Our findings showed that the LPS group did not exhibit any statistically significant changes in either spontaneous locomotion or in exploratory behavior. However, we report lasting cognitive deficits in the DSP4+LPS group, potentially due to the reduction of NE levels in hippocampus caused by the DSP4 administration in the DSP4-treated rats (Srinivasan and Schmidt 2004). The lack of behavioral effects in the LPS group differs from Haba et al. (2012) who showed that LPS administration reduced novel object exploration in mice for at least 24 hours after injection, but this difference can be explained by our use of a different animal model and our extended time between LPS administration and cognitive testing. Indeed, Czerniawski et al. (2015) report that LPS administration does not impair novel object recognition in rats 6 hours after LPS administration, suggesting that there are still conflicting results in animal models regarding short- or long-term effects of LPS on behavioral measures.

LPS influence on neuroinflammation has been largely attributed to the upregulation of cytokines that cross the BBB and interact with CNS tissue (Banks and Erickson 2010). These LPS-induced pro-inflammatory cytokines can suppress the expression or release of BDNF (Guan and Fang 2006), which is important for the survival of LC-NE neurons. Our results indicate that LPS treatment combined with DSP4 in aged rats can further reduce BDNF levels in the hippocampus, at least initially. The reduction of hippocampal BDNF protein levels was negatively correlated with the extent of neuroinflammation induced by the LPS administration. Interestingly, after the resolution of inflammation (i.e., seven days post-LPS administration), both serum and hippocampal BDNF levels

in the DSP4+LPS-treated group were returned to levels similar to the control group. This reduction suggests that after the inflammatory state is resolved and pro-inflammatory cytokines are no longer suppressing BDNF, BDNF levels are upregulated both centrally and peripherally as a compensation to return function to normal. A similar compensatory increase in BDNF levels has been observed in other neuronal lesion models (Li et al. 2001), lending support to the notion of BDNF increase due to lesions in the brain. It is possible that the relative reduction in BDNF levels in serum acutely after DSP4+LPS treatment result from the systemic administration of the NE neurotoxin affecting the sympathetic nervous system and therefore cause degenerative changes in BDNF in serum. As shown by Ross and Stenfors (Ross et al. 2015), NE levels in the periphery were reduced as well after systemic DSP4 lesions but recovered fully within a week of injection, which could explain why peripheral cytokines and BDNF levels are affected by the NE neurotoxin. Similar to the pro-inflammatory effects of NE degeneration in the brain (Kalinin et al. 2006), the peripheral sympathetic system acts to suppress inflammatory reactions in the peripheral nervous system (Roggero et al. 2016). It is therefore not unlikely that the DSP4 lesions could have given rise to sympathetic changes in the short term which would have affected the expression of BDNF and initiated peripheral inflammation, aggravated by LPS. An interaction between the peripheral and central NE transmitter systems may be important especially with aging, since studies have shown not only age-related degeneration of the LC-NE nucleus but also reduced sympathetic nervous system innervation in target tissues in the periphery with

aging (Rengo et al. 2016). This interesting connection between aging effects in peripheral versus central NE afferents will be pursued in future studies. In conclusion, our results indicate that NE depletion increases susceptibility to neuroinflammation induced by systemic inflammation in aged rats. This result is relevant to the aging community where NE levels are decreased due to natural and pathology-associated degeneration of the LC-NE pathways (Mann 1983, Grudzien et al. 2007). An age-related reduced LC-NE function leading to an increased inflammatory reaction both in the periphery and in the brain could aggravate severe effects of immune challenges in the elderly (Bardou et al. 2014). Epidemiological studies have shown strong influence of peripheral inflammation on cognitive performance in elderly subjects (Duarte et al. 2017, Handing et al. 2017, Kesse-Guyot et al. 2017). For example, Kesse-Guyot et al. (2017) suggested that a pro-inflammatory diet at midlife might be associated with subsequent lower cognitive functioning. Our findings in aged rats support this previous suggestion and also point to a complicated relationship between inflammation and NE innervation that affects both peripheral and central systems. Our findings suggest future treatment options, such as using NE-enhancing drugs that may affect not only neuronal components of the brain but may also mitigate ongoing inflammatory processes both in the brain and peripherally.

4.5 Acknowledgement

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CHAPTER 5.

SERUM BDNF LEVELS AS A PREDICTOR FOR CHANGES IN NEUROPSYCHOLOGICAL PERFORMANCE AND NEUROIMAGING MEASURES IN HEALTHY OLDER ADULTS

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5.1 Introduction

Part of the challenge in AD research is identifying the mechanistic shift from healthy brain aging to neurodegeneration, which may occur decades before the development of symptoms (Dubois et al. 2007, Sperling et al. 2011, Jack et al. 2013). In this chapter, my goal is to investigate whether serum BDNF can predict subtle changes in neuropsychological performance and neuroimaging measures related to AD over time in healthy older adults. There are many possible confounders of the relationship between BDNF and our measures of interest that need to be accounted for when interpreting our results. Other than age and level of education which are commonly examined in interpreting neuropsychological performance, we paid special attention to inflammatory markers based on our results in **Chapters 3** and **4.** We also look into differences between women and men in our cohort based on a wealth of literature supporting sex differences in incidence of AD and in BDNF levels. The following is a review of literature specific for this chapter.

5.1.1 Sex differences in AD and BDNF

AD disproportionately affects women, but a definitive biological reason for this finding remains elusive (Carter et al. 2012, Hebert et al. 2013, Association 2018). One possible reason AD prevalence is higher in women than men is due to the fact that women live longer than men on average (Hebert et al. 2001, Chene et al. 2015). However, women with AD also have greater impairment across a range of cognitive domains than men, and this finding may be attributed

to hormonal differences (Mielke et al. 2014, Laws et al. 2016). Estrogen loss adversely affects cognition in both postpartum and postmenopausal women, and estrogen therapy improves cognitive performance in women with AD (Laws et al. 2016). Estrogen also has neuroprotective properties that can drive APP processing by α-secretase instead of β-secretase to reduce amyloid plaque production and prevent hyperphosphorylation of tau to reduce tangle formation (Wharton et al. 2009). In addition, several studies have shown that women with the ε4 allele of the *APOE* gene have an increased risk of progressing from MCI to AD and experience a more precipitous clinical and pathologic decline than men with the same allele (Mielke et al. 2014, Kim et al. 2015). Furthermore, a recent meta-analysis showed that the Val66Met genetic variation on the BDNF gene conferred increased susceptibility to AD in women but not men (Fukumoto et al. 2010). This dimorphic sexual effect is paralleled in blood BDNF protein levels which have been found to be higher in women than in men (Trajkovska et al. 2007, Bus et al. 2011, Shimada et al. 2014), and women with BDNF levels in the top quintile were less likely to develop dementia and AD (Weinstein et al. 2014). These reports all support the concept that women with AD may have a different disease course than men due to a) estrogen effects on AD pathology and b) differential production and function of BDNF. **Further research is needed into how serum BDNF levels differentially predict subtle AD-related changes between sexes in healthy older adults.**

5.1.2 Neurocognitive correlates of BDNF

A wide range of studies has shown that serum BDNF levels affect cognitive function in various populations (Gunstad et al. 2008, Erickson et al. 2010, Nettiksimmons et al. 2014, Shimada et al. 2014, Dols et al. 2015, Piepmeier and Etnier 2015, Buchman et al. 2016). BDNF is highly concentrated in the hippocampus and has been shown to be vital for memory processes (Tapia-Arancibia et al. 2008, Cunha et al. 2010). In older adults, lower BDNF levels have been linked to poorer memory and smaller hippocampal size (Erickson et al. 2010, von Bohlen und Halbach 2010, Dols et al. 2015). A carefully performed mediation analysis on cross-sectional data from older adults between 59 and 81 years old additionally revealed that age-related decline in BDNF levels partially contributes to volumetric shrinkage of the hippocampus associated with advancing age (Erickson et al. 2010). Evidence also indicates that associations between hippocampal volume, memory, and blood BDNF levels are different in men and women. Women with lower serum BDNF levels were associated with greater decline in memory (Weinstein et al. 2014). These results strongly suggest a link between BDNF, memory, and hippocampal volume, and, **in this study, we tested whether serum BDNF levels are correlated with changes in memory performance and hippocampal volume.** In addition, a recent study found that lower BDNF expression in *post mortem* human dorsolateral prefrontal cortex (DLPFC) has also been related to greater *ante mortem* cognitive decline in AD patients compared to controls (Buchman et al. 2016). The prefrontal cortex is associated with executive function (Yuan and Raz

2014), and, to parallel our investigation of BDNF levels in BA46 in **Chapter 3**, **we tested whether serum BDNF levels are correlated with changes in executive function performance and DLPFC thickness.**

5.1.3 Associations between BDNF and white matter

While volumetric shrinkage represents neuronal loss in the region of interest, subtle changes in the brain associated with astrocytic gliosis and myelin loss are better detected through diffusion analysis (Larsson et al. 2004, Hugenschmidt et al. 2008), and diffusion metrics are more sensitive in studying healthy populations where early signs of pathology are not easily detectable (Fellgiebel and Yakushev 2011). Although several studies (Montag et al. 2010, Chiang et al. 2011, Tost et al. 2013) have investigated the relationship between diffusion metrics of the brain and the Val66Met variant in the BDNF gene, which is associated with lower secretion of BDNF (Egan et al. 2003), there are a limited number of studies relating blood BDNF levels to diffusion metrics in older adults. In one study, investigators found that higher plasma BDNF levels reflected a higher degree of white matter lesions and lower white matter integrity (i.e., fractional anisotropy) in a manually defined prefrontal region (Dalby et al. 2013). In another study, it was found that serum BDNF levels negatively associated with mean diffusivity in the hippocampus (Manna et al. 2015). However, **the relationship between serum BDNF and the white matter microstructure of the limbic system, which highly interconnects the prefrontal cortex and the hippocampus, has not been reported and was investigated in this study.**

5.1.4 Diffusion tensor imaging

To study the white matter microstructure of the limbic system, we used a magnetic resonance imaging (MRI) modality called diffusion tensor imaging (DTI). DTI characterizes the microstructure properties of tissue by detecting the micro-scale displacement of water (Basser and Pierpaoli 1996). Fractional anisotropy (FA) and mean diffusivity (MD) are the most commonly reported DTI metric. FA describes the degree of directional variability of water diffusion in a voxel. FA ranges from a score of 0 to 1, where a score of 0 indicates unrestricted (or equally restricted) diffusion such that a molecule can diffuse equally in all directions (i.e., isotropic), and a score of 1 indicates restricted diffusion in a certain axis (i.e., anisotropic). Values closer to 1 are commonly found in white matter fiber bundles where diffusion in certain directions is restricted by myelin. MD is the average of the magnitude of diffusion in three orthogonal directions. MD is highest in CSF where there are few barriers to diffusion. In older adults, FA decreases and MD increases with increasing age, and these changes are thought to be due to myelin loss (Falangola et al. 2008).

5.1.5 Current investigation

In this study, we complement the aforementioned research by investigating the potential of baseline serum BDNF to predict changes in neuropsychological performance (executive function and memory) and neuroimaging measures (cortical thickness of the DLPFC, hippocampal volume,

and white matter microstructure of the limbic tracts) in a cohort of healthy older adults with particular attention to sex differences. This study is similar to the study done by Driscoll et al. (2012), which found that lower baseline plasma BDNF levels were associated with steeper frontal white matter volume decline in women but not men (n=59). In contrast to Driscoll's study, we used different neuroimaging regions of interest, and we used serum BDNF levels instead of plasma BDNF levels for reasons explained in **Chapter 1 Section 1.6.1**. We hypothesized that lower baseline serum BDNF levels were related to lower cognitive functioning, thinner DLPFC, smaller hippocampal volume, and abnormal white matter microstructure in limbic tracts as well as declines in these measures over time and that these effects will be more pronounced in women than men. As previously described in **Chapter 1** and supported in the results of **Chapters 3** and **4**, inflammation and BDNF are highly interconnected, and measures of inflammation along with age, education, and sex were accounted for when assessing the relationship between serum BDNF levels and neuropsychological performance or neuroimaging measures.

5.2 Methods

5.2.1 Participants

The MR Imaging of Neurodegeneration (MIND) study at the Medical University of South Carolina recruited cognitively healthy older adults (ages 60-85) to participate in annual neuropsychological tests, MRI scans, and a fasting blood draw. Neuropsychological testing was performed under the supervision of

a board-certified neuropsychologist. Brain MRI was obtained the same day as neuropsychology testing and was stored in an encrypted and de-identified database for subsequent analysis. No more than 50 ml of blood was extracted and stored from each participant by the South Carolina Translation Research (SCTR) Nexus. The Institutional Review Board at the Medical University of South Carolina approved this study (Pro00028302), and all participants gave written informed consent. A total of 65 participants were enrolled from October 2013 to April 2015, and 39 of these participants returned for a follow-up after 15.2 ± 3.2 months. This report is based on the baseline and follow-up data from these 39 subjects.

5.2.2 Quantification of BDNF

BDNF levels in serum samples were measured in duplicates by ELISA using the human BDNF Quantikine kit (R&D Systems, Minneapolis, USA), as described in **Chapter 2 Section 2.4.**

5.2.3 Quantification of cytokine and chemokine

Quantification of serum levels of cytokines IL-1β and IL-10 were determined using the 14-plex Human High Sensitivity T-Cell Discovery Array (HDHSTC14) by Eve Technologies (Canada) and a serum cytokine score for the first time point (referred to as basal inflammation) was calculated as described in **Chapter 2 Section 2.6**.

5.2.4 Neuropsychological assessment

A trained neuropsychologist or technician administered neuropsychological testing on the same day as MRI scanning. Raw scores from nine neuropsychological measures were each converted into standard z-scores based on the group mean and standard deviation at each time point. Average z-score composites were calculated for the following two neuropsychological domains: Executive Function (WMS-R Logical Memory – Digit Span Backward Total, Trail Making B, F-A-S Verbal Fluency) and Memory (WMS-R Logical Memory IIa, RAVLT – Long Delay Recall).

5.2.5 MRI acquisition

Scanning was performed on a 3T TIM Trio MR system (Siemens Medical Solutions, Erlangen, Germany) with the parameters previously reported (Fieremans et al. 2013). The following protocols were used 1) 3D T1-weighted imaging using an MPRAGE sequence with these parameters: TR/TI/TE=1900/900/2.26 ms, FOV=256x256 mm^2, a generalized auto-calibrating partially parallel acquisition (GRAPPA) factor of 2, 1 x 1 x 1 mm^3 voxels, scan duration 4 min, 26 s. 2) T2-FLAIR sequence with these parameters: TR/TI/TE = 9000/2500/79.0 ms, FOV=220x220 mm^2, a GRAPPA factor of 2, voxel size 0.9 x 0.9 x 3 mm^3, scan duration 4 min, 14 s. 3) DKI was acquired using single-shot, twice-refocused echo planar imaging, with these parameters: 3 b-values (0, 1000, 2000 s/mm^2) along 64 diffusion-encoding directions, one average, TR/TE = 8300/103 ms, FOV=220x220 mm^2, a GRAPPA factor of two, scan duration 18

min, 17 s. There were a total of 25 separate acquisitions with the b-value set to zero and identical image parameters (scan duration 3 min, 46 s).

5.2.6 Imaging processing

Cortical thickness and hippocampal volume values were estimated in both hemispheres using the Freesurfer package (surfer.nmr.mgh.harvard.edu). Bilateral hippocampal volumes were averaged together and normalized for intracranial volume (ICV) through proportion (i.e., hippocampal volume / ICV). DLPFC thickness was estimated as the average of gray matter thickness of regions labeled as middle frontal gyrus, middle frontal sulcus, superior frontal sulcus, inferior frontal sulcus, and superior frontal gyrus as previously described (Yamagishi et al. 2016).

Raw diffusion images were first visually inspected for image quality and then de-noised with a principal components analysis technique (Veraart et al. 2016). Gibbs ringing artifact reduction (Kellner et al. 2016) was then applied prior to additional processing utilizing in-house software (diffusional kurtosis estimator, DKE, (Tabesh et al. 2011) that was used for motion correction and registration and for estimating the diffusion and diffusional kurtosis tensors. The tensors were then used to derive the DKI-acquired parametric maps for diffusivity metrics (i.e., Fractional Anisotropy [FA] and Mean Diffusivity [MD]). Non-brain tissue was removed using the Brain Extraction Tool from FSL (FMRIB Software Library, Oxford, UK, http://www.fmrib.ox.ac.uk/fsl/). A fractional intensity threshold of 0.3 resulted in optimum non-brain tissue extraction, verified via visual inspection.

Region of interest (ROI) analysis was conducted on maps normalized via FSL's tract-based spatial statistics pipeline (TBSS). In TBSS, all subject FA images were non-linearly registered into standard FMRIB58_FA space, and a mean FA image was generated and thresholded to create the mean FA skeleton. All other parametric maps of each subject were then projected onto this FA skeleton with a threshold of FA=0.2. The TBSS-processed maps were then subjected to ROI analyses, extracting metric values using the Johns Hopkins ICBM-DTI-81 atlas (Mori, Wakana, Nagae-Poetscher, & van Zijl, 2005). A composite limbic tract variable was created by averaging the metric values of three tracts bilaterally: cres of the fornix, uncinate fasciculus, and cingulum [hippocampus]).

5.2.7 Statistical analysis

Statistical analysis was completed in IBM SPSS Statistics for Windows, version 24 (IBM Corp., Armonk, N.Y., USA). Serum BDNF measurements have been previously reported to have a non-normal distribution (Ziegenhorn et al. 2007, Balietti et al. 2018); however, visualization of BDNF levels in the data set used in this study indicated a reasonably normal distribution, and transformation to normalize the distribution of BDNF levels was not done to make interpretations of our results easier. Differences between sexes in demographics and baseline serum BDNF levels were tested using independent-samples t-tests. Differences between sexes and changes over time in neuropsychological performance and neuroimaging measures were tested using a mixed ANOVA with sex as a

between-subject factor (women and men) and time as a within-subject factor (baseline and follow-up). To assess whether baseline serum BDNF levels can predict changes over time in neuropsychological performance and neuroimaging measures, first, annualized change scores for our variables of interest were calculated by dividing the difference of the follow-up (FU) score, and baseline (BL) score by the follow-up interval measured in years (i.e., [FU – BL]/Interval). Then, multiple linear regressions were used to test whether BDNF at baseline interacts with sex to predict changes in neuropsychological performance or neuroimaging measures over time. Based on previous studies, BDNF levels are affected by age, education, and inflammation, and these terms were included in our model (Ziegenhorn et al. 2007, Bus et al. 2011, Bus et al. 2012, Calabrese et al. 2014). Six regression models were created with the change score in neuropsychological domains (executive function or memory) or neuroimaging measures (DLPFC thickness, hippocampal volume, limbic FA, and limbic MD) as the dependent variable (DV). Independent variables included age, education, sex, basal inflammation, baseline DV score, baseline BDNF, and an interaction term for BDNF and sex. A two-tailed p-value <0.05 was regarded as a significant result.

5.3 Results

5.3.1 Baseline serum BDNF levels were higher in women than in men

Baseline sample characteristics (Means ± SD) are reported in **Table 5-1**. Serum BDNF and cytokine values, neuropsychological performance scores, and

neuroimaging measures from 39 older adults (28 women) were used in this study. Results from independent-sample t-tests indicated that there were no statistical differences between women and men for age, education and basal inflammation. Baseline serum BDNF levels were statistically higher ($t_{33.2}$=2.075, p=0.045) in women (21.3 ± 8.3 ng/ml) than in men (17.1 ± 4.5 ng/ml). This finding of sex differences in baseline serum BDNF levels further supported our rationale to investigate differences between sex in our neuropsychological and neuroimaging measures of interest.

Table 5-1. Summary of baseline variables. Data represented as Mean (SD). Differences between women and men were calculated through independent-samples t-test, and p-values from this test are reported under the significance (Sig) column.

Characteristic	Total	Women	Men	Sig
Demographics				
Sample Size	39	28	11	
Age (years)	67.7 (5.2)	68 (5.6)	66.9 (4.2)	0.564
Education (years)	16.5 (2.5)	16.3 (2.5)	17.2 (2.2)	0.295
Basal Inflammation	0.00 (0.61)	0.05 (0.66)	-0.13 (0.48)	0.409
Serum BDNF (ng/ml)	20.1 (7.6)	21.3 (8.3)	17.1 (4.5)	0.046*

*p<0.05

5.3.2 Neuropsychological performance differed by sex over time

Mixed ANOVAs were used to investigate the sex differences in neuropsychological performance over time. The results of this mixed ANOVA on neuropsychological performance showed that there was a marginally significant interaction between sex and time on executive function performance ($F_{1,37}$=3.072, p=0.098, η_p^2=0.077) and a significant interaction between sex and time ($F_{1,37}$=6.166, p=0.018, η_p^2=0.143) on memory performance (**Figure 5-1**). For women, executive function performance increased and memory performance decreased between baseline and follow-up. In men, the opposite trend was found: men exhibited decreased executive function performance and increased memory performance between baseline and follow-up. However, it is important to note that these changes in composite z-scores were less than 1 SD and are unlikely to be clinically significant.

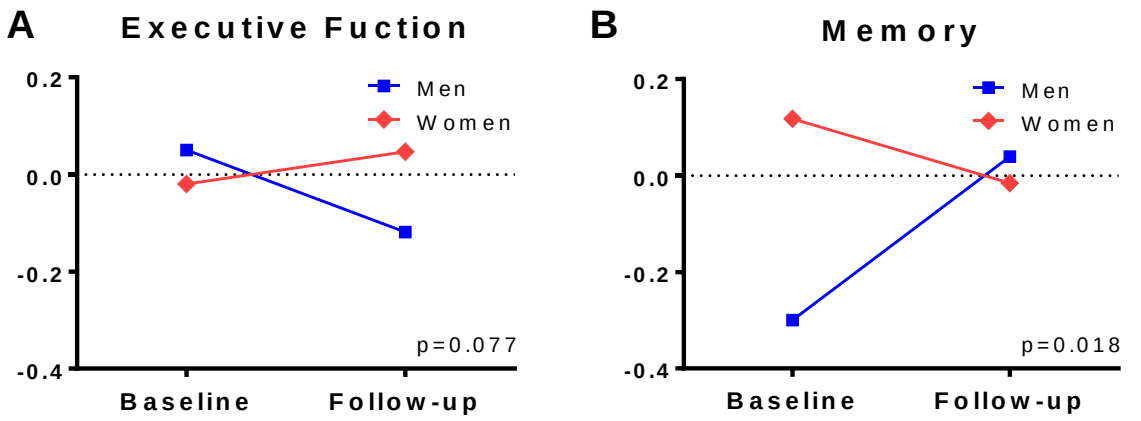

Figure 5-1. Interaction between sex and time in neuropsychological performance. Mean values of baseline and follow-up neuropsychological performance scores in women and men are graphed. Results from a mixed ANOVA indicated a marginally significant interaction between sex and time on executive function performance and a significant interaction between sex and time on memory performance. In executive function performance, composite z-scores for women increased while z-scores for men decreased between baseline and follow-up. In memory performance, z-scores for women decreased while scores for men z-increased between baseline and follow-up.

5.3.3 White matter microstructure decreased over time

Mixed ANOVAs were used to investigate the sex differences in neuroimaging measures over time. The results of the mixed ANOVA on neuroimaging measures showed that there was no significant interaction between sex and time on all four neuroimaging measures (all p>0.5). This result suggests that sex did not affect the change of these neuroimaging measures between baseline and follow-up. A significant main effect was found in limbic FA ($F_{1,37}$=7.500, p=0.009, η_p^2=0.169) and limbic MD ($F_{1,37}$=4.977, p=0.032, η_p^2=0.119), but not in DLPFC thickness ($F_{1,37}$=0.287, p=0.595, η_p^2=0.008) or hippocampal volume ($F_{1,37}$=0.125, p=0.725, η_p^2=0.003). Visualization of these results indicates that, for both women and men, limbic FA decreased and limbic

MD increased between baseline and follow-up (**Figure 5-2**), but DLPFC thickness and hippocampal volume did not significantly change between baseline and follow-up.

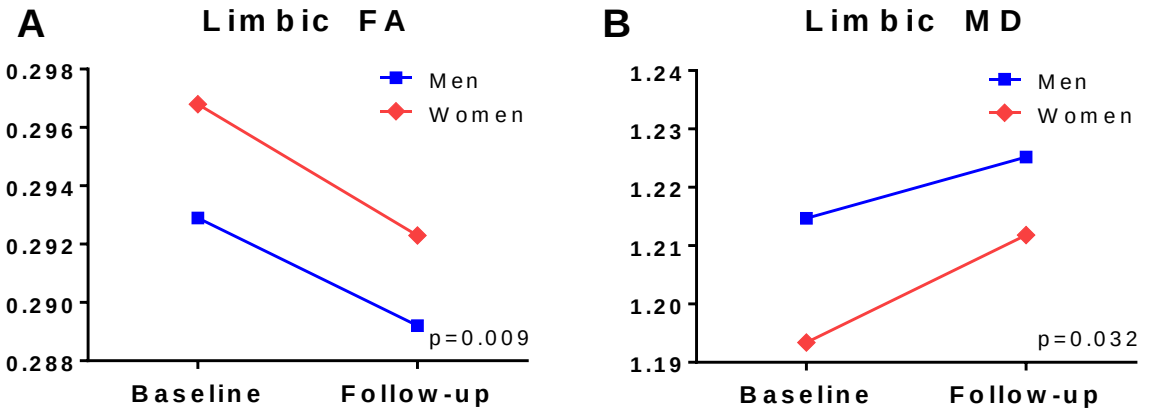

Figure 5-2. Changes in limbic white matter microstructure over time. Results from a mixed ANOVA indicated a significant main effect of time for limbic fractional anisotropy (FA) and limbic mean diffusivity (MD). For both women and men, limbic FA significantly decreased, and limbic MD significantly increased between baseline and follow-up.

5.3.4 Serum BDNF levels are marginally significant as predictors of changes in neuroimaging measures

Results from the multiple linear regression models are reported in **Table 5-2**. Overall, our models failed to reject the null hypothesis that none of the independent variables are related to change scores of neuropsychological domain or neuroimaging measures (all $p>0.05$). Our model for memory change scores was marginally significant ($F_{7,31}= 2.261$, $p=0.056$); however, only age was statistically related to the change in memory score, and our variables of interest, BDNF and sex*BDNF, were not significantly related to memory change scores. A negative β value for age in this model suggests that increasing age was associated with steeper declines in memory over time. The interaction between

baseline BDNF and sex were found to be marginally significant as a predictor for changes in hippocampal volume (p=0.085) and limbic FA (p=0.070). Visualization of the relationship of baseline BDNF levels and the change in hippocampal volume and limbic FA by sex suggests that higher serum BDNF levels are related to greater declines in hippocampal volume and limbic FA in men, but not women (**Figure 5-3**). However, it is important to note that these z-score changes were less than 1 SD and are unlikely to be clinically significant.

Table 5-2. Results from multiple linear regression models. Data represented as β (SE). BL = Baseline; DLPFC = Dorsolateral Prefrontal Cortex Thickness; DV = dependent variable; Educ = Education. EF = Executive Function; FA = Fractional Anisotropy; HIP = Hippocampus Volume; Inflam = Basal Inflammation; MD = Mean Diffusivity.

IV	EF	Memory	HIP	DLPFC	Limbic FA	Limbic MD
Constant	0.915 (1.097)	1.857 (1.424)	-0.015 (0.036)	0.191 (0.298)	-0.026 (0.034)	0.014 (0.119)
Age	-0.016 (0.013)	-0.039* (0.016)	0.000 (0.000)	-0.001 (0.002)	0.000 (0.000)	0.000 (0.001)
Educ	0.031 (0.022)	0.023 (0.036)	0.001 (0.001)	0.003 (0.003)	0.000 (0.000)	0.002 (0.002)
Sex	-0.226 (0.433)	0.281 (0.619)	0.016 (0.01)	-0.07 (0.069)	0.018† (0.01)	-0.056 (0.049)
Inflam	0.013 (0.093)	-0.077 (0.134)	0.000 (0.002)	0.001 (0.014)	0.002 (0.002)	-0.006 (0.009)
BL DV	-0.164† (0.088)	-0.107 (0.112)	-0.049 (0.077)	-0.032 (0.076)	0.023 (0.053)	-0.005 (0.09)
BL BDNF	0 (0.027)	0.002 (0.04)	0.001† (0.001)	-0.005 (0.004)	0.001 (0.001)	-0.003 (0.003)
Sex*BL BDNF	-0.001 (0.024)	-0.001 (0.035)	-0.001† (0.001)	0.004 (0.004)	-0.001† (0.001)	0.003 (0.003)
R^2	0.225	0.338	0.115	0.075	0.195	0.107
F	1.283	2.261	0.557	0.359	1.074	0.533

*p<0.05, †p<0.10

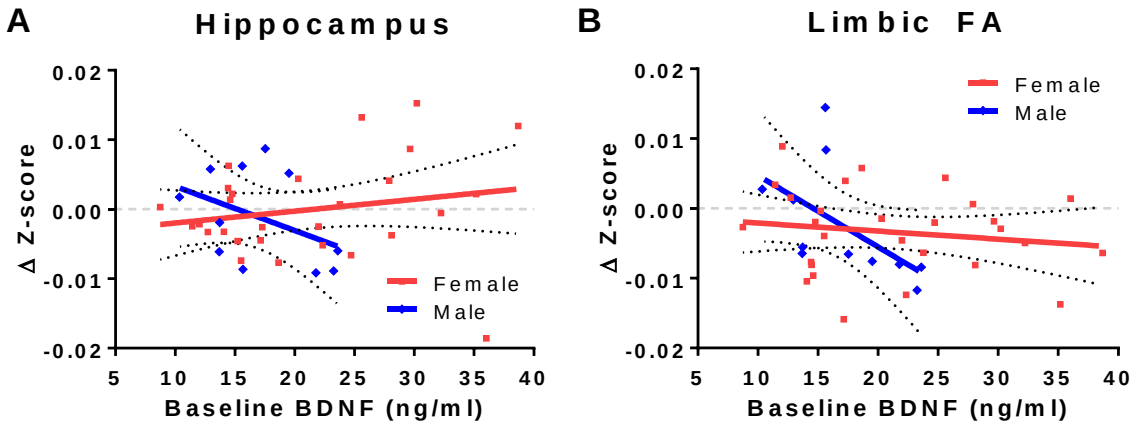

Figure 5-3. Relationship between baseline BDNF levels and changes in neuroimaging measures over time by sex. Although the overall linear regression model was not significant, the interaction between baseline BDNF and sex was found to be marginally significant as a predictor for changes in hippocampal volume (p=0.085) and limbic FA (p=0.070). Visualization of the relationship between baseline BDNF and changes in hippocampal volume (A) and limbic FA (B) suggest that higher baseline BDNF levels are associated with greater declines in these neuroimaging measures in men but not women.

5.4 Discussion

In our healthy older adult cohort, we found that baseline serum BDNF levels were higher in women compared to men, that executive function scores declined in men over time, and that memory scores declined in women over time. In addition, we found marginally significant relationships between baseline serum BDNF levels and changes in hippocampal volume and limbic FA indicating that higher serum BDNF levels in men were associated with greater declines in hippocampal volume and limbic FA. Our results are contrary to Driscoll et al. (2012) where higher BDNF levels in plasma were associated with slower changes in white matter volume in women but not in men. Nonetheless, our results suggest that sex differences in the relationship between BDNF neuropsychological performance, and neuroimaging measures should be

investigated further. Consideration of sex differences in BDNF and AD research is especially important considering that older adult women with mild cognitive impairment decline faster than men (Lin et al. 2015), and interventions to increase BDNF levels, such as exercise, may have a lower effect in women than in men (Szuhany et al. 2015).

We examined whether serum BDNF levels correlated with three neuroimaging measures sensitive to aging and dementia: cortical thickness of the DLPFC, hippocampal volume, and white matter microstructure of the limbic tracts. Based on previous literature, we hypothesized that lower serum BDNF levels would indicate to smaller hippocampal volumes, thinner DLPFC, and abnormal white matter microstructure in the limbic tracts and that this association would be greater in women than men. We did not find a significant relationship between serum BDNF levels and changes in these neuroimaging measures changes over time. Our results instead indicated that higher serum BDNF levels in men are associated with abnormal changes in neuroimaging measures (i.e., greater declines in hippocampal volume and limbic FA) which is the opposite relationship we hypothesized. On the one hand, this result may be interpreted as these men having a compensatory increase of serum BDNF to help ameliorate some underlying pathology that is affecting the volume of their hippocampus. Further follow-up on these men as they possibly convert to MCI could support this theory. In addition, this relationship between higher blood BDNF levels and abnormal neuroimaging findings is mirrored by Dalby et al. (2013) findings of higher plasma BDNF levels reflecting higher prefrontal white matter lesions and

lower white matter integrity (FA). On the other hand, there are important limitations to this study, including a small sample size, which make our result difficult to interpret.

Major strengths of our study include the use of neuropsychological domains composed of scores from several neuropsychological tests, the use of multiple neuroimaging techniques to assess subtle changes in the brain, and the use of a longitudinal design to assess prospective change. Although we did not find a significant change in DLPFC thickness and hippocampal volume between timepoints, it is remarkable that we found changes in white matter microstructure in our cohort despite the short time interval. This suggests that changes in white matter microstructure may be more sensitive than measures of DLPFC thickness and hippocampal volume to aging. Our finding of a marginal relationship between baseline serum BDNF levels and limbic FA support the rationale to further investigate serum BDNF levels and white matter structure with more advanced techniques.

In addition to its major strengths, this study has some limitations. Participants were volunteers recruited by the University and may not be representative for the general older adult population. Our study also has a small, unbalanced sample size with more women than men which may have biased our results. The changes between baseline and follow-up visits we noted in neuropsychological performance and neuroimaging measures over time were subtle and did not have clinical significance or affect activities of daily lviing in these healthy older adults. In addition, serum BDNF levels assessed from an

ELISA can be difficult to interpret since they represent a mixture of mature and proBDNF (Michalski and Fahnestock 2003, Yoshida 2012, Lim et al. 2015, Polacchini et al. 2015). Although both forms of BDNF are decreased in AD (Michalski and Fahnestock 2003, Peng et al. 2005), they act on different receptors (mature BDNF on TrkB and proBDNF on p75) to drive neuronal survival vs death (Teng et al. 2005, Tapia-Arancibia et al. 2008), and we could not assess the contribution of either form to our measure of total BDNF. In addition, BDNF is produced in peripheral tissue (e.g., endothelial cells and smooth muscles), and BDNF measured in serum reflect BDNF from neuronal and non-neuronal sources (Donovan et al. 1995, Fujimura et al. 2002, Michalski and Fahnestock 2003). Nevertheless, the association between serum BDNF levels and brain BDNF levels is supported by reports demonstrating changes in BDNF levels in blood from people with various psychiatric and neurological disorders (Carlino et al. 2011, Yoshida 2012, Fernandes et al. 2015) and by a human study where BDNF levels were found to be higher in blood from the internal jugular vein compared to blood from the brachial arterial, suggesting that BDNF levels in venous blood are supplemented by BDNF produced in the brain (Dawood et al. 2007). Finally, our multiple linear regression models were found to not significantly predict our dependent variables, suggesting optimization of our predictor variables is needed to assess better the relationship between serum BDNF levels and our variable of interest.

Overall our results suggest that a relationship between serum BDNF levels and both neuropsychological performance and neuroimaging measures

are limited in healthy older adults. This result somewhat differs with the literature where lower blood BDNF levels are related to smaller hippocampi, cognitive decline, and associations with AD status (Connor et al. 1997, Laske et al. 2006, Yasutake et al. 2006, Erickson et al. 2010, Nettiksimmons et al. 2014). Our result may differ from the literature due to our use of healthy older adults, and further follow-up on these older adults as they age may further differentiate serum BDNF levels and neuropsychological performance or neuroimaging measures more apparent between those with MCI and healthy controls. In this study, we found interesting differences between men and women in serum BDNF levels, in changes of neuropsychological scores over time, and in the relationship between serum BDNF levels and changes in neuroimaging measures over time. These results serve to support rationale to investigate sex differences in future studies relating BDNF to longitudinal changes in older adults. Understanding the differential role of BDNF between women and men can also inform future interventional studies that modulate BDNF through diet, exercise, or pharmaceutical intervention.

CHAPTER 6.

GENERAL DISCUSSION

6.1 Overview of results

The primary goal of my studies was to investigate the biomarker potential of BDNF in the context of AD. Because of the many different, sometimes conflicting results in the literature regarding BDNF levels in serum and their relationship to AD pathology, there was a need to clarify this issue in order to move the field forward. This is especially important in light of a recent clinical Phase I trial focused on replacing BDNF levels in the brain of patients with AD using viral vectors (Tuszynski 2017, Tuszynski et al. 2017). I hypothesized that BDNF fluctuates with disease course with high levels of BDNF in early stages of the disease course and low levels of BDNF in late stages of disease course. In order to examine this hypothesis, I utilized three different systems: A) *post mortem* brain tissue and blood, B) an aged rat model with and without LC degeneration, and C) blood, neuropsychological scores, and neuroimaging measures from healthy older adults. Each of these models contributed significantly to the overall goals of my thesis.

In **Chapter 3**, I describe the results of the first aim of my dissertation where I investigated whether serum BDNF levels were correlated with brain BDNF levels and AD pathology using *post mortem* human samples. I found that serum proBDNF levels were inversely related to hippocampal levels of total BDNF (tBDNF) and proBDNF. I also identified subgroups of AD cases with high pathology (High AD) and low pathology (Low AD). The Low AD subgroup had lower amyloid and tau pathology than the High AD subgroup, as well as less GFAP immunostaining. In addition, the Low AD subgroup had higher tBDNF

levels compared to the control and High AD subgroups, indicating a compensatory increase in BDNF to ameliorate neuropathology. I also discovered that the high-affinity BDNF TrkB receptor was negatively correlated with both amyloid and pTau staining in *post mortem* sections of the hippocampus. This result underscores the disruption of the neuroprotective effects of BDNF by AD pathology.

In **Chapter 4**, I sought to investigate whether BDNF levels were altered, either in serum or brain tissue, with an inflammatory challenge in aged rats with and without a selective degeneration of the LC-NE neurons. The rationale for investigating the combination of LPS and LC-NE degeneration was that LC-NE neurons degenerate early in the AD disease process and aged rats with LC-NE degeneration may model older adults at the beginning stages of MCI and AD {Ross, 2015 #550}. I found that an LPS challenge in aged rats resulted in increased peripheral and hippocampal inflammation and decreased serum and hippocampal BDNF levels after 4 hours. These acute effects were more severe in rats with an LC-NE lesion, presumably due to the loss of anti-inflammatory protection of NE. Protective effects of NE innervation against glial activation has been shown by others in both AD mouse models and aged rats and mice by us and others (Kalinin et al. 2006, Kalinin et al. 2007, Fortress et al. 2015). Seven days after the LPS challenge, inflammation in serum and the hippocampus had been resolved, but aged rats with an LC-NE lesion had increased serum BDNF levels when compared with aged rats without an LC-NE lesion, suggesting a

protective compensatory increase in peripheral BDNF in the LC-NE lesioned group.

These two aims established that serum BDNF levels can reflect pathology due to chronic AD neurodegeneration (**Chapter 3**) and can be sensitive to acute challenges in aged rats (**Chapter 4**). These results indicate that serum BDNF levels can reflect AD pathology and can be modified by acute neuropathological insults, features that support the rationale of using serum BDNF levels as a biomarker. My next goal was to test how sensitive serum BDNF levels are to subtle changes in older adult neuropsychological performance and neuroimaging measures.

In **Chapter 5**, I investigated whether serum BDNF levels could predict changes in neuropsychological performance and neuroimaging measures in cognitively normal older adults who completed two visits approximately 15 months apart. In this cohort, serum BDNF levels were higher in women than men, memory scores declined in men over time, and executive function declined in women over time. Although we did not find significant declines in DLPFC thickness and hippocampal volume over time in our cohort, it was remarkable that we found significant changes in white matter microstructure of the limbic system even during this short 15-month interval. To address our main goal of assessing the ability of serum BDNF levels to predict changes in neuropsychological performance and neuroimaging measures, linear regression models were used which controls for possible confounders: age, education, and serum cytokine score. Although our models were not found to be statistically

significant overall, we found marginally significant interactions between BDNF and sex in predicting changes in hippocampal volume and limbic FA. This result suggested that higher baseline BDNF levels were associated with greater declines in hippocampal volume and limbic FA in men, but not in women. Results from this aim overall support the rationale of further investigating how serum BDNF levels reflect changes in white matter microstructure differently in women and men using advance MRI modalities and longer time course in future studies. In summary, my dissertation work demonstrates that serum BDNF levels are related to the severity of AD pathology, inversely fluctuate with inflammation in an aged rat model with LC-NE neurodegeneration, and have potential to predict hippocampal and limbic white matter changes in cognitively normal older adults.

6.2 Measuring BDNF in Fluids

Several different peripheral fluid sources were considered for biomarker analysis in this dissertation. Measuring BDNF in saliva samples was an attractive objective considering the ease of obtaining saliva compared to obtaining blood or CSF. However, Mandel et al. (2009) found that not every sample collected contained both pro- and mature forms of BDNF, and BDNF in saliva was considered to be too unreliable. In **Chapter 3**, we showed for the first time that BDNF levels in *post mortem* CSF samples were not reliably detectable using a commercially available ELISA from R&D Systems. Although we were able to detect BDNF levels in the CSF more often in control samples compared to AD samples, our findings highlight the difficulty of measuring and interpreting the

significance of BDNF levels in CSF, which has also been pointed out previously by others using *pre mortem* CSF (Blasko et al. 2006, Laske et al. 2006, Laske et al. 2007, Jiao et al. 2016). BDNF measurement in plasma and whole blood was also considered, but we ultimately decided to focus on BDNF levels in serum for reasons described in **Chapter 1 Section 1.6.1**.

6.3 Brain and Serum BDNF

In **Chapter 4**, we found that serum BDNF levels positively correlated with brain BDNF levels in our aged rat model, reproducing results from studies done by others (Karege et al. 2002, Sartorius et al. 2009, Klein et al. 2011). In **Chapter 3**, we uniquely investigated the relationship between brain and serum BDNF levels in human samples. A positive correlation between human brain and serum BDNF levels was expected due to results found in other animal models (Karege et al. 2002, Sartorius et al. 2009, Klein et al. 2011), due to several reports relating blood BDNF to various psychiatric and neurological diseases (Carlino et al. 2011, Yoshida 2012, Fernandes et al. 2015), and due to an interesting study completed by Dawood et al. (2007) where BDNF levels in venous blood from the jugular vein were higher than arterial blood from the brachial artery, suggesting that BDNF levels in venous blood were supplemented by BDNF produced in the brain. We used two of the most commonly used commercial ELISA to measure total BDNF levels, one from R&D Systems and one from Promega. As discussed in **Chapter 2 section 2.4.1**, the R&D BDNF ELISA kit was not appropriate to use on human brain samples due to tissue matrix interference, and the Promega

BDNF ELISA kit was used to quantify brain BDNF levels. Although we did not find a correlation between serum and brain BDNF levels in human samples, this may be due to the fact that we used the R&D ELISA kit to measure serum BDNF levels and the Promega ELISA kit to measure brain BDNF levels. We did find a positive correlation between serum total BDNF levels and hippocampal staining of HLA-DR, suggesting that peripheral BDNF levels increased to compensate for increasing neuroinflammation. To the best of my knowledge, this is the first study to investigate directly the relationship between brain and serum BDNF levels in humans, and our results warrant further replication in larger and more diverse sample sizes. One of the possible reasons this study may have not been done before is due to the limited availability of *post mortem* human brain and fluid samples, and increased support to human brain banks will make studies like these more possible.

We recognize that BDNF levels measured from ELISA represent total BDNF composed of both proBDNF and mBDNF, and interpretation of total BDNF may be convoluted by the disease prejudicially altering levels of either proBDNF or mBDNF. The importance of carefully interpreting results from BDNF ELISA is further highlighted in **Chapter 2 Section 2.4.1** where I show that high levels of proBDNF levels in biological samples can influence quantification of BDNF using the R&D BDNF ELISA kit such that one can overestimate the amount of mature BDNF in a sample if they adopt the assumption that the kit only measures mature BDNF. Accordingly, we referred to BDNF levels measured by ELISA as total BDNF, made up of both mature and proBDNF. In **Chapter 3**, we further

investigated the different forms of BDNF using western blots and found a negative correlation between serum proBDNF levels and hippocampal total BDNF levels as well as hippocampal proBDNF levels although we had hypothesized a positive correlation between brain and serum mature BDNF levels. Possible reasons for this counterintuitive result are discussed in further detail in **Chapter 3**. The fact that we found a) serum BDNF levels positively correlated with brain BDNF levels in rats but not in humans and b) human serum proBDNF levels negatively correlated with brain BDNF levels, further underscores the difficulty of using serum BDNF levels as a clinical biomarker without consideration of both the forms of BDNF: proBDNF and mBDNF. A recent report indicated that serum BDNF isoforms may also differ across ethnic backgrounds (Hashimoto 2016). These results warrant further investigation specifically into the differences between mature and proBDNF across demographic factors and clinical status. In addition, quantification of factors that process proBDNF into mature BDNF and/or facilitate the production or clearance of mature and proBDNF may further facilitate interpretation of how the disease course affects total BDNF levels. Innovations into multiplex assays that can accurately measure BDNF isoforms and relevant modifier molecules in small amounts of samples would greatly facilitate progress in this area. Although several multiplex systems currently exist, it is unclear whether these systems are tested for use in human brain samples, and validation experiments similar to the ones completed in **Chapter 2** on the R&D BDNF ELISA kits will be important for the future of biomarker studies with human brain samples.

6.4 BDNF and Inflammation

In addition to considering the pro- versus mature forms of BDNF, the strong antagonistic relationship between BDNF and inflammation should also be considered when interpreting BDNF measurements in a clinical setting. In **Chapter 3**, we found that staining for activated microglia increased with increasing AD pathology (amyloid and pTau) and that the AD case with the highest activated microglia staining had the lowest levels of BDNF, suggesting that BDNF plays a protective role against pTau and amyloid accumulation. Furthermore, in **Chapter 4**, we found that higher hippocampal IL-1β levels and lower BDNF levels in aged rats were correlated with increased activation of astrocytes in the hippocampus and that aged rats with the highest microglial and astroglial activation in the hippocampus had the poorest cognitive performance. These findings have been supported by others, showing a strong correlation between neuroinflammation and cognitive performance, both with aging and in AD (Bettcher and Kramer 2014). In **Chapter 5**, we analyzed the relationship between serum BDNF levels and changes in neuropsychological performance and neuroimaging measures while controlling for basal inflammation status assessed by cytokine levels in serum. In this chapter, our linear regression models did not indicate that inflammation was an important predictor for changes in neuropsychological scores or neuroimaging measures. However, this was most likely due to our use of healthy older adults.

6.5 BDNF as a Biomarker

A summary of major questions and answers addressed in my dissertation is presented in **Figure 6-1**. In **Chapter 3**, I predicted that inflammation would be higher and serum BDNF levels would be lower in AD compared to control. In the brain, we found increased neuroinflammation and decreased BDNF levels in the High AD subgroup compared to control. In serum, however, we did not find a significant difference in serum cytokines and BDNF levels between AD and control. This finding in serum most likely is due to the high variability in cytokines measurements in *post mortem* samples. In addition, others have shown that BDNF levels deteriorate in blood even when stored at -80^{o}C (Trajkovska et al. 2007, Zuccato et al. 2011), and this may have influenced our findings. We did find a slight decrease in mean serum total BDNF levels in the High AD subgroup compared to control, but this was found to be not statistically significant, possibly due to our small sample size. Interestingly, we found that serum proBDNF levels were increased in AD compared to control which complements several studies that found increased serum total BDNF levels in AD compared to control (Laske et al. 2006, Angelucci et al. 2010, Konukoglu et al. 2012, Sonali et al. 2013). We additionally found that serum proBDNF levels were negatively correlated with BDNF levels in brain tissue, suggesting either a compensatory increase in serum proBDNF to replace depleting brain BDNF or disease-related interference of processing of proBDNF to mBDNF. Although the cleavage of proBDNF to the mature form has not been examined in AD patients previously, to our knowledge, our group recently showed a deficient cleavage of proNGF to its mature form in

patients with Down syndrome and AD, suggesting that the cleavage process of neurotrophins may be affected by the AD pathology (Iulita et al. 2014).

Interestingly, we found an increase of brain BDNF levels in the Low AD subgroup compared to both the High AD subgroup and control, suggesting an upregulation of brain BDNF to theoretically compensate and counteract the early effects of the pathology. Increased BDNF levels in serum were previously reported in early AD compared to control and late AD (Laske et al. 2006, Konukoglu et al. 2012), and increased total BDNF and proBDNF levels were reported in the hippocampus of AD compared to control (Durany et al. 2000, Lim et al. 2015). Our results support the hypothesis first proposed by Laske et al. (2006) that BDNF has a dynamic relationship with disease course characterized by an early compensatory increase and subsequent reduction of BDNF levels as the disease progresses. These results in human samples are further supported by our findings from **Chapter 4** where after an inflammatory challenge causes an immediate drop in serum BDNF in aged rats, a compensatory increase in serum BDNF is found in aged rats with an AD-related neurodegeneration. These results are depicted in the proposed model in **Figure 6-1**.

In **Chapter 5**, we tested whether serum BDNF levels can predict changes in neuropsychological performance and neuroimaging measures in cognitively healthy older adults while controlling for inflammation. In this preliminary investigation with a modest sample size, we found significant differences between women and men in their baseline serum BDNF levels and in the progression of memory and executive function performance over the course of

15 months. In addition, high serum BDNF levels in men were associated with steeper declines in hippocampal volume and white matter microstructure of the limbic system. Although these relationships were marginally significant due to our small and unbalanced sample size, this study lays the foundation for follow up studies to investigate further how serum BDNF levels predict changes over time in older women and men especially in more at-risk populations such as patients with MCI or AD.

Overall, our results indicate that serum BDNF is sensitive to AD pathology and has some predictive ability for changes related to AD pathology; however, our results do not assess whether changes in BDNF are specific to AD pathology since we did not include other neurodegenerative pathologies (e.g., PD or HD) in our comparison. Changes in serum BDNF may be due to changes related to AD pathology, as supported by this dissertation, or due to changes in diet, exercise, infection, or other neuropsychiatric disorders. Nevertheless, serum BDNF levels reflect clinically-relevant changes in older adults and should be considered to be included in routine blood workup in a clinical setting. Ideally, dramatic changes in serum BDNF levels in older adults will prompt the clinician to ask relevant questions about lifestyle changes or neuropsychological changes to determine whether additional testing or intervention is needed.

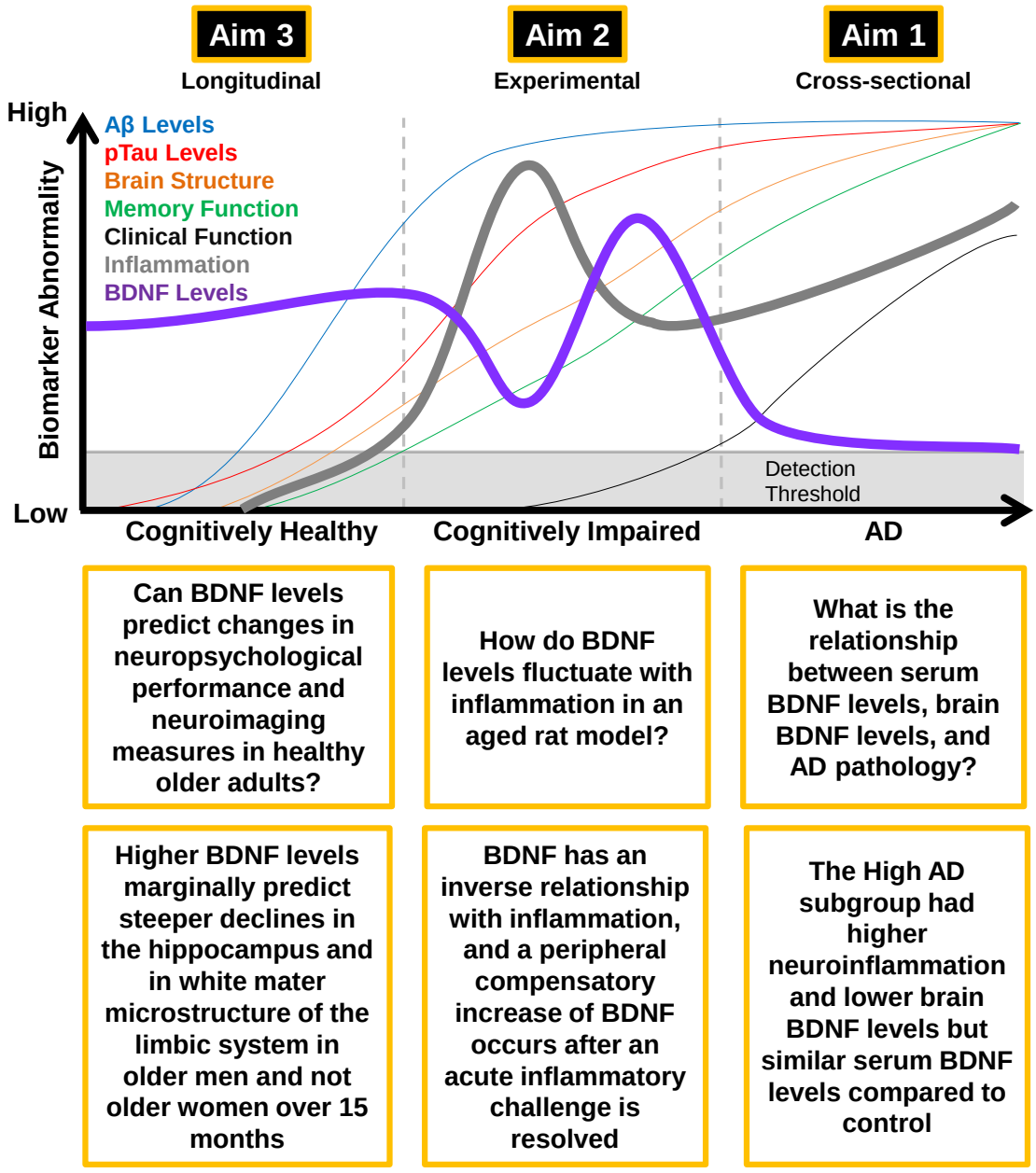

Figure 6-1. Framework of major dissertation questions and answers. Answers to major dissertation questions based on aims are added to the model and questions presented in Figure 1-5. Only results from Aim 1 did not support the hypothetical changes of BDNF and inflammation plotted in the model. Results from Aim 1 indicate that the High AD subgroup had higher neuroinflammation and lower brain BDNF levels compared to control but equivalent serum tBDNF levels and cytokine levels compared to control. This is contrary to our expected finding of high inflammation and low BDNF in serum in AD compared to cognitively healthy control.

6.6 Future Directions

BDNF levels in serum have neuronal and non-neuronal sources, and future studies on how to better isolate and measure BDNF from only neuronal sources would theoretically better reflect neurocognitive or neuropathological changes. Exosomes are extra-cellular microvesicles found in biological fluids that are produced by cells through the endocytic pathway. Using a novel technique developed by Dr. Granholm's collaborator, Dr. Ed Goetzl, we were able to isolate specific exosomes of neuronal origin from plasma, referred to as Neuron-Derived Exosomes or NDEs (Fiandaca et al. 2015, Goetzl et al. 2015, Hamlett et al. 2018). The Granholm laboratory has utilized this method to track AD biomarkers in NDEs in individuals with Down syndrome (DS), a condition with a high incidence of early-onset AD (Hamlett

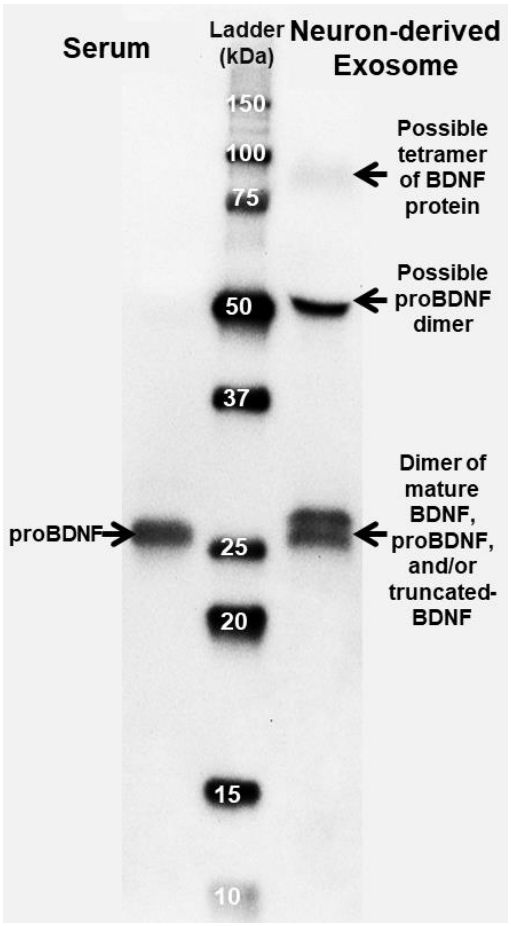

Figure 6-2. Representative BDNF western blot on human serum and neuron-derived exosomes. Two proteins with higher molecular mass (~50 kDa, ~100 kDa) and a doublet (>25 kDa) are seen in 20 µg of neuronal-derived exosome but not in 20 µg of human serum after probing with anti-BDNF antibody (Alomone, ANT-010). The identity of the higher molecular weight proteins in exosomes may be dimers or tetramers of BDNF species.

et al. 2018). Considering that serum BDNF may reflect non-neuronal BDNF, BDNF levels in NDEs may better reflect BDNF levels in the human brain and better correlate with neuropathological changes. In a preliminary study, I purified NDEs from older adult blood samples and performed a western blot analysis of BDNF in serum and exosomes. These studies show remarkable differences in BDNF species, with two higher molecular weight bands (~50 kDa and ~100 kDa) found in exosomes but not in serum (**Figure 6-2**). These bands may represent dimers or higher order organization of proBDNF, which are normally digested by proteases present in serum. These preliminary results suggest that BDNF composition in NDEs is more complex than those found in serum and is worthwhile investigating further to understand the function of BDNF in NDEs and their possible biomarker potential, since the neuronal origin of the purified NDEs may be better predictors of what is going on in the brain.

6.7. BDNF as a neuroprotective therapy for AD

In addition to having potential as a biomarker for AD, several investigators have suggested that elevating BDNF levels in the brain may have value as a preventative or interventional drug for AD patients. For example, Alvarez et al. (2016) recently demonstrated that a combination treatment consisting of Cerebrolysin and Donepezil gave rise to synergistic increases in serum BDNF levels in patients with AD. The combination therapy was more effective than each drug alone, and BDNF serum levels were investigated at weeks 16 and 28. In addition, this group showed an increased BDNF response in apolipoprotein E

epsilon-4 allele carriers, and higher BDNF levels were also associated with better cognitive improvements in apolipoprotein E epsilon-4 allele patients treated with Cerebrolysin and the combined therapy. There are other drugs with proven positive effects on serum BDNF levels, including memantine (Amidfar et al. 2017) and caffeine (Lao-Peregrín et al. 2017), suggesting that drug administration or intake of caffeine can increase BDNF systems in the brain and lead to protection against AD pathology. Another way to increase BDNF levels and/or expression is via exercise. Multiple studies, both from others and from our group, have shown that moderate levels of exercise increase BDNF levels which, in turn, increase cognitive performance (Rasmussen et al. 2009, Lafenetre et al. 2010, Heijnen et al. 2015, Hakansson et al. 2017). Finally, a more extreme way to significantly increase BDNF expression in the brain is to administer intracranial AAV-BDNF vectors. This is now in Phase I clinical trials lead by Dr. Mark Tusczynski at UCSD (Tuszynski 2017, Tuszynski et al. 2017). Gene therapy experiments will finally confirm whether BDNF plays a major role in protecting the brain from AD pathology. These kinds of findings could motivate the population to engage in life style changes that may prove to be protective for dementia.

6.8 Conclusion

This dissertation led to significant observations of the biomarker potential of serum BDNF in *post mortem* human brain samples, in aged rat models with neurodegeneration, and in a longitudinal study on older adults. Through the experiments conducted in this dissertation, I have had the unique opportunity to

collaborate with experts across disciplines and learn about the strengths and challenges of techniques used in neuropathology, neuroimaging, and neuropsychology. In addition to developing a professional network at MUSC, my work and training has allowed me to travel to Boston, Chicago, Denver, and D.C., where I received additional training and guidance from leaders in the aging and AD research field including Drs. Cynthia Lemere, Dean Hartley, and Sangram Sisodia. I do not doubt that the skills and connections I have developed through this dissertation will guide my career as a physician-scientist and neuropathologist.

APPENDIX 1. BDNF LEVELS IN AD HUMAN SERUM SAMPLES

AD = Alzheimer's disease, Con = Control, ELISA = Enzyme-linked immunosorbent assay, MCI = Mild Cognitive Impairment

Appendix 1. Studies on serum BDNF levels in AD

Result	Author	Year	Origin	Sample Size	Method	Notes
Decreased BDNF levels in AD compared to Con	Borba et al.	2016	Brazil	13 AD 10 MCI 10 Con	ELISA kit (R&D Systems)	AD and MCI had lower BDNF levels than Con
	Curto et al.	2014	Italy	30 AD 16 Con	Emax ImmunoAssay System (Promega)	AD had lower BDNF levels than Con
	Forlenza et al.	2010	Brazil	30 AD 71 MCI 59 Con	ELISA (DuoSet R&D Systems)	AD and MCI had similar BDNF levels that were lower than Con
	Forlenza et al.	2015b	Brazil	26 AD 62 MCI 46 Con	ELISA (DuoSet R&D Systems)	AD and MCI had lower BDNF levels than Con and MCI with lower BDNF levels progressed to AD
	Gezen-Ak et al.	2013	Turkey	76 AD 30 MCI 50 Con	ELISA (ChemiKineTM, CYT306, Millipore)	Early and late-onset AD had lower BDNF levels compared to Con and MCI

Appendix 1. Studies on serum BDNF levels in AD

Result	Author	Year	Origin	Sample Size	Method	Notes
	Jiao et al.	2016	China	44 AD 54 Con	ELISA (Abexa)	AD had lower BDNF levels than age- and sex-matched Con
	Laske et al.	2007	Germany	27 AD 28 Con	ELISA (R&D Systems)	AD had lower BDNF levels compared to Con in elderly
	Laske et al.	2011	Germany	40 AD	ELISA (R&D Systems)	AD patients with fast cognitive decline (n=12) had lower BDNF levels than those with slow cognitive decline (n=28)
	Laske et al.	2006b	Germany	28 AD 10 Con	ELISA (R&D Systems)	AD had lower BDNF levels than Con
	Lee et al.	2009	Korea	47 AD 41 MCI 39 Con	ELISA (Emax Promega)	AD and MCI had similar BDNF levels which was lower than Con

Appendix 1. Studies on serum BDNF levels in AD

Result	Author	Year	Origin	Sample Size	Method	Notes
	Leyhe et al.	2008	Germany	19 AD 20 Con	ELISA (R&D Systems)	AD had lower BDNF levels than Con which was rectified by 15 months of donepezil
	Leyhe et al.	2009	Germany	27 AD 15 Con	ELISA (R&D Systems)	AD had lower BDNF levels than Con which was rectified by 6 weeks of lithium treatment
	Liu et al.	2015	China	110 AD 120 Con	Raybio human BDNF ELISA	AD had lower BDNF levels than Con in elderly Han Chinese
	Ventriglia et al.	2013	Italy	266 AD 169 Con	ELISA (Quantikine R&D Systems)	AD had lower BDNF levels than Con
	Wang et al.	2015	China	64 AD 68 MCI 90 Con	ELISA (R&D Systems)	AD and MCI had lower BDNF levels than Con and MCI with lower BDNF levels progressed to AD

Appendix 1. Studies on serum BDNF levels in AD

Result	Author	Year	Origin	Sample Size	Method	Notes
	Weinstein et al.	2014	USA	2131 Con	ELISA (R&D Systems)	Higher BDNF in Con associated with lower risk to develop AD or dementia
	Yasutake et al.	2006	Japan	60 AD 33 Con	ELISA (Quantikine R&D Systems)	AD had lower BDNF levels than Con and vascular dementia
Increased BDNF levels in AD compared to Con	Angelucci et al.	2010	Italy	89 AD 54 MCI 27 Con	ELISA (R&D Systems)	AD and MCI had higher BDNF levels compared to Con
	Konukoglu et al.	2012	Turkey	12 AD 20 Con	ELISA (Emax Promega)	Early AD had higher BDNF level than Con and late AD
	Laske et al.	2006a	Germany	15 AD 10 Con	ELISA (R&D Systems)	Early AD had higher BDNF level than Con and late AD
	Sonali et al.	2013	India	63 AD 15 MCI 63 Con	Raybio human BDNF ELISA	AD and MCI had higher BDNF levels compared to Con

Appendix 1. Studies on serum BDNF levels in AD

Result	Author	Year	Origin	Sample Size	Method	Notes
No difference in BDNF levels between AD and Con	Alvarez et al.	2014	Spain	252 AD 48 MCI 62 Con	ELISA (R&D Systems)	BDNF levels were not different between AD, MCI and Con
	Konukoglu et al.	2012	Turkey	10 AD 20 Con	ELISA (Emax Promega)	Late AD had similar BDNF levels to Con but both were lower than early AD
	Laske et al.	2006a	Germany	15 AD 10 Con	ELISA (R&D Systems)	Late AD had similar BDNF levels to Con but both were lower than early AD
	O'Bryant et al.	2009	USA	98 AD 98 Con	Multi-Analyte Profile (Rule Based Medicine)	BDNF levels were not different between AD and Con
	O'Bryant et al.	2011	USA	198 AD 201 Con	Multi-Analyte Profile (Rule Based Medicine)	BDNF levels were not different between AD and Con
	Woolley et al.	2012	USA	34 AD 30 MCI 38 Con	ELISA (R&D Systems)	BDNF levels were not different between AD, Con, and MCI

APPENDIX 2. BDNF LEVELS IN AD HUMAN BRAIN SAMPLES

AD = Alzheimer's disease, BA = Brodmann Area, BF = Basal forebrain, BG = Basal Ganglia, CBL = Cerebellar cortex, CG = Cingulate gyrus, Con = Control, DG = Dentate gyrus, ELISA = Enzyme-linked immunosorbent assay, ENT = Entorhinal cortex, FC = Frontal cortex, HIP = Hippocampus, IHC = Immunohistochemistry, mBDNF = mature BDNF, MC = Motor Cortex, MCI = Mild Cognitive Impairment, OC = Occipital cortex, PC = Parietal cortex, PUT = Putamen, TC = Temporal cortex, THL = Thalamus, WB = Western Blot

Appendix 2. Studies on brain BDNF levels in AD

Result	Author	Year	Region	Method	Notes
Decreased BDNF levels in AD compared to Con	Connor et al.	1997	HIP (9 AD 8 Con) TC (9 AD 8 Con)	IHC (Amgen)	AD had lower BDNF-immunopositive cell bodies in the HIP CA1 region and TC
	Ferrer et al.	1999	FC (4 AD 4 Con) HIP (6 AD 4 Con)	IHC (sc N-20)	AD had reduced BDNF immunoreactivity in FC and HIP neurons
	Hock et al.	2000	HIP (9AD 9 Con) PC (8 AD 5 Con)	ELISA (Emax Promega)	AD had lower levels of BDNF in HIP and PC but not FC or CBL
	Jiao et al.	2016	PC (12 AD 12 Con)	WB (ab72439) ELISA (Abexa)	AD had ~50% lower BDNF levels in PC compared to age- and sex-matched controls
	Lee et al.	2005	TC (57 AD 19 Con)	ELISA (R&D MAB848)	AD had ~33% lower BDNF levels in TC but not in FC or CBL
	Lim et al.	2015	HIP (10 AD 10 Con)	WB (GeneCopoeia, and sc N-20)	AD had 6-fold lower mBDNF and 16-fold higher proBDNF levels than Con

Appendix 2. Studies on brain BDNF levels in AD

Result	Author	Year	Region	Method	Notes
	Michalski et al.	2003	PC (7 AD 8 Con)	WB (sc N-20)	AD had 40% lower pro-BDNF levels in PC
	Narisawa-Saito et al.	1996	DG (10 AD 10 Con) ENT (10 AD 10 Con) MC (10 AD 10 Con)	ELISA	AD had lower BDNF level in ENT but not MC or DG
	Peng et al.	2005	PC (17 AD 20 Con 17 MCI)	WB (sc N-20)	AD and MCI had lower proBDNF and mature BDNF levels in PC compared to Con
Increased BDNF levels in AD compared to Con	Durany et al.	2000	HIP (9 AD 9 Con) PC (12 AD 9 Con)	ELISA (Emax Promega)	Higher BDNF concentration found in AD compared to Con in HIP and PC but not CG, FC, OC, PUT, TC, THAL
	Lim et al.	2015	HIP (10 AD 10 Con)	WB (Gene Copoeia, and sc N-20)	AD had 6-fold lower mBDNF and 16-fold higher proBDNF levels than Con
No difference in BDNF levels between AD and Con	Durany et al.	2000	CG (8 AD 8 Con) FC (12 AD 12 Con) OC (9 AD 9 Con) PUT (8 AD 8 Con) TC (13 AD 13 Con) THL (6 AD 6 Con)	ELISA (Emax Promega)	Higher BDNF concentration found in AD compared to Con in HIP and PC but not CG, FC, OC, PUT, TC, THAL
	Hock et al.	2000	CBL (7 AD 5 Con) FC (19AD 9 Con)	ELISA (Emax Promega)	AD had lower levels of BDNF in HIP and PC but not FC or CBL

Appendix 2. Studies on brain BDNF levels in AD

Result	Author	Year	Region	Method	Notes
	Kao et al.	2012	HIP (2 AD 1 Con)	IHC (sc N-20)	BDNF immunoreactivity in the HIP CA1 region was similar between the three groups: 1) control, 2) early AD with no cognitive deficit, and 3) early AD with mild cognitive deficit
	Lee et al.	2005	CBL (54 AD 16 Con) FC (50 AD 13 Con)	ELISA (R&D MAB848)	AD had ~33% lower BDNF levels in TC but not in FC or CBL
	Michalski et al.	2015	PC (28 AD 22 Con)	ELISA (DuoSet R&D Systems)	BDNF levels were similar between four groups all aged 90 years or older: 1) Con, 2) Con w/ dementia, 3) AD w/o dementia, and 4) AD w/ dementia
	Murer et al.	1999	HIP (3 AD 4 Con) BG (3 AD 4 Con) BF (3 AD 4 Con)	IHC	BDNF staining pattern and labeling intensity was similar between AD and Con

LIST OF REFERENCES

Abe, K., M. Horiuchi and K. Yoshimura (1997). "Potentiation by DSP-4 of EEG slowing and memory impairment in basal forebrain-lesioned rats." Eur J Pharmacol **321**(2): 149-155. PMC: 9063682.

Albert, M. S., S. T. DeKosky, D. Dickson, B. Dubois, H. H. Feldman, N. C. Fox, A. Gamst, D. M. Holtzman, W. J. Jagust, R. C. Petersen, P. J. Snyder, M. C. Carrillo, B. Thies and C. H. Phelps (2011). "The diagnosis of mild cognitive impairment 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): 270-279. doi: 10.1016/j.jalz.2011.03.008. PMC: 21514249. PMCID: PMC3312027.

Allen, S. J., G. K. Wilcock and D. Dawbarn (1999). "Profound and selective loss of catalytic TrkB immunoreactivity in Alzheimer's disease." Biochem Biophys Res Commun **264**(3): 648-651. doi: 10.1006/bbrc.1999.1561. PMC: 10543986.

Alonso, M., M. R. Vianna, A. M. Depino, T. Mello e Souza, P. Pereira, G. Szapiro, H. Viola, F. Pitossi, I. Izquierdo and J. H. Medina (2002). "BDNF-triggered events in the rat hippocampus are required for both short- and long-term memory formation." Hippocampus **12**(4): 551-560. doi: 10.1002/hipo.10035. PMC: 12201640.

Altar, C. A., N. Cai, T. Bliven, M. Juhasz, J. M. Conner, A. L. Acheson, R. M. Lindsay and S. J. Wiegand (1997). "Anterograde transport of brain-derived neurotrophic factor and its role in the brain." Nature **389**(6653): 856-860. doi: 10.1038/39885. PMC: 9349818.

Alvarez, A., M. Aleixandre, C. Linares, E. Masliah and H. Moessler (2014). "Apathy and APOE4 are associated with reduced BDNF levels in Alzheimer's disease." J Alzheimers Dis **42**(4): 1347-1355. doi: 10.3233/JAD-140849. PMC: 25024337. PMCID: PMC4931817.

Alvarez, X. A., I. Alvarez, O. Iglesias, I. Crespo, J. Figueroa, M. Aleixandre, C. Linares, E. Granizo, M. Garcia-Fantini, J. Marey, E. Masliah, S. Winter, D. Muresanu and H. Moessler (2016). "Synergistic Increase of Serum BDNF in Alzheimer Patients Treated with Cerebrolysin and Donepezil: Association with Cognitive Improvement in ApoE4 Cases." Int J Neuropsychopharmacol. doi: 10.1093/ijnp/pyw024. PMC: 27207906. PMCID: PMC4926802.

Alzheimer, A. (1907). "Über eine eigenartige Erkrankung der Hirnrinde." Allgemeine Zeitschrift für Psychiatrie Psychisch-Gerichtlich Medizine **64**: 146-148.

Amidfar, M., Y.-K. Kim and O. Wiborg (2017). "Effectiveness of memantine on depression-like behavior, memory deficits and brain mRNA levels of BDNF and TrkB in rats subjected to repeated unpredictable stress." Pharmacological Reports.

Amor, S., L. A. Peferoen, D. Y. Vogel, M. Breur, P. van der Valk, D. Baker and J. M. van Noort (2014). "Inflammation in neurodegenerative diseases--an update." Immunology **142**(2): 151-166. doi: 10.1111/imm.12233. PMC: 24329535. PMCID: PMC4008224.

Andorfer, C., Y. Kress, M. Espinoza, R. De Silva, K. L. Tucker, Y.-A. Barde, K. Duff and P. Davies (2003). "Hyperphosphorylation and aggregation of tau in mice expressing normal human tau isoforms." Journal of Neurochemistry **86**(3): 582-590. doi: 10.1046/j.1471-4159.2003.01879.x.

Angelucci, F., S. Brene and A. A. Mathe (2005). "BDNF in schizophrenia, depression and corresponding animal models." Mol Psychiatry **10**(4): 345-352. doi: 10.1038/sj.mp.4001637. PMC: 15655562.

Angelucci, F., G. Spalletta, F. Iulio, A. Ciaramella, F. Salani, A. Varsi, W. Gianni, G. Sancesario, C. Caltagirone and P. Bossu (2010). "Alzheimers Disease (AD) and Mild Cognitive Impairment (MCI) Patients are Characterized by Increased BDNF Serum Levels." Current Alzheimer Research **7**(1): 15-20. doi: 10.2174/156720510790274473.

Antunes, M. and G. Biala (2012). "The novel object recognition memory: neurobiology, test procedure, and its modifications." Cogn Process **13**(2): 93-110. doi: 10.1007/s10339-011-0430-z. PMC: 22160349. PMCID: PMC3332351.

Arancibia, S., M. Silhol, F. Mouliere, J. Meffre, I. Hollinger, T. Maurice and L. Tapia-Arancibia (2008). "Protective effect of BDNF against beta-amyloid induced neurotoxicity in vitro and in vivo in rats." Neurobiol Dis **31**(3): 316-326. doi: 10.1016/j.nbd.2008.05.012. PMC: 18585459.

Association, A. (2018). "2018 Alzheimer's disease facts and figures." Alzheimer's & Dementia **14**(3): 367-429. doi: 10.1016/j.jalz.2018.02.001.

Association, A. P. (2013). Diagnostic and statistical manual of mental disorders (DSM-5®), American Psychiatric Pub.

Augustinack, J. C., A. Schneider, E.-M. Mandelkow and B. T. Hyman (2001). "Specific tau phosphorylation sites correlate with severity of neuronal

cytopathology in Alzheimer's disease." Acta Neuropathologica **103**(1): 26-35. doi: 10.1007/s004010100423.

Balietti, M., C. Giuli and F. Conti (2018). "Peripheral Blood Brain-Derived Neurotrophic Factor as a Biomarker of Alzheimer's Disease: Are There Methodological Biases?" Mol Neurobiol. doi: 10.1007/s12035-017-0866-y. PMC: 29330839.

Ballatore, C., V. M. Lee and J. Q. Trojanowski (2007). "Tau-mediated neurodegeneration in Alzheimer's disease and related disorders." Nat Rev Neurosci **8**(9): 663-672. doi: 10.1038/nrn2194. PMC: 17684513.

Banks, W. A. and M. A. Erickson (2010). "The blood-brain barrier and immune function and dysfunction." Neurobiol Dis **37**(1): 26-32. doi: 10.1016/j.nbd.2009.07.031. PMC: 19664708.

Banks, W. A. and S. M. Robinson (2010). "Minimal penetration of lipopolysaccharide across the murine blood-brain barrier." Brain Behav Immun **24**(1): 102-109. doi: 10.1016/j.bbi.2009.09.001. PMC: 19735725. PMCID: PMC2789209.

Baranello, R. J., K. L. Bharani, V. Padmaraju, N. Chopra, D. K. Lahiri, N. H. Greig, M. A. Pappolla and K. Sambamurti (2015). "Amyloid-beta protein clearance and degradation (ABCD) pathways and their role in Alzheimer's disease." Curr Alzheimer Res **12**(1): 32-46. PMC: 25523424. PMCID: PMC4820400.

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. doi: 10.1016/j.neurobiolaging.2013.11.006. PMC: 24315728. PMCID: PMC3946275.

Barrientos, R. M., M. G. Frank, A. M. Hein, E. A. Higgins, L. R. Watkins, J. W. Rudy and S. F. Maier (2009). "Time course of hippocampal IL-1 beta and memory consolidation impairments in aging rats following peripheral infection." Brain Behav Immun **23**(1): 46-54. doi: 10.1016/j.bbi.2008.07.002. PMC: 18664380. PMCID: PMC2630971.

Barrientos, R. M., D. B. Sprunger, S. Campeau, L. R. Watkins, J. W. Rudy and S. F. Maier (2004). "BDNF mRNA expression in rat hippocampus following contextual learning is blocked by intrahippocampal IL-1beta administration." J Neuroimmunol **155**(1-2): 119-126. doi: 10.1016/j.jneuroim.2004.06.009. PMC: 15342202.

Bartus, R. T., R. L. Dean, B. Beer and A. S. Lippa (1982). "The cholinergic hypothesis of geriatric memory dysfunction." Science **217**(4558): 408-414.

Basser, P. J. and C. Pierpaoli (1996). "Microstructural and Physiological Features of Tissues Elucidated by Quantitative-Diffusion-Tensor MRI." Journal of Magnetic Resonance, Series B **111**(3): 209-219. doi: 10.1006/jmrb.1996.0086.

Beach, T. G., S. E. Monsell, L. E. Phillips and W. Kukull (2012). "Accuracy of the clinical diagnosis of Alzheimer disease at National Institute on Aging Alzheimer Disease Centers, 2005-2010." J Neuropathol Exp Neurol **71**(4): 266-273. doi: 10.1097/NEN.0b013e31824b211b. PMC: 22437338. PMCID: PMC3331862.

Begni, V., M. A. Riva and A. Cattaneo (2017). "Cellular and molecular mechanisms of the brain-derived neurotrophic factor in physiological and pathological conditions." Clinical Science **131**(2): 123-138.

Bekris, L. M., C. E. Yu, T. D. Bird and D. W. Tsuang (2010). "Genetics of Alzheimer disease." J Geriatr Psychiatry Neurol **23**(4): 213-227. doi: 10.1177/0891988710383571. PMC: 21045163. PMCID: PMC3044597.

Bettcher, B. M. and J. H. Kramer (2014). "Longitudinal inflammation, cognitive decline, and Alzheimer's disease: a mini-review." Clin Pharmacol Ther **96**(4): 464-469. doi: 10.1038/clpt.2014.147. PMC: 25009982. PMCID: PMC4224964.

Bharani, K. L., R. Derex, A. C. Granholm and A. Ledreux (2017). "A noradrenergic lesion aggravates the effects of systemic inflammation on the hippocampus of aged rats." PLoS One **12**(12): e0189821. doi: 10.1371/journal.pone.0189821. PMC: 29261743. PMCID: PMC5736222.

Billingsley, M. L. and R. L. Kincaid (1997). "Regulated phosphorylation and dephosphorylation of tau protein: effects on microtubule interaction, intracellular trafficking and neurodegeneration." Biochem J **323 (Pt 3)**: 577-591. PMC: 9169588. PMCID: PMC1218358.

Binder, D. K. and H. E. Scharfman (2004). "Brain-derived neurotrophic factor." Growth Factors **22**(3): 123-131. doi: 10.1080/08977190410001723308. PMC: 15518235. PMCID: PMC2504526.

Blasko, I., W. Lederer, H. Oberbauer, T. Walch, G. Kemmler, H. Hinterhuber, J. Marksteiner and C. Humpel (2006). "Measurement of thirteen biological markers in CSF of patients with Alzheimer's disease and other dementias." Dement Geriatr Cogn Disord **21**(1): 9-15. doi: 10.1159/000089137. PMC: 16244482.

Bliss, T. V. and S. F. Cooke (2011). "Long-term potentiation and long-term depression: a clinical perspective." Clinics (Sao Paulo) **66 Suppl 1**: 3-17. PMC: 21779718. PMCID: PMC3118435.

Boelen, A. (1995). "Soluble cytokine receptors and the low 3,5,3'-triiodothyronine syndrome in patients with nonthyroidal disease." Journal of Clinical Endocrinology & Metabolism **80**(3): 971-976. doi: 10.1210/jc.80.3.971.

Borba, E. M., J. A. Duarte, G. Bristot, E. Scotton, A. L. Camozzato and M. L. Chaves (2016). "Brain-Derived Neurotrophic Factor Serum Levels and Hippocampal Volume in Mild Cognitive Impairment and Dementia due to Alzheimer Disease." Dement Geriatr Cogn Dis Extra **6**(3): 559-567. doi: 10.1159/000450601. PMC: 28101102. PMCID: PMC5216193.

Bortel, A. (2014). Nature of DSP-4-Induced Neurotoxicity. Handbook of Neurotoxicity**:** 219-236.

Bowen, D. and A. Davison (1980). "Biochemical changes in the cholinergic system of the ageing brain and in senile dementia." Psychological medicine **10**(2): 315-319.

Braak, H. and E. Braak (1991). "Neuropathological stageing of Alzheimer-related changes." Acta Neuropathol **82**(4): 239-259. PMC: 1759558.

Braak, H. and K. Del Tredici (2011). "The pathological process underlying Alzheimer's disease in individuals under thirty." Acta Neuropathol **121**(2): 171-181. doi: 10.1007/s00401-010-0789-4. PMC: 21170538.

Braun, D., J. L. Madrigal and D. L. Feinstein (2014). "Noradrenergic regulation of glial activation: molecular mechanisms and therapeutic implications." Curr Neuropharmacol **12**(4): 342-352. doi: 10.2174/1570159X12666140828220938. PMC: 25342942. PMCID: PMC4207074.

Buchman, A. S., L. Yu, P. A. Boyle, J. A. Schneider, P. L. De Jager and D. A. Bennett (2016). "Higher brain BDNF gene expression is associated with slower cognitive decline in older adults." Neurology **86**(8): 735-741. doi: 10.1212/WNL.0000000000002387. PMC: 26819457. PMCID: PMC4763800.

Budni, J., T. Bellettini-Santos, F. Mina, M. L. Garcez and A. I. Zugno (2015). "The involvement of BDNF, NGF and GDNF in aging and Alzheimer's disease." Aging Dis **6**(5): 331-341. doi: 10.14336/AD.2015.0825. PMC: 26425388. PMCID: PMC4567216.

Buee, L., T. Bussiere, V. Buee-Scherrer, A. Delacourte and P. R. Hof (2000). "Tau protein isoforms, phosphorylation and role in neurodegenerative disorders." Brain Res Brain Res Rev **33**(1): 95-130. PMC: 10967355.

Bus, B. A., M. L. Molendijk, B. J. Penninx, J. K. Buitelaar, G. Kenis, J. Prickaerts, B. M. Elzinga and R. C. Voshaar (2011). "Determinants of serum brain-derived neurotrophic factor." Psychoneuroendocrinology **36**(2): 228-239. doi: 10.1016/j.psyneuen.2010.07.013. PMC: 20702043.

Bus, B. A., I. Tendolkar, B. Franke, J. de Graaf, M. den Heijer, J. K. Buitelaar and R. C. Oude Voshaar (2012). "Serum brain-derived neurotrophic factor: determinants and relationship with depressive symptoms in a community population of middle-aged and elderly people." World J Biol Psychiatry **13**(1): 39-47. doi: 10.3109/15622975.2010.545187. PMC: 21247257. PMCID: PMC3279138.

Busse, S., J. Steiner, J. Alter, H. Dobrowolny, C. Mawrin, B. Bogerts, R. Hartig and M. Busse (2015). "Expression of HLA-DR, CD80, and CD86 in Healthy Aging and Alzheimer's Disease." J Alzheimers Dis **47**(1): 177-184. doi: 10.3233/JAD-150217. PMC: 26402766.

Cagnin, A., D. J. Brooks, A. M. Kennedy, R. N. Gunn, R. Myers, F. E. Turkheimer, T. Jones and R. B. Banati (2001). "In-vivo measurement of activated microglia in dementia." Lancet **358**(9280): 461-467. doi: 10.1016/S0140-6736(01)05625-2. PMC: 11513911.

Calabrese, F., A. C. Rossetti, G. Racagni, P. Gass, M. A. Riva and R. Molteni (2014). "Brain-derived neurotrophic factor: a bridge between inflammation and neuroplasticity." Front Cell Neurosci **8**: 430. doi: 10.3389/fncel.2014.00430. PMC: 25565964. PMCID: PMC4273623.

Carlino, D., E. Leone, F. Di Cola, G. Baj, R. Marin, G. Dinelli, E. Tongiorgi and M. De Vanna (2011). "Low serum truncated-BDNF isoform correlates with higher cognitive impairment in schizophrenia." J Psychiatr Res **45**(2): 273-279. doi: 10.1016/j.jpsychires.2010.06.012. PMC: 20630543.

Carter, C. L., E. M. Resnick, M. Mallampalli and A. Kalbarczyk (2012). "Sex and gender differences in Alzheimer's disease: recommendations for future research." J Womens Health (Larchmt) **21**(10): 1018-1023. doi: 10.1089/jwh.2012.3789. PMC: 22917473.

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. doi: 10.1126/scitranslmed.3002156. PMC: 21715678. PMCID: PMC3192364.

Chakravarthy, B., M. Menard, S. Ito, C. Gaudet, I. Dal Pra, U. Armato and J. Whitfield (2012). "Hippocampal membrane-associated p75NTR levels are increased in Alzheimer's disease." J Alzheimers Dis **30**(3): 675-684. doi: 10.3233/JAD-2012-120115. PMC: 22451321.

Chalermpalanupap, T., B. Kinkead, W. T. Hu, M. P. Kummer, T. Hammerschmidt, M. T. Heneka, D. Weinshenker and A. I. Levey (2013). "Targeting norepinephrine in mild cognitive impairment and Alzheimer's disease." Alzheimers Res Ther **5**(2): 21. doi: 10.1186/alzrt175. PMC: 23634965. PMCID: PMC3706916.

Chen, W., E. A. Abud, S. T. Yeung, A. Lakatos, T. Nassi, J. Wang, D. Blum, L. Buee, W. W. Poon and M. Blurton-Jones (2016). "Increased tauopathy drives microglia-mediated clearance of beta-amyloid." Acta Neuropathol Commun **4**(1): 63. doi: 10.1186/s40478-016-0336-1. PMC: 27339073. PMCID: PMC4918195.

Chene, G., A. Beiser, R. Au, S. R. Preis, P. A. Wolf, C. Dufouil and S. Seshadri (2015). "Gender and incidence of dementia in the Framingham Heart Study from mid-adult life." Alzheimers Dement **11**(3): 310-320. doi: 10.1016/j.jalz.2013.10.005. PMC: 24418058. PMCID: PMC4092061.

Chiang, M. C., M. Barysheva, A. W. Toga, S. E. Medland, N. K. Hansell, M. R. James, K. L. McMahon, G. I. de Zubicaray, N. G. Martin, M. J. Wright and P. M. Thompson (2011). "BDNF gene effects on brain circuitry replicated in 455 twins." Neuroimage **55**(2): 448-454. doi: 10.1016/j.neuroimage.2010.12.053. PMC: 21195196. PMCID: PMC3192852.

Combs, C. K., J. C. Karlo, S. C. Kao and G. E. Landreth (2001). "beta-Amyloid stimulation of microglia and monocytes results in TNFalpha-dependent expression of inducible nitric oxide synthase and neuronal apoptosis." J Neurosci **21**(4): 1179-1188. PMC: 11160388.

Connor, B., D. Young, P. Lawlor, W. Gai, H. Waldvogel, R. L. M. Faull and M. Dragunow (1996). "Trk receptor alterations in Alzheimer's disease." Molecular Brain Research **42**(1): 1-17. doi: 10.1016/s0169-328x(96)00040-x.

Connor, B., D. Young, Q. Yan, R. L. Faull, B. Synek and M. Dragunow (1997). "Brain-derived neurotrophic factor is reduced in Alzheimer's disease." Brain Res Mol Brain Res **49**(1-2): 71-81. PMC: 9387865.

Counts, S. E. and E. J. Mufson (2010). "Noradrenaline activation of neurotrophic pathways protects against neuronal amyloid toxicity." J Neurochem **113**(3): 649-660. doi: 10.1111/j.1471-4159.2010.06622.x. PMC: 20132474. PMCID: PMC2913691.

Crary, J. F. (2016). "Primary age-related tauopathy and the amyloid cascade hypothesis: the exception that proves the rule?" J Neurol Neuromedicine **1**(6): 53-57. PMC: 27819070. PMCID: PMC5094182.

Crary, J. F., J. Q. Trojanowski, J. A. Schneider, J. F. Abisambra, E. L. Abner, I. Alafuzoff, S. E. Arnold, J. Attems, T. G. Beach, E. H. Bigio, N. J. Cairns, D. W. Dickson, M. Gearing, L. T. Grinberg, P. R. Hof, B. T. Hyman, K. Jellinger, G. A. Jicha, G. G. Kovacs, D. S. Knopman, J. Kofler, W. A. Kukull, I. R. Mackenzie, E. Masliah, A. McKee, T. J. Montine, M. E. Murray, J. H. Neltner, I. Santa-Maria, W. W. Seeley, A. Serrano-Pozo, M. L. Shelanski, T. Stein, M. Takao, D. R. Thal, J. B. Toledo, J. C. Troncoso, J. P. Vonsattel, C. L. White, 3rd, T. Wisniewski, R. L. Woltjer, M. Yamada and P. T. Nelson (2014). "Primary age-related tauopathy (PART): a common pathology associated with human aging." Acta Neuropathol **128**(6): 755-766. doi: 10.1007/s00401-014-1349-0. PMC: 25348064. PMCID: PMC4257842.

Culley, D. J., M. Snayd, M. G. Baxter, Z. Xie, I. H. Lee, J. Rudolph, S. K. Inouye, E. R. Marcantonio and G. Crosby (2014). "Systemic inflammation impairs attention and cognitive flexibility but not associative learning in aged rats: possible implications for delirium." Front Aging Neurosci **6**: 107. doi: 10.3389/fnagi.2014.00107. PMC: 24959140. PMCID: PMC4050637.

Cunha, C., R. Brambilla and K. L. Thomas (2010). "A simple role for BDNF in learning and memory?" Front Mol Neurosci **3**: 1. doi: 10.3389/neuro.02.001.2010. PMC: 20162032. PMCID: PMC2821174.

Cunningham, C., S. Campion, K. Lunnon, C. L. Murray, J. F. Woods, R. M. Deacon, J. N. Rawlins and V. H. Perry (2009). "Systemic inflammation induces acute behavioral and cognitive changes and accelerates neurodegenerative disease." Biol Psychiatry **65**(4): 304-312. doi: 10.1016/j.biopsych.2008.07.024. PMC: 18801476. PMCID: PMC2633437.

Cunningham, C. and E. Hennessy (2015). "Co-morbidity and systemic inflammation as drivers of cognitive decline: new experimental models adopting a broader paradigm in dementia research." Alzheimers Res Ther **7**(1): 33. doi: 10.1186/s13195-015-0117-2. PMC: 25802557. PMCID: PMC4369837.

Curto, M., A. Martocchia, F. Comite, S. Scaccianoce, D. Xenos, C. Nasca, S. Ferracuti, P. Girardi, F. Nicoletti and P. Falaschi (2014). "The presence of depressive symptoms in comorbidity with Alzheimer's disease does not influence changes in serum brain-derived neurotrophic factor levels in older patients." Int J Geriatr Psychiatry **29**(4): 439-440. doi: 10.1002/gps.4067. PMC: 24599735.

Czerniawski, J., T. Miyashita, G. Lewandowski and J. F. Guzowski (2015). "Systemic lipopolysaccharide administration impairs retrieval of context-object discrimination, but not spatial, memory: Evidence for selective disruption of specific hippocampus-dependent memory functions during acute neuroinflammation." Brain Behav Immun **44**: 159-166. doi: 10.1016/j.bbi.2014.09.014. PMC: 25451612. PMCID: PMC4358899.

Dalby, R. B., B. Elfving, P. H. Poulsen, L. Foldager, J. Frandsen, P. Videbech and R. Rosenberg (2013). "Plasma brain-derived neurotrophic factor and prefrontal white matter integrity in late-onset depression and normal aging." Acta Psychiatr Scand **128**(5): 387-396. doi: 10.1111/acps.12085. PMC: 23350796.

Davatzikos, C., P. Bhatt, L. M. Shaw, K. N. Batmanghelich and J. Q. Trojanowski (2011). "Prediction of MCI to AD conversion, via MRI, CSF biomarkers, and pattern classification." Neurobiol Aging **32**(12): 2322 e2319-2327. doi: 10.1016/j.neurobiolaging.2010.05.023. PMC: 20594615. PMCID: PMC2951483.

Davies, P. and A. Maloney (1976). "Selective loss of central cholinergic neurons in Alzheimer's disease." The Lancet **308**(8000): 1403.

Dawood, T., J. Anderson, D. Barton, E. Lambert, M. Esler, E. Hotchkin, D. Haikerwal, D. Kaye and G. Lambert (2007). "Reduced overflow of BDNF from the brain is linked with suicide risk in depressive illness." Mol Psychiatry **12**(11): 981-983. doi: 10.1038/sj.mp.4002059. PMC: 17957235.

Devi, L. and M. Ohno (2015). "TrkB reduction exacerbates Alzheimer's disease-like signaling aberrations and memory deficits without affecting beta-amyloidosis in 5XFAD mice." Transl Psychiatry **5**: e562. doi: 10.1038/tp.2015.55. PMC: 25942043. PMCID: PMC4471286.

Di Benedetto, S., L. Muller, E. Wenger, S. Duzel and G. Pawelec (2017). "Contribution of neuroinflammation and immunity to brain aging and the mitigating effects of physical and cognitive interventions." Neurosci Biobehav Rev **75**: 114-128. doi: 10.1016/j.neubiorev.2017.01.044. PMC: 28161508.

Diniz, B. S. and A. L. Teixeira (2011). "Brain-derived neurotrophic factor and Alzheimer's disease: physiopathology and beyond." Neuromolecular Med **13**(4): 217-222. doi: 10.1007/s12017-011-8154-x. PMC: 21898045.

Dols, A., C. S. Thesing, F. Bouckaert, R. C. Oude Voshaar, H. C. Comijs and M. L. Stek (2015). "BDNF serum levels are not related to cognitive functioning in older depressed patients and controls." Int Psychogeriatr **27**(4): 649-656. doi: 10.1017/S1041610214002622. PMC: 25519687.

Donoso, V., C. R. Gomez, M. A. Orriantia, V. Perez, C. Torres, C. Coddou, P. Nelson, K. Maisey, B. Morales, R. Fernandez, M. Imarai, J. P. Huidobro-Toro, F. Sierra and C. Acuna-Castillo (2008). "The release of sympathetic neurotransmitters is impaired in aged rats after an inflammatory stimulus: a possible link between cytokine production and sympathetic transmission." Mech Ageing Dev **129**(12): 728-734. doi: 10.1016/j.mad.2008.09.018. PMC: 18973771. PMCID: PMC2650491.

Donovan, M. J., R. C. Miranda, R. Kraemer, T. A. McCaffrey, L. Tessarollo, D. Mahadeo, S. Sharif, D. R. Kaplan, P. Tsoulfas, L. Parada and et al. (1995). "Neurotrophin and neurotrophin receptors in vascular smooth muscle cells. Regulation of expression in response to injury." Am J Pathol **147**(2): 309-324. PMC: 7639328. PMCID: PMC1869811.

Driscoll, I., B. Martin, Y. An, S. Maudsley, L. Ferrucci, M. P. Mattson and S. M. Resnick (2012). "Plasma BDNF is associated with age-related white matter atrophy but not with cognitive function in older, non-demented adults." PLoS One **7**(4): e35217. doi: 10.1371/journal.pone.0035217. PMC: 22523577. PMCID: PMC3327651.

Duarte, P. O., M. G. F. Duarte, A. Pelichek, K. Pfrimer, E. Ferriolli, J. C. Moriguti and N. K. C. Lima (2017). "Cardiovascular risk factors and inflammatory activity among centenarians with and without dementia." Aging Clin Exp Res **29**(3): 411-417. doi: 10.1007/s40520-016-0603-9. PMC: 27377066.

Dubois, B., H. H. Feldman, C. Jacova, S. T. DeKosky, P. Barberger-Gateau, J. Cummings, A. Delacourte, D. Galasko, S. Gauthier, G. Jicha, K. Meguro, J. O'Brien, F. Pasquier, P. Robert, M. Rossor, S. Salloway, Y. Stern, P. J. Visser and P. Scheltens (2007). "Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS–ADRDA criteria." The Lancet Neurology **6**(8): 734-746. doi: 10.1016/s1474-4422(07)70178-3.

Dubois, B., H. Hampel, H. H. Feldman, P. Scheltens, P. Aisen, S. Andrieu, H. Bakardjian, H. Benali, L. Bertram, K. Blennow, K. Broich, E. Cavedo, S. Crutch, J. F. Dartigues, C. Duyckaerts, S. Epelbaum, G. B. Frisoni, S. Gauthier, R. Genthon, A. A. Gouw, M. O. Habert, D. M. Holtzman, M. Kivipelto, S. Lista, J. L. Molinuevo, S. E. O'Bryant, G. D. Rabinovici, C. Rowe, S. Salloway, L. S. Schneider, R. Sperling, M. Teichmann, M. C. Carrillo, J. Cummings, C. R. Jack, Jr., G. Proceedings of the Meeting of the International Working, A. D. the American Alzheimer's Association on "The Preclinical State of, July and U. S. A. Washington Dc (2016). "Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria." Alzheimers Dement **12**(3): 292-323. doi: 10.1016/j.jalz.2016.02.002. PMC: 27012484.

Dulin, F., F. Leveille, J. B. Ortega, J. P. Mornon, A. Buisson, I. Callebaut and N. Colloc'h (2008). "P3 peptide, a truncated form of A beta devoid of synaptotoxic effect, does not assemble into soluble oligomers." FEBS Lett **582**(13): 1865-1870. doi: 10.1016/j.febslet.2008.05.002. PMC: 18474239.

Durany, N., T. Michel, J. Kurt, F. F. Cruz-Sanchez, J. Cervas-Navarro and P. Riederer (2000). "Brain-derived neurotrophic factor and neurotrophin-3 levels in Alzheimer's disease brains." Int J Dev Neurosci **18**(8): 807-813. PMC: 11156744.

Egan, M. F., M. Kojima, J. H. Callicott, T. E. Goldberg, B. S. Kolachana, A. Bertolino, E. Zaitsev, B. Gold, D. Goldman, M. Dean, B. Lu and D. R. Weinberger (2003). "The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function." Cell **112**(2): 257-269. PMC: 12553913.

Egensperger, R., S. Weggen, N. Ida, G. Multhaup, R. Schnabel, K. Beyreuther and T. A. Bayer (1999). "Reverse relationship between beta-amyloid precursor protein and beta-amyloid peptide plaques in Down's syndrome versus sporadic/familial Alzheimer's disease." Acta Neuropathol **97**(2): 113-118. PMC: 9928821.

Elfving, B., P. H. Plougmann, H. K. Muller, A. A. Mathe, R. Rosenberg and G. Wegener (2010). "Inverse correlation of brain and blood BDNF levels in a genetic rat model of depression." Int J Neuropsychopharmacol **13**(5): 563-572. doi: 10.1017/S1461145709990721. PMC: 19796445.

Elfving, B., P. H. Plougmann and G. Wegener (2010). "Detection of brain-derived neurotrophic factor (BDNF) in rat blood and brain preparations using ELISA: pitfalls and solutions." J Neurosci Methods **187**(1): 73-77. doi: 10.1016/j.jneumeth.2009.12.017. PMC: 20043947.

Elliott, E., R. Atlas, A. Lange and I. Ginzburg (2005). "Brain-derived neurotrophic factor induces a rapid dephosphorylation of tau protein through a PI-3 Kinase signalling mechanism." Eur J Neurosci **22**(5): 1081-1089. doi: 10.1111/j.1460-9568.2005.04290.x. PMC: 16176349.

Erickson, K. I., D. L. Miller and K. A. Roecklein (2012). "The aging hippocampus: interactions between exercise, depression, and BDNF." Neuroscientist **18**(1): 82-97. doi: 10.1177/1073858410397054. PMC: 21531985. PMCID: PMC3575139.

Erickson, K. I., R. S. Prakash, M. W. Voss, L. Chaddock, S. Heo, M. McLaren, B. D. Pence, S. A. Martin, V. J. Vieira, J. A. Woods, E. McAuley and A. F. Kramer (2010). "Brain-derived neurotrophic factor is associated with age-related decline in hippocampal volume." J Neurosci **30**(15): 5368-5375.

doi: 10.1523/JNEUROSCI.6251-09.2010. PMC: 20392958. PMCID: PMC3069644.

Fahnestock, M., D. Garzon, R. M. Holsinger and B. Michalski (2002). "Neurotrophic factors and Alzheimer's disease: are we focusing on the wrong molecule?" J Neural Transm Suppl(62): 241-252. PMC: 12456067.

Falangola, M. F., J. H. Jensen, J. S. Babb, C. Hu, F. X. Castellanos, A. Di Martino, S. H. Ferris and J. A. Helpern (2008). "Age-related non-Gaussian diffusion patterns in the prefrontal brain." J Magn Reson Imaging **28**(6): 1345-1350. doi: 10.1002/jmri.21604. PMC: 19025941. PMCID: PMC2669671.

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. doi: 10.1111/jnc.13447. PMC: 26968403.

Fellgiebel, A. and I. Yakushev (2011). "Diffusion tensor imaging of the hippocampus in MCI and early Alzheimer's disease." J Alzheimers Dis **26 Suppl 3**: 257-262. doi: 10.3233/JAD-2011-0001. PMC: 21971465.

Fernandes, B. S., M. L. Molendijk, C. A. Kohler, J. C. Soares, C. M. Leite, R. Machado-Vieira, T. L. Ribeiro, J. C. Silva, P. M. Sales, J. Quevedo, V. Oertel-Knochel, E. Vieta, A. Gonzalez-Pinto, M. Berk and A. F. Carvalho (2015). "Peripheral brain-derived neurotrophic factor (BDNF) as a biomarker in bipolar disorder: a meta-analysis of 52 studies." BMC Med **13**: 289. doi: 10.1186/s12916-015-0529-7. PMC: 26621529. PMCID: PMC4666054.

Ferreira-Vieira, T. H., I. M. Guimaraes, F. R. Silva and F. M. Ribeiro (2016). "Alzheimer's disease: Targeting the Cholinergic System." Curr Neuropharmacol **14**(1): 101-115. PMC: 26813123. PMCID: PMC4787279.

Ferrer, I., C. Marin, M. J. Rey, T. Ribalta, E. Goutan, R. Blanco, E. Tolosa and E. Marti (1999). "BDNF and full-length and truncated TrkB expression in Alzheimer disease. Implications in therapeutic strategies." J Neuropathol Exp Neurol **58**(7): 729-739. PMC: 10411343.

Ferrer, I., A. Martinez, S. Boluda, P. Parchi and M. Barrachina (2008). "Brain banks: benefits, limitations and cautions concerning the use of post-mortem brain tissue for molecular studies." Cell Tissue Bank **9**(3): 181-194. doi: 10.1007/s10561-008-9077-0. PMC: 18543077.

Fiandaca, M. S., D. Kapogiannis, M. Mapstone, A. Boxer, E. Eitan, J. B. Schwartz, E. L. Abner, R. C. Petersen, H. J. Federoff, B. L. Miller and E. J. Goetzl (2015). "Identification of preclinical Alzheimer's disease by a profile

of pathogenic proteins in neurally derived blood exosomes: A case-control study." Alzheimers Dement **11**(6): 600-607 e601. doi: 10.1016/j.jalz.2014.06.008. PMC: 25130657. PMCID: PMC4329112.

Fieremans, E., A. Benitez, J. H. Jensen, M. F. Falangola, A. Tabesh, R. L. Deardorff, M. V. Spampinato, J. S. Babb, D. S. Novikov, S. H. Ferris and J. A. Helpern (2013). "Novel white matter tract integrity metrics sensitive to Alzheimer disease progression." AJNR Am J Neuroradiol **34**(11): 2105-2112. doi: 10.3174/ajnr.A3553. PMC: 23764722. PMCID: PMC3962262.

Fjell, A. M., L. McEvoy, D. Holland, A. M. Dale, K. B. Walhovd and I. Alzheimer's Disease Neuroimaging (2014). "What is normal in normal aging? Effects of aging, amyloid and Alzheimer's disease on the cerebral cortex and the hippocampus." Prog Neurobiol **117**: 20-40. doi: 10.1016/j.pneurobio.2014.02.004. PMC: 24548606. PMCID: PMC4343307.

Forlenza, O. V., B. S. Diniz, A. L. Teixeira, E. B. Ojopi, L. L. Talib, V. A. Mendonca, G. Izzo and W. F. Gattaz (2010). "Effect of brain-derived neurotrophic factor Val66Met polymorphism and serum levels on the progression of mild cognitive impairment." World J Biol Psychiatry **11**(6): 774-780. doi: 10.3109/15622971003797241. PMC: 20491609.

Forlenza, O. V., B. S. Diniz, A. L. Teixeira, M. Radanovic, L. L. Talib, N. P. Rocha and W. F. Gattaz (2015). "Lower Cerebrospinal Fluid Concentration of Brain-Derived Neurotrophic Factor Predicts Progression from Mild Cognitive Impairment to Alzheimer's Disease." Neuromolecular Med **17**(3): 326-332. doi: 10.1007/s12017-015-8361-y. PMC: 26138246.

Forlenza, O. V., A. S. Miranda, I. Guimar, L. L. Talib, B. S. Diniz, W. F. Gattaz and A. L. Teixeira (2015). "Decreased Neurotrophic Support is Associated with Cognitive Decline in Non-Demented Subjects." J Alzheimers Dis **46**(2): 423-429. doi: 10.3233/JAD-150172. PMC: 25737042.

Fortress, A. M., E. D. Hamlett, E. M. Vazey, G. Aston-Jones, W. A. Cass, H. A. Boger and A. C. Granholm (2015). "Designer receptors enhance memory in a mouse model of Down syndrome." J Neurosci **35**(4): 1343-1353. doi: 10.1523/JNEUROSCI.2658-14.2015. PMC: 25632113. PMCID: PMC4308587.

Franceschi, C., M. Bonafe, S. Valensin, F. Olivieri, M. De Luca, E. Ottaviani and G. De Benedictis (2000). "Inflamm-aging. An evolutionary perspective on immunosenescence." Ann N Y Acad Sci **908**: 244-254. doi: 10.1111/j.1749-6632.2000.tb06651.x

PMC: 10911963.

Franceschi, C., M. BonafÈ, S. Valensin, F. Olivieri, M. De Luca, E. Ottaviani and G. De Benedictis (2006). "Inflamm-aging: An Evolutionary Perspective on Immunosenescence." Annals of the New York Academy of Sciences **908**(1): 244-254. doi: 10.1111/j.1749-6632.2000.tb06651.x.

Franceschi, C. and J. Campisi (2014). "Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases." J Gerontol A Biol Sci Med Sci **69 Suppl 1**: S4-9. doi: 10.1093/gerona/glu057. PMC: 24833586.

Francis, P. T., A. M. Palmer, M. Snape and G. K. Wilcock (1999). "The cholinergic hypothesis of Alzheimer's disease: a review of progress." Journal of Neurology, Neurosurgery & Psychiatry **66**(2): 137-147. doi: 10.1136/jnnp.66.2.137.

Fu, H. Q., T. Yang, W. Xiao, L. Fan, Y. Wu, N. Terrando and T. L. Wang (2014). "Prolonged neuroinflammation after lipopolysaccharide exposure in aged rats." PLoS One **9**(8): e106331. doi: 10.1371/journal.pone.0106331. PMC: 25170959. PMCID: PMC4149545.

Fujimura, H., C. A. Altar, R. Chen, T. Nakamura, T. Nakahashi, J. Kambayashi, B. Sun and N. N. Tandon (2002). "Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation." Thromb Haemost **87**(4): 728-734. PMC: 12008958.

Fukumoto, N., T. Fujii, O. Combarros, M. I. Kamboh, S. J. Tsai, S. Matsushita, B. Nacmias, D. E. Comings, H. Arboleda, M. Ingelsson, B. T. Hyman, H. Akatsu, A. Grupe, A. L. Nishimura, M. Zatz, K. M. Mattila, J. Rinne, Y. Goto, T. Asada, S. Nakamura and H. Kunugi (2010). "Sexually dimorphic effect of the Val66Met polymorphism of BDNF on susceptibility to Alzheimer's disease: New data and meta-analysis." Am J Med Genet B Neuropsychiatr Genet **153B**(1): 235-242. doi: 10.1002/ajmg.b.30986. PMC: 19504537.

Furman, J. L., D. M. Sama, J. C. Gant, T. L. Beckett, M. P. Murphy, A. D. Bachstetter, L. J. Van Eldik and C. M. Norris (2012). "Targeting astrocytes ameliorates neurologic changes in a mouse model of Alzheimer's disease." J Neurosci **32**(46): 16129-16140. doi: 10.1523/JNEUROSCI.2323-12.2012. PMC: 23152597. PMCID: PMC3506017.

Geinisman, Y., L. de Toledo-Morrell, F. Morrell, I. S. Persina and M. Rossi (1992). "Age-related loss of axospinous synapses formed by two afferent systems in the rat dentate gyrus as revealed by the unbiased stereological dissector technique." Hippocampus **2**(4): 437-444.

Gezen-Ak, D., E. Dursun, H. Hanagasi, B. Bilgic, E. Lohman, O. S. Araz, I. L. Atasoy, M. Alaylioglu, B. Onal, H. Gurvit and S. Yilmazer (2013). "BDNF, TNFalpha, HSP90, CFH, and IL-10 serum levels in patients with early or late onset Alzheimer's disease or mild cognitive impairment." J Alzheimers Dis **37**(1): 185-195. doi: 10.3233/JAD-130497. PMC: 23948885.

Ginsberg, S. D., S. Che, J. Wuu, S. E. Counts and E. J. Mufson (2006). "Down regulation of trk but not p75NTR gene expression in single cholinergic basal forebrain neurons mark the progression of Alzheimer's disease." J Neurochem **97**(2): 475-487. doi: 10.1111/j.1471-4159.2006.03764.x. PMC: 16539663.

Goetzl, E. J., A. Boxer, J. B. Schwartz, E. L. Abner, R. C. Petersen, B. L. Miller, O. D. Carlson, M. Mustapic and D. Kapogiannis (2015). "Low neural exosomal levels of cellular survival factors in Alzheimer's disease." Ann Clin Transl Neurol **2**(7): 769-773. doi: 10.1002/acn3.211. PMC: 26273689. PMCID: PMC4531059.

Gomar, J. J., M. T. Bobes-Bascaran, C. Conejero-Goldberg, P. Davies, T. E. Goldberg and I. Alzheimer's Disease Neuroimaging (2011). "Utility of combinations of biomarkers, cognitive markers, and risk factors to predict conversion from mild cognitive impairment to Alzheimer disease in patients in the Alzheimer's disease neuroimaging initiative." Arch Gen Psychiatry **68**(9): 961-969. doi: 10.1001/archgenpsychiatry.2011.96. PMC: 21893661.

Gonzalez, A., G. Moya-Alvarado, C. Gonzalez-Billaut and F. C. Bronfman (2016). "Cellular and molecular mechanisms regulating neuronal growth by brain-derived neurotrophic factor." Cytoskeleton (Hoboken) **73**(10): 612-628. doi: 10.1002/cm.21312. PMC: 27223597.

Granholm, A.-C., G. Gerhardt, M. Bygdeman and I. Strömberg (1992). "Human fetal xenografts of brainstem tissue containing locus coeruleus neurons: functional and structural studies of intraocular grafts in athymic nude rats." Experimental neurology **118**(1): 7-17.

Griffin, W. S., L. C. Stanley, C. Ling, L. White, V. MacLeod, L. J. Perrot, C. L. White, 3rd and C. Araoz (1989). "Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease." Proc Natl Acad Sci U S A **86**(19): 7611-7615. PMC: 2529544. PMCID: PMC298116.

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. doi: 10.1016/j.neurobiolaging.2006.02.007. PMC: 16574280.

Guan, Z. and J. Fang (2006). "Peripheral immune activation by lipopolysaccharide decreases neurotrophins in the cortex and hippocampus in rats." Brain Behav Immun **20**(1): 64-71. doi: 10.1016/j.bbi.2005.04.005. PMC: 15922558.

Gunstad, J., A. Benitez, J. Smith, E. Glickman, M. B. Spitznagel, T. Alexander, J. Juvancic-Heltzel and L. Murray (2008). "Serum brain-derived neurotrophic factor is associated with cognitive function in healthy older adults." J Geriatr Psychiatry Neurol **21**(3): 166-170. doi: 10.1177/0891988708316860. PMC: 18503034.

Haba, R., N. Shintani, Y. Onaka, H. Wang, R. Takenaga, A. Hayata, A. Baba and H. Hashimoto (2012). "Lipopolysaccharide affects exploratory behaviors toward novel objects by impairing cognition and/or motivation in mice: Possible role of activation of the central amygdala." Behav Brain Res **228**(2): 423-431. doi: 10.1016/j.bbr.2011.12.027. PMC: 22209851.

Hager, K., A. S. Baseman, J. S. Nye, H. R. Brashear, J. Han, M. Sano, B. Davis and H. M. Richards (2014). "Effects of galantamine in a 2-year, randomized, placebo-controlled study in Alzheimer's disease." Neuropsychiatr Dis Treat **10**: 391-401. doi: 10.2147/NDT.S57909. PMC: 24591834. PMCID: PMC3937252.

Hakansson, K., A. Ledreux, K. Daffner, Y. Terjestam, P. Bergman, R. Carlsson, M. Kivipelto, B. Winblad, A. C. Granholm and A. K. Mohammed (2017). "BDNF Responses in Healthy Older Persons to 35 Minutes of Physical Exercise, Cognitive Training, and Mindfulness: Associations with Working Memory Function." J Alzheimers Dis **55**(2): 645-657. doi: 10.3233/JAD-160593. PMC: 27716670.

Hamlett, E. D., A. Ledreux, H. Potter, H. J. Chial, D. Patterson, J. M. Espinosa, B. M. Bettcher and A. C. Granholm (2018). "Exosomal biomarkers in Down syndrome and Alzheimer's disease." Free Radic Biol Med **114**: 110-121. doi: 10.1016/j.freeradbiomed.2017.08.028. PMC: 28882786.

Han, W., T. Ji, B. Mei and J. Su (2011). "Peptide p3 may play a neuroprotective role in the brain." Med Hypotheses **76**(4): 543-546. doi: 10.1016/j.mehy.2010.12.013. PMC: 21255941.

Handing, E. P., B. J. Small, R. Andel, C. L. McEvoy and N. Kumar (2017). "Can Nutrition or Inflammation Moderate the Age-Cognition Association Among Older Adults?" J Gerontol B Psychol Sci Soc Sci. doi: 10.1093/geronb/gbx054. PMC: 28482093.

Harada, C. N., M. C. Natelson Love and K. L. Triebel (2013). "Normal cognitive aging." Clin Geriatr Med **29**(4): 737-752. doi: 10.1016/j.cger.2013.07.002. PMC: 24094294. PMCID: PMC4015335.

Hashimoto, K. (2016). "Ethnic differences in the serum levels of proBDNF, a precursor of brain-derived neurotrophic factor (BDNF), in mood disorders." Eur Arch Psychiatry Clin Neurosci **266**(3): 285-287. doi: 10.1007/s00406-015-0641-x. PMC: 26338800.

Hashimoto, K. (2016). "Regulation of brain-derived neurotrophic factor (BDNF) and its precursor proBDNF in the brain by serotonin." Eur Arch Psychiatry Clin Neurosci **266**(3): 195-197. doi: 10.1007/s00406-016-0682-9. PMC: 26899501.

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. doi: 10.1007/s12402-012-0076-4. PMC: 22585442. PMCID: PMC3359455.

Hauss-Wegrzyniak, B., P. Dobrzanski, J. D. Stoehr and G. L. Wenk (1998). "Chronic neuroinflammation in rats reproduces components of the neurobiology of Alzheimer's disease." Brain Res **780**(2): 294-303. PMC: 9507169.

Hauss-Wegrzyniak, B., M. A. Lynch, P. D. Vraniak and G. L. Wenk (2002). "Chronic Brain Inflammation Results in Cell Loss in the Entorhinal Cortex and Impaired LTP in Perforant Path-Granule Cell Synapses." Experimental Neurology **176**(2): 336-341. doi: 10.1006/exnr.2002.7966.

Hauss-Wegrzyniak, B., P. Vraniak and G. L. Wenk (1999). "The effects of a novel NSAID on chronic neuroinflammation are age dependent." Neurobiol Aging **20**(3): 305-313. PMC: 10588578.

He, W., D. Goodkind, P. R. Kowal and U. S. B. o. t. Census (2016). An Aging World : 2015, United States Census Bureau.

Head, E., I. T. Lott, D. M. Wilcock and C. A. Lemere (2016). "Aging in Down Syndrome and the Development of Alzheimer's Disease Neuropathology." Curr Alzheimer Res **13**(1): 18-29. PMC: 26651341. PMCID: PMC4948181.

Hebert, L. E., P. A. Scherr, J. J. McCann, L. A. Beckett and D. A. Evans (2001). "Is the Risk of Developing Alzheimer's Disease Greater for Women than for Men?" American Journal of Epidemiology **153**(2): 132-136. doi: 10.1093/aje/153.2.132.

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. doi: 10.1212/WNL.0b013e31828726f5. PMC: 23390181. PMCID: PMC3719424.

Heijnen, S., B. Hommel, A. Kibele and L. S. Colzato (2015). "Neuromodulation of Aerobic Exercise-A Review." Front Psychol **6**: 1890. doi: 10.3389/fpsyg.2015.01890. PMC: 26779053. PMCID: PMC4703784.

Hendrickx, D. A. E., C. G. van Eden, K. G. Schuurman, J. Hamann and I. Huitinga (2017). "Staining of HLA-DR, Iba1 and CD68 in human microglia reveals partially overlapping expression depending on cellular morphology and pathology." J Neuroimmunol **309**: 12-22. doi: 10.1016/j.jneuroim.2017.04.007. PMC: 28601280.

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 (2015). "Neuroinflammation in Alzheimer's disease." The Lancet Neurology **14**(4): 388-405. doi: 10.1016/s1474-4422(15)70016-5.

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. doi: 20026222. PMC: 11923407.

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. doi: 10.1073/pnas.0909586107. PMC: 20231476. PMCID: PMC2851853.

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. doi: 10.1038/nrn3880. PMC: 25991443.

Hill, B. M., R. H. Laessig, D. D. Koch and D. J. Hassemer (1992). "Comparison of plastic vs. glass evacuated serum-separator (SST) blood-drawing tubes for common clinical chemistry determinations." Clin Chem **38**(8 Pt 1): 1474-1478. PMC: 1643717.

Hock, C., K. Heese, C. Hulette, C. Rosenberg and U. Otten (2000). "Region-Specific Neurotrophin Imbalances in Alzheimer Disease." Archives of Neurology **57**(6). doi: 10.1001/archneur.57.6.846.

Hol, E. M. and M. Pekny (2015). "Glial fibrillary acidic protein (GFAP) and the astrocyte intermediate filament system in diseases of the central nervous system." Curr Opin Cell Biol **32**: 121-130. doi: 10.1016/j.ceb.2015.02.004. PMC: 25726916.

Holleran, L., J. H. Kim, M. Gangolli, T. Stein, V. Alvarez, A. McKee and D. L. Brody (2017). "Axonal disruption in white matter underlying cortical sulcus tau pathology in chronic traumatic encephalopathy." Acta Neuropathol **133**(3): 367-380. doi: 10.1007/s00401-017-1686-x. PMC: 28214960.

Holmes, C. (2013). "Review: systemic inflammation and Alzheimer's disease." Neuropathol Appl Neurobiol **39**(1): 51-68. doi: 10.1111/j.1365-2990.2012.01307.x. PMC: 23046210.

Howard, R., R. McShane, J. Lindesay, C. Ritchie, A. Baldwin, R. Barber, A. Burns, T. Dening, D. Findlay, C. Holmes, A. Hughes, R. Jacoby, R. Jones, R. Jones, I. McKeith, A. Macharouthu, J. O'Brien, P. Passmore, B. Sheehan, E. Juszczak, C. Katona, R. Hills, M. Knapp, C. Ballard, R. Brown, S. Banerjee, C. Onions, M. Griffin, J. Adams, R. Gray, T. Johnson, P. Bentham and P. Phillips (2012). "Donepezil and memantine for moderate-to-severe Alzheimer's disease." N Engl J Med **366**(10): 893-903. doi: 10.1056/NEJMoa1106668. PMC: 22397651.

Hu, X.-Y., H.-Y. Zhang, S. Qin, H. Xu, D. F. Swaab and J.-N. Zhou (2002). "Increased p75NTR Expression in Hippocampal Neurons Containing Hyperphosphorylated τ in Alzheimer Patients." Experimental Neurology **178**(1): 104-111. doi: 10.1006/exnr.2002.8018.

Huang, E. J. and L. F. Reichardt (2001). "Neurotrophins: roles in neuronal development and function." Annu Rev Neurosci **24**: 677-736. doi: 10.1146/annurev.neuro.24.1.677. PMC: 11520916. PMCID: PMC2758233.

Hugenschmidt, C. E., A. M. Peiffer, R. A. Kraft, R. Casanova, A. R. Deibler, J. H. Burdette, J. A. Maldjian and P. J. Laurienti (2008). "Relating imaging indices of white matter integrity and volume in healthy older adults." Cereb Cortex **18**(2): 433-442. doi: 10.1093/cercor/bhm080. PMC: 17575289.

Hung, S. Y. and W. M. Fu (2017). "Drug candidates in clinical trials for Alzheimer's disease." J Biomed Sci **24**(1): 47. doi: 10.1186/s12929-017-0355-7. PMC: 28720101. PMCID: PMC5516350.

Hyman, B. T., C. H. Phelps, T. G. Beach, E. H. Bigio, N. J. Cairns, M. C. Carrillo, D. W. Dickson, C. Duyckaerts, M. P. Frosch, E. Masliah, S. S. Mirra, P. T. Nelson, J. A. Schneider, D. R. Thal, B. Thies, J. Q. Trojanowski, H. V. Vinters and T. J. Montine (2012). "National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease." Alzheimers Dement **8**(1): 1-13. doi: 10.1016/j.jalz.2011.10.007. PMC: 22265587. PMCID: PMC3266529.

Ihara, M., T. M. Polvikoski, R. Hall, J. Y. Slade, R. H. Perry, A. E. Oakley, E. Englund, J. T. O'Brien, P. G. Ince and R. N. Kalaria (2010). "Quantification of myelin loss in frontal lobe white matter in vascular dementia, Alzheimer's disease, and dementia with Lewy bodies." Acta Neuropathol **119**(5): 579-589. doi: 10.1007/s00401-009-0635-8. PMC: 20091409. PMCID: PMC2849937.

Iulita, M. F., S. Do Carmo, A. K. Ower, A. M. Fortress, L. Flores Aguilar, M. Hanna, T. Wisniewski, A. C. Granholm, M. Buhusi, J. Busciglio and A. C. Cuello (2014). "Nerve growth factor metabolic dysfunction in Down's syndrome brains." Brain **137**(Pt 3): 860-872. doi: 10.1093/brain/awt372. PMC: 24519975. PMCID: PMC3927704.

Iyer, S. S., A. A. Ghaffari and G. Cheng (2010). "Lipopolysaccharide-mediated IL-10 transcriptional regulation requires sequential induction of type I IFNs and IL-27 in macrophages." J Immunol **185**(11): 6599-6607. doi: 10.4049/jimmunol.1002041. PMC: 21041726. PMCID: PMC4103176.

Jack, C. R., Jr., D. A. Bennett, K. Blennow, M. C. Carrillo, B. Dunn, S. B. Haeberlein, D. M. Holtzman, W. Jagust, F. Jessen, J. Karlawish, E. Liu, J. L. Molinuevo, T. Montine, C. Phelps, K. P. Rankin, C. C. Rowe, P. Scheltens, E. Siemers, H. M. Snyder, R. Sperling and Contributors (2018). "NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease." Alzheimers Dement **14**(4): 535-562. doi: 10.1016/j.jalz.2018.02.018. PMC: 29653606.

Jack, C. R., Jr. and D. M. Holtzman (2013). "Biomarker modeling of Alzheimer's disease." Neuron **80**(6): 1347-1358. doi: 10.1016/j.neuron.2013.12.003. PMC: 24360540. PMCID: PMC3928967.

Jack, C. R., D. S. Knopman, W. J. Jagust, R. C. Petersen, M. W. Weiner, P. S. Aisen, L. M. Shaw, P. Vemuri, H. J. Wiste, S. D. Weigand, T. G. Lesnick, V. S. Pankratz, M. C. Donohue and J. Q. Trojanowski (2013). "Update on hypothetical model of Alzheimer's disease biomarkers." The Lancet Neurology **12**(2): 207-216. doi: 10.1016/s1474-4422(12)70291-0.

Jeong, H. K., I. Jou and E. H. Joe (2010). "Systemic LPS administration induces brain inflammation but not dopaminergic neuronal death in the substantia

nigra." Exp Mol Med **42**(12): 823-832. doi: 10.3858/emm.2010.42.12.085. PMC: 20962566. PMCID: PMC3015156.

Jiao, S. S., L. L. Shen, C. Zhu, X. L. Bu, Y. H. Liu, C. H. Liu, X. Q. Yao, L. L. Zhang, H. D. Zhou, D. G. Walker, J. Tan, J. Gotz, X. F. Zhou and Y. J. Wang (2016). "Brain-derived neurotrophic factor protects against tau-related neurodegeneration of Alzheimer's disease." Transl Psychiatry **6**(10): e907. doi: 10.1038/tp.2016.186. PMC: 27701410. PMCID: PMC5315549.

Jin, K., A. L. Peel, X. O. Mao, L. Xie, B. A. Cottrell, D. C. Henshall and D. A. Greenberg (2004). "Increased hippocampal neurogenesis in Alzheimer's disease." Proc Natl Acad Sci U S A **101**(1): 343-347. doi: 10.1073/pnas.2634794100. PMC: 14660786. PMCID: PMC314187.

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. doi: 10.1111/j.1460-9568.2006.05223.x. PMC: 17229089.

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. doi: 10.1016/j.neurobiolaging.2006.06.003. PMC: 16837104.

Kao, P. F., M. G. Banigan, C. R. Vanderburg, A. C. McKee, P. R. Polgar, S. Seshadri and I. Delalle (2012). "Increased expression of TrkB and Capzb2 accompanies preserved cognitive status in early Alzheimer disease pathology." J Neuropathol Exp Neurol **71**(7): 654-664. doi: 10.1097/NEN.0b013e31825d06b7. PMC: 22710966. PMCID: PMC3398703.

Karege, F., M. Schwald and M. Cisse (2002). "Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets." Neurosci Lett **328**(3): 261-264. PMC: 12147321.

Karran, E., M. Mercken and B. De Strooper (2011). "The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics." Nat Rev Drug Discov **10**(9): 698-712. doi: 10.1038/nrd3505. PMC: 21852788.

Kawano, T., A. Morikawa, S. Imori, S. Waki, T. Tamura, D. Yamanaka, F. Yamazaki and M. Yokoyama (2014). "Preventive effects of multisensory rehabilitation on development of cognitive dysfunction following systemic

inflammation in aged rats." J Anesth **28**(5): 780-784. doi: 10.1007/s00540-013-1786-2. PMC: 24442128.

Kazim, S. F. and K. Iqbal (2016). "Neurotrophic factor small-molecule mimetics mediated neuroregeneration and synaptic repair: emerging therapeutic modality for Alzheimer's disease." Mol Neurodegener **11**(1): 50. doi: 10.1186/s13024-016-0119-y. PMC: 27400746. PMCID: PMC4940708.

Kellner, E., B. Dhital, V. G. Kiselev and M. Reisert (2016). "Gibbs-ringing artifact removal based on local subvoxel-shifts." Magnetic resonance in medicine **76**(5): 1574-1581.

Kelly, S. C., B. He, S. E. Perez, S. D. Ginsberg, E. J. Mufson and S. E. Counts (2017). "Locus coeruleus cellular and molecular pathology during the progression of Alzheimer's disease." Acta Neuropathol Commun **5**(1): 8. doi: 10.1186/s40478-017-0411-2. PMC: 28109312. PMCID: PMC5251221.

Kesse-Guyot, E., K. E. Assmann, V. A. Andreeva, M. Touvier, L. Neufcourt, N. Shivappa, J. R. Hebert, M. D. Wirth, S. Hercberg, P. Galan and C. Julia (2017). "Long-term association between the dietary inflammatory index and cognitive functioning: findings from the SU.VI.MAX study." Eur J Nutr **56**(4): 1647-1655. doi: 10.1007/s00394-016-1211-3. PMC: 27055851.

Kim, B. Y., S. H. Lee, P. L. Graham, F. Angelucci, A. Lucia, H. Pareja-Galeano, T. Leyhe, Y. Turana, I. R. Lee, J. H. Yoon and J. I. Shin (2017). "Peripheral Brain-Derived Neurotrophic Factor Levels in Alzheimer's Disease and Mild Cognitive Impairment: a Comprehensive Systematic Review and Meta-analysis." Mol Neurobiol **54**(9): 7297-7311. doi: 10.1007/s12035-016-0192-9. PMC: 27815832.

Kim, L. H., K. Plaza, S. R. Thomas, C. Draijer, K. Radford, M. Peters-Golden, M. Mukherjee and P. Nair (2018). "Endogenous peroxidases in sputum interfere with horse-radish peroxidase-based ELISAs." J Immunol Methods **454**: 76-79. doi: 10.1016/j.jim.2017.11.005. PMC: 29154772. PMCID: PMC5863226.

Kim, S., M. J. Kim, S. Kim, H. S. Kang, S. W. Lim, W. Myung, Y. Lee, C. H. Hong, S. H. Choi, D. L. Na, S. W. Seo, B. D. Ku, S. Y. Kim, S. Y. Kim, J. H. Jeong, S. A. Park, B. J. Carroll and D. K. Kim (2015). "Gender differences in risk factors for transition from mild cognitive impairment to Alzheimer's disease: A CREDOS study." Compr Psychiatry **62**: 114-122. doi: 10.1016/j.comppsych.2015.07.002. PMC: 26343475.

Klein, A. B., R. Williamson, M. A. Santini, C. Clemmensen, A. Ettrup, M. Rios, G. M. Knudsen and S. Aznar (2011). "Blood BDNF concentrations reflect

brain-tissue BDNF levels across species." Int J Neuropsychopharmacol **14**(3): 347-353. doi: 10.1017/S1461145710000738. PMC: 20604989.

Knopman, D. S., S. T. DeKosky, J. L. Cummings, H. Chui, J. Corey-Bloom, N. Relkin, G. W. Small, B. Miller and J. C. Stevens (2001). "Practice parameter: diagnosis of dementia (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology." Neurology **56**(9): 1143-1153. PMC: 11342678.

Kohman, R. A., A. J. Tarr, N. L. Sparkman, C. E. Day, A. Paquet, G. R. Akkaraju and G. W. Boehm (2007). "Alleviation of the effects of endotoxin exposure on behavior and hippocampal IL-1beta by a selective non-peptide antagonist of corticotropin-releasing factor receptors." Brain Behav Immun **21**(6): 824-835. doi: 10.1016/j.bbi.2007.01.009. PMC: 17339098.

Konukoglu, D., G. Andican, S. Firtina, G. Erkol and A. Kurt (2012). "Serum brain-derived neurotrophic factor, nerve growth factor and neurotrophin-3 levels in dementia." Acta Neurol Belg **112**(3): 255-260. doi: 10.1007/s13760-012-0101-6. PMC: 22740022.

Krstic, D., A. Madhusudan, J. Doehner, P. Vogel, T. Notter, C. Imhof, A. Manalastas, M. Hilfiker, S. Pfister, C. Schwerdel, C. Riether, U. Meyer and I. Knuesel (2012). "Systemic immune challenges trigger and drive Alzheimer-like neuropathology in mice." J Neuroinflammation **9**: 151. doi: 10.1186/1742-2094-9-151. PMC: 22747753. PMCID: PMC3483167.

Lafenetre, P., O. Leske, Z. Ma-Hogemeie, A. Haghikia, Z. Bichler, P. Wahle and R. Heumann (2010). "Exercise can rescue recognition memory impairment in a model with reduced adult hippocampal neurogenesis." Front Behav Neurosci **3**: 34. doi: 10.3389/neuro.08.034.2009. PMC: 20204139. PMCID: PMC2831627.

Laflamme, N. and S. Rivest (1999). "Effects of systemic immunogenic insults and circulating proinflammatory cytokines on the transcription of the inhibitory factor kappaB alpha within specific cellular populations of the rat brain." J Neurochem **73**(1): 309-321. PMC: 10386984.

Lai, A. Y. and J. McLaurin (2012). "Clearance of amyloid-beta peptides by microglia and macrophages: the issue of what, when and where." Future Neurol **7**(2): 165-176. doi: 10.2217/fnl.12.6. PMC: 22737039. PMCID: PMC3380064.

Lao-Peregrín, C., J. J. Ballesteros, M. Fernández, A. Zamora-Moratalla, A. Saavedra, M. Gómez Lázaro, E. Pérez-Navarro, D. Burks and E. D. Martín (2017). "Caffeine-mediated BDNF release regulates long-term

synaptic plasticity through activation of IRS2 signaling." Addiction biology **22**(6): 1706-1718.

Lapiz, M., Y. Mateo, S. Durkin, T. Parker and C. 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 **155**(3): 251-259. doi: 10.1007/s002130100702.

Larsson, E. M., E. Englund, M. Sjobeck, J. Latt and S. Brockstedt (2004). "MRI with diffusion tensor imaging post-mortem at 3.0 T in a patient with frontotemporal dementia." Dement Geriatr Cogn Disord **17**(4): 316-319. doi: 10.1159/000077162. PMC: 15178944.

Laske, C., K. Stellos, N. Hoffmann, E. Stransky, G. Straten, G. W. Eschweiler and T. Leyhe (2011). "Higher BDNF serum levels predict slower cognitive decline in Alzheimer's disease patients." Int J Neuropsychopharmacol **14**(3): 399-404. doi: 10.1017/S1461145710001008. PMC: 20860877.

Laske, C., E. Stransky, T. Leyhe, G. W. Eschweiler, W. Maetzler, A. Wittorf, S. Soekadar, E. Richartz, N. Koehler, M. Bartels, G. Buchkremer and K. Schott (2007). "BDNF serum and CSF concentrations in Alzheimer's disease, normal pressure hydrocephalus and healthy controls." J Psychiatr Res **41**(5): 387-394. doi: 10.1016/j.jpsychires.2006.01.014. PMC: 16554070.

Laske, C., E. Stransky, T. Leyhe, G. W. Eschweiler, K. Schott, H. Langer and M. Gawaz (2006). "Decreased brain-derived neurotrophic factor (BDNF)- and beta-thromboglobulin (beta-TG)- blood levels in Alzheimer's disease." Thromb Haemost **96**(1): 102-103. doi: 10.1160/TH06-03-0173. PMC: 16807663.

Laske, C., E. Stransky, T. Leyhe, G. W. Eschweiler, A. Wittorf, E. Richartz, M. Bartels, G. Buchkremer and K. Schott (2006). "Stage-dependent BDNF serum concentrations in Alzheimer's disease." J Neural Transm (Vienna) **113**(9): 1217-1224. doi: 10.1007/s00702-005-0397-y. PMC: 16362629.

Laws, K. R., K. Irvine and T. M. Gale (2016). "Sex differences in cognitive impairment in Alzheimer's disease." World J Psychiatry **6**(1): 54-65. doi: 10.5498/wjp.v6.i1.54. PMC: 27014598. PMCID: PMC4804268.

Ledreux, A., E. D. Hamlett, J. Lockrow, K. L. Bharani and A. C. Granholm (2016). Neurodegeneration and Memory Loss in Down Syndrome: Perspectives on Potential Therapeutics. Research Progress in Alzheimer's Disease and Dementia. . M.-K. Sun, Nova Publishers. **6:** 169-194.

Lee, J., H. Fukumoto, J. Orne, J. Klucken, S. Raju, C. R. Vanderburg, M. C. Irizarry, B. T. Hyman and M. Ingelsson (2005). "Decreased levels of BDNF

protein in Alzheimer temporal cortex are independent of BDNF polymorphisms." Exp Neurol **194**(1): 91-96. doi: 10.1016/j.expneurol.2005.01.026. PMC: 15899246.

Lee, J. G., B. S. Shin, Y. S. You, J. E. Kim, S. W. Yoon, D. W. Jeon, J. H. Baek, S. W. Park and Y. H. Kim (2009). "Decreased serum brain-derived neurotrophic factor levels in elderly korean with dementia." Psychiatry Investig **6**(4): 299-305. doi: 10.4306/pi.2009.6.4.299. PMC: 20140129. PMCID: PMC2808800.

Lessmann, V., K. Gottmann and M. Malcangio (2003). "Neurotrophin secretion: current facts and future prospects." Progress in Neurobiology **69**(5): 341-374. doi: 10.1016/s0301-0082(03)00019-4.

Levin, R., A. E. Dor-Abarbanel, S. Edelman, A. R. Durrant, K. Hashimoto, D. C. Javitt and U. Heresco-Levy (2015). "Behavioral and cognitive effects of the N-methyl-D-aspartate receptor co-agonist D-serine in healthy humans: initial findings." J Psychiatr Res **61**: 188-195. doi: 10.1016/j.jpsychires.2014.12.007. PMC: 25554623.

Lewis, J. and D. W. Dickson (2016). "Propagation of tau pathology: hypotheses, discoveries, and yet unresolved questions from experimental and human brain studies." Acta Neuropathol **131**(1): 27-48. doi: 10.1007/s00401-015-1507-z. PMC: 26576562.

Leyhe, T., G. W. Eschweiler, E. Stransky, T. Gasser, P. Annas, H. Basun and C. Laske (2009). "Increase of BDNF serum concentration in lithium treated patients with early Alzheimer's disease." J Alzheimers Dis **16**(3): 649-656. doi: 10.3233/JAD-2009-1004. PMC: 19276559.

Leyhe, T., E. Stransky, G. W. Eschweiler, G. Buchkremer and C. Laske (2008). "Increase of BDNF serum concentration during donepezil treatment of patients with early Alzheimer's disease." Eur Arch Psychiatry Clin Neurosci **258**(2): 124-128. doi: 10.1007/s00406-007-0764-9. PMC: 17990049.

Li, G., E. R. Peskind, S. P. Millard, P. Chi, I. Sokal, C. E. Yu, L. M. Bekris, M. A. Raskind, D. R. Galasko and T. J. Montine (2009). "Cerebrospinal fluid concentration of brain-derived neurotrophic factor and cognitive function in non-demented subjects." PLoS One **4**(5): e5424. doi: 10.1371/journal.pone.0005424. PMC: 19412541. PMCID: PMC2671606.

Li, Y. X., W. Tokuyama, H. Okuno, Y. Miyashita and T. Hashimoto (2001). "Differential induction of brain-derived neurotrophic factor mRNA in rat inferior olive subregions following unilateral labyrinthectomy." Neuroscience **106**(2): 385-394. PMC: 11566508.

Lim, J. Y., C. P. Reighard and D. C. Crowther (2015). "The pro-domains of neurotrophins, including BDNF, are linked to Alzheimer's disease through a toxic synergy with Abeta." Hum Mol Genet **24**(14): 3929-3938. doi: 10.1093/hmg/ddv130. PMC: 25954034. PMCID: PMC4476443.

Lim, Y., J. H. Zhong and X. F. Zhou (2015). "Development of mature BDNF-specific sandwich ELISA." J Neurochem **134**(1): 75-85. doi: 10.1111/jnc.13108. PMC: 25824396.

Lim, Y. Y., J. Hassenstab, C. Cruchaga, A. Goate, A. M. Fagan, T. L. Benzinger, P. Maruff, P. J. Snyder, C. L. Masters, R. Allegri, J. Chhatwal, M. R. Farlow, N. R. Graff-Radford, C. Laske, J. Levin, E. McDade, J. M. Ringman, M. Rossor, S. Salloway, P. R. Schofield, D. M. Holtzman, J. C. Morris, R. J. Bateman and N. Dominantly Inherited Alzheimer (2016). "BDNF Val66Met moderates memory impairment, hippocampal function and tau in preclinical autosomal dominant Alzheimer's disease." Brain **139**(Pt 10): 2766-2777. doi: 10.1093/brain/aww200. PMC: 27521573. PMCID: PMC5815565.

Lin, K. A., K. R. Choudhury, B. G. Rathakrishnan, D. M. Marks, J. R. Petrella, P. M. Doraiswamy and I. Alzheimer's Disease Neuroimaging (2015). "Marked gender differences in progression of mild cognitive impairment over 8 years." Alzheimers Dement (N Y) **1**(2): 103-110. doi: 10.1016/j.trci.2015.07.001. PMC: 26451386. PMCID: PMC4593067.

Linnarsson, S., A. Bjorklund and P. Ernfors (1997). "Learning deficit in BDNF mutant mice." Eur J Neurosci **9**(12): 2581-2587. PMC: 9517463.

Liu, Y. H., S. S. Jiao, Y. R. Wang, X. L. Bu, X. Q. Yao, Y. Xiang, Q. H. Wang, L. Wang, J. Deng, J. Li, X. F. Zhou, H. D. Zhou and Y. J. Wang (2015). "Associations Between ApoEepsilon4 Carrier Status and Serum BDNF Levels--New Insights into the Molecular Mechanism of ApoEepsilon4 Actions in Alzheimer's Disease." Mol Neurobiol **51**(3): 1271-1277. doi: 10.1007/s12035-014-8804-8. PMC: 24986007.

Lockrow, J., H. Boger, G. Gerhardt, G. Aston-Jones, D. Bachman and A. C. Granholm (2011). "A noradrenergic lesion exacerbates neurodegeneration in a Down syndrome mouse model." J Alzheimers Dis **23**(3): 471-489. doi: 10.3233/JAD-2010-101218. PMC: 21098982. PMCID: PMC3991557.

Lommatzsch, M., D. Zingler, K. Schuhbaeck, K. Schloetcke, C. Zingler, P. Schuff-Werner and J. C. Virchow (2005). "The impact of age, weight and gender on BDNF levels in human platelets and plasma." Neurobiol Aging **26**(1): 115-123. doi: 10.1016/j.neurobiolaging.2004.03.002. PMC: 15585351.

Lue, L. F., L. Brachova, W. H. Civin and J. Rogers (1996). "Inflammation, A beta deposition, and neurofibrillary tangle formation as correlates of Alzheimer's disease neurodegeneration." J Neuropathol Exp Neurol **55**(10): 1083-1088. PMC: 8858005.

Lyons, W. E., L. A. Mamounas, G. A. Ricaurte, V. Coppola, S. W. Reid, S. H. Bora, C. Wihler, V. E. Koliatsos and L. Tessarollo (1999). "Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities." Proc Natl Acad Sci U S A **96**(26): 15239-15244. PMC: 10611369. PMCID: PMC24804.

Malkki, H. (2015). "Alzheimer disease: possible prion-like transmission of AD-like pathology in humans." Nature reviews Neurology **11**(11): 612.

Mandel, A. L., H. Ozdener and V. Utermohlen (2009). "Identification of pro- and mature brain-derived neurotrophic factor in human saliva." Arch Oral Biol **54**(7): 689-695. doi: 10.1016/j.archoralbio.2009.04.005. PMC: 19467646. PMCID: PMC2716651.

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

Manna, A., F. Piras, C. Caltagirone, P. Bossu, S. L. Sensi and G. Spalletta (2015). "Left hippocampus-amygdala complex macro- and microstructural variation is associated with BDNF plasma levels in healthy elderly individuals." Brain Behav **5**(7): e00334. doi: 10.1002/brb3.334. PMC: 26221568. PMCID: PMC4511284.

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. doi: 10.1016/j.brainresrev.2004.02.002. PMC: 15063099.

Matrone, C., M. T. Ciotti, D. Mercanti, R. Marolda and P. Calissano (2008). "NGF and BDNF signaling control amyloidogenic route and Abeta production in hippocampal neurons." Proc Natl Acad Sci U S A **105**(35): 13139-13144. doi: 10.1073/pnas.0806133105. PMC: 18728191. PMCID: PMC2525562.

Maurer, K., S. Volk and H. Gerbaldo (1997). "Auguste D and Alzheimer's disease." The Lancet **349**(9064): 1546-1549. doi: 10.1016/s0140-6736(96)10203-8.

McGeer, P. L. and E. G. McGeer (2007). "NSAIDs and Alzheimer disease: epidemiological, animal model and clinical studies." Neurobiol Aging **28**(5): 639-647. doi: 10.1016/j.neurobiolaging.2006.03.013. PMC: 16697488.

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. doi: 10.1016/j.jalz.2011.03.005. PMC: 21514250. PMCID: PMC3312024.

Medeiros, R. and F. M. LaFerla (2013). "Astrocytes: Conductors of the Alzheimer disease neuroinflammatory symphony." Experimental Neurology **239**: 133-138. doi: https://doi.org/10.1016/j.expneurol.2012.10.007.

Meeker, R. and K. Williams (2014). "Dynamic nature of the p75 neurotrophin receptor in response to injury and disease." J Neuroimmune Pharmacol **9**(5): 615-628. doi: 10.1007/s11481-014-9566-9. PMC: 25239528. PMCID: PMC4243516.

Mello-Carpes, P. B., L. da Silva de Vargas, M. C. Gayer, R. Roehrs and I. Izquierdo (2016). "Hippocampal noradrenergic activation is necessary for object recognition memory consolidation and can promote BDNF increase and memory persistence." Neurobiol Learn Mem **127**: 84-92. doi: 10.1016/j.nlm.2015.11.014. PMC: 26691781.

Michalski, B., M. M. Corrada, C. H. Kawas and M. Fahnestock (2015). "Brain-derived neurotrophic factor and TrkB expression in the "oldest-old," the 90+ Study: correlation with cognitive status and levels of soluble amyloid-beta." Neurobiol Aging **36**(12): 3130-3139. doi: 10.1016/j.neurobiolaging.2015.08.022. PMC: 26410307. PMCID: PMC4756909.

Michalski, B. and M. Fahnestock (2003). "Pro-brain-derived neurotrophic factor is decreased in parietal cortex in Alzheimer's disease." Molecular Brain Research **111**(1-2): 148-154. doi: 10.1016/s0169-328x(03)00003-2.

Mielke, M. M., P. Vemuri and W. A. Rocca (2014). "Clinical epidemiology of Alzheimer's disease: assessing sex and gender differences." Clin Epidemiol **6**: 37-48. doi: 10.2147/CLEP.S37929. PMC: 24470773. PMCID: PMC3891487.

Mineyko, A., A. Narendran, M. L. Fritzler, X. C. Wei, H. Schmeling and A. Kirton (2012). "Inflammatory biomarkers of pediatric focal cerebral arteriopathy." Neurology **79**(13): 1406-1408. doi: 10.1212/WNL.0b013e31826c199e. PMC: 22914842.

Mitchell, A. J. and M. Shiri-Feshki (2009). "Rate of progression of mild cognitive impairment to dementia--meta-analysis of 41 robust inception cohort

studies." Acta Psychiatr Scand **119**(4): 252-265. doi: 10.1111/j.1600-0447.2008.01326.x. PMC: 19236314.

Molendijk, M. L., B. A. Bus, P. Spinhoven, A. Kaimatzoglou, R. C. Oude Voshaar, B. W. Penninx, I. M. H. van and B. M. Elzinga (2012). "A systematic review and meta-analysis on the association between BDNF val(66)met and hippocampal volume--a genuine effect or a winners curse?" Am J Med Genet B Neuropsychiatr Genet **159B**(6): 731-740. doi: 10.1002/ajmg.b.32078. PMC: 22815222.

Montag, C., J. C. Schoene-Bake, J. Faber, M. Reuter and B. Weber (2010). "Genetic variation on the BDNF gene is not associated with differences in white matter tracts in healthy humans measured by tract-based spatial statistics." Genes Brain Behav **9**(8): 886-891. doi: 10.1111/j.1601-183X.2010.00626.x. PMC: 20662942.

Montine, T. J., C. H. Phelps, T. G. Beach, E. H. Bigio, N. J. Cairns, D. W. Dickson, C. Duyckaerts, M. P. Frosch, E. Masliah, S. S. Mirra, P. T. Nelson, J. A. Schneider, D. R. Thal, J. Q. Trojanowski, H. V. Vinters, B. T. Hyman, A. National Institute on and A. Alzheimer's (2012). "National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach." Acta Neuropathol **123**(1): 1-11. doi: 10.1007/s00401-011-0910-3. PMC: 22101365. PMCID: PMC3268003.

Morales, I., J. M. Jiménez, M. Mancilla and R. B. Maccioni (2013). "Tau oligomers and fibrils induce activation of microglial cells." Journal of Alzheimer's Disease **37**(4): 849-856.

Morimoto, K., J. Horio, H. Satoh, L. Sue, T. Beach, S. Arita, I. Tooyama and Y. Konishi (2011). "Expression profiles of cytokines in the brains of Alzheimer's disease (AD) patients compared to the brains of non-demented patients with and without increasing AD pathology." J Alzheimers Dis **25**(1): 59-76. doi: 10.3233/JAD-2011-101815. PMC: 21368376. PMCID: PMC3572941.

Mufson, E. J., S. E. Counts, S. E. Perez and S. D. Ginsberg (2008). "Cholinergic system during the progression of Alzheimer's disease: therapeutic implications." Expert Rev Neurother **8**(11): 1703-1718. doi: 10.1586/14737175.8.11.1703. PMC: 18986241. PMCID: PMC2631573.

Muir, J., S. Dunnett, T. Robbins and B. Everitt (1992). "Attentional functions of the forebrain cholinergic systems: effects of intraventricular hemicholinium, physostigmine, basal forebrain lesions and intracortical grafts on a multiple-choice serial reaction time task." Experimental Brain Research **89**(3): 611-622.

Murer, M. G., F. Boissiere, Q. Yan, S. Hunot, J. Villares, B. Faucheux, Y. Agid, E. Hirsch and R. Raisman-Vozari (1999). "An immunohistochemical study of the distribution of brain-derived neurotrophic factor in the adult human brain, with particular reference to Alzheimer's disease." Neuroscience **88**(4): 1015-1032. PMC: 10336117.

Murer, M. G., Q. Yan and R. Raisman-Vozari (2001). "Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease." Prog Neurobiol **63**(1): 71-124. PMC: 11040419.

Murray, C. A. and M. A. Lynch (1998). "Evidence that increased hippocampal expression of the cytokine interleukin-1 beta is a common trigger for age- and stress-induced impairments in long-term potentiation." J Neurosci **18**(8): 2974-2981. PMC: 9526014.

Nabavi, S., R. Fox, C. D. Proulx, J. Y. Lin, R. Y. Tsien and R. Malinow (2014). "Engineering a memory with LTD and LTP." Nature **511**(7509): 348-352. doi: 10.1038/nature13294. PMC: 24896183. PMCID: PMC4210354.

Nagahara, A. H. and M. H. Tuszynski (2011). "Potential therapeutic uses of BDNF in neurological and psychiatric disorders." Nat Rev Drug Discov **10**(3): 209-219. doi: 10.1038/nrd3366. PMC: 21358740.

Nagahara, A. H., B. R. Wilson, I. Ivasyk, I. Kovacs, S. Rawalji, J. R. Bringas, P. J. Pivirotto, W. S. Sebastian, L. Samaranch, K. S. Bankiewicz and M. H. Tuszynski (2018). "MR-guided delivery of AAV2-BDNF into the entorhinal cortex of non-human primates." Gene Ther **25**(2): 104-114. doi: 10.1038/s41434-018-0010-2. PMC: 29535375. PMCID: PMC5924461.

Nakai, S., W. Matsunaga, Y. Ishida, K. Isobe and T. Shirokawa (2006). "Effects of BDNF infusion on the axon terminals of locus coeruleus neurons of aging rats." Neurosci Res **54**(3): 213-219. doi: 10.1016/j.neures.2005.12.001. PMC: 16406148.

Narisawa-Saito, M., K. Wakabayashi, S. Tsuji, H. Takahashi and H. Nawa (1996). "Regional specificity of alterations in NGF, BDNF and NT-3 levels in Alzheimer's disease." Neuroreport **7**(18): 2925-2928. PMC: 9116211.

Nelson, P. T., I. Alafuzoff, E. H. Bigio, C. Bouras, H. Braak, N. J. Cairns, R. J. Castellani, B. J. Crain, P. Davies, K. Del Tredici, C. Duyckaerts, M. P. Frosch, V. Haroutunian, P. R. Hof, C. M. Hulette, B. T. Hyman, T. Iwatsubo, K. A. Jellinger, G. A. Jicha, E. Kovari, W. A. Kukull, J. B. Leverenz, S. Love, I. R. Mackenzie, D. M. Mann, E. Masliah, A. C. McKee, T. J. Montine, J. C. Morris, J. A. Schneider, J. A. Sonnen, D. R. Thal, J. Q. Trojanowski, J. C. Troncoso, T. Wisniewski, R. L. Woltjer and T. G. Beach (2012). "Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature." J Neuropathol Exp Neurol

71(5): 362-381. doi: 10.1097/NEN.0b013e31825018f7. PMC: 22487856. PMCID: PMC3560290.

Nettiksimmons, J., E. M. Simonsick, T. Harris, S. Satterfield, C. Rosano, K. Yaffe and A. B. C. S. Health (2014). "The associations between serum brain-derived neurotrophic factor, potential confounders, and cognitive decline: a longitudinal study." PLoS One **9**(3): e91339. doi: 10.1371/journal.pone.0091339. PMC: 24670553. PMCID: PMC3966768.

Nockher, W. A. and H. Renz (2005). "Neurotrophins in clinical diagnostics: pathophysiology and laboratory investigation." Clin Chim Acta **352**(1-2): 49-74. doi: 10.1016/j.cccn.2004.10.002. PMC: 15653100.

Nolan, Y., F. O. Maher, D. S. Martin, R. M. Clarke, M. T. Brady, A. E. Bolton, K. H. Mills and M. A. Lynch (2005). "Role of interleukin-4 in regulation of age-related inflammatory changes in the hippocampus." J Biol Chem **280**(10): 9354-9362. doi: 10.1074/jbc.M412170200. PMC: 15615726.

O'Bryant, S. E., V. L. Hobson, J. R. Hall, R. C. Barber, S. Zhang, L. Johnson, R. Diaz-Arrastia and C. Texas Alzheimer's Research (2011). "Serum brain-derived neurotrophic factor levels are specifically associated with memory performance among Alzheimer's disease cases." Dement Geriatr Cogn Disord **31**(1): 31-36. doi: 10.1159/000321980. PMC: 21135555. PMCID: PMC3019366.

O'Donnell, J., D. Zeppenfeld, E. McConnell, S. Pena and M. Nedergaard (2012). "Norepinephrine: a neuromodulator that boosts the function of multiple cell types to optimize CNS performance." Neurochem Res **37**(11): 2496-2512. doi: 10.1007/s11064-012-0818-x. PMC: 22717696. PMCID: PMC3548657.

Osborn, L. M., W. Kamphuis, W. J. Wadman and E. M. Hol (2016). "Astrogliosis: An integral player in the pathogenesis of Alzheimer's disease." Prog Neurobiol **144**: 121-141. doi: 10.1016/j.pneurobio.2016.01.001. PMC: 26797041.

Osuchowski, M. F., F. Craciun, K. M. Weixelbaumer, E. R. Duffy and D. G. Remick (2012). "Sepsis chronically in MARS: systemic cytokine responses are always mixed regardless of the outcome, magnitude, or phase of sepsis." J Immunol **189**(9): 4648-4656. doi: 10.4049/jimmunol.1201806. PMC: 23008446. PMCID: PMC3478412.

Osuchowski, M. F., F. L. Craciun, E. Schuller, C. Sima, R. Gyurko and D. G. Remick (2010). "Untreated type 1 diabetes increases sepsis-induced mortality without inducing a prelethal cytokine response." Shock **34**(4): 369-376. doi: 10.1097/SHK.0b013e3181dc40a8. PMC: 20610941. PMCID: PMC2941557.

Pan, W., W. A. Banks, M. B. Fasold, J. Bluth and A. J. Kastin (1998). "Transport of brain-derived neurotrophic factor across the blood-brain barrier." Neuropharmacology **37**(12): 1553-1561. PMC: 9886678.

Parkhurst, C. N., G. Yang, I. Ninan, J. N. Savas, J. R. Yates, 3rd, J. J. Lafaille, B. L. Hempstead, D. R. Littman and W. B. Gan (2013). "Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor." Cell **155**(7): 1596-1609. doi: 10.1016/j.cell.2013.11.030. PMC: 24360280. PMCID: PMC4033691.

Patterson, S. L. (2015). "Immune dysregulation and cognitive vulnerability in the aging brain: Interactions of microglia, IL-1beta, BDNF and synaptic plasticity." Neuropharmacology **96**(Pt A): 11-18. doi: 10.1016/j.neuropharm.2014.12.020. PMC: 25549562. PMCID: PMC4475415.

Peng, S., J. Wuu, E. J. Mufson and M. Fahnestock (2005). "Precursor form of brain-derived neurotrophic factor and mature brain-derived neurotrophic factor are decreased in the pre-clinical stages of Alzheimer's disease." J Neurochem **93**(6): 1412-1421. doi: 10.1111/j.1471-4159.2005.03135.x. PMC: 15935057.

Perini, G., V. Della-Bianca, V. Politi, G. Della Valle, I. Dal-Pra, F. Rossi and U. Armato (2002). "Role of p75 neurotrophin receptor in the neurotoxicity by beta-amyloid peptides and synergistic effect of inflammatory cytokines." J Exp Med **195**(7): 907-918. PMC: 11927634. PMCID: PMC2193732.

Perry, E. K., P. H. Gibson, G. Blessed, R. H. Perry and B. E. Tomlinson (1977). "Neurotransmitter enzyme abnormalities in senile dementia." Journal of the Neurological Sciences **34**(2): 247-265. doi: 10.1016/0022-510x(77)90073-9.

Perry, V. H. (2010). "Contribution of systemic inflammation to chronic neurodegeneration." Acta Neuropathol **120**(3): 277-286. doi: 10.1007/s00401-010-0722-x. PMC: 20644946.

Perry, V. H., C. Cunningham and C. Holmes (2007). "Systemic infections and inflammation affect chronic neurodegeneration." Nat Rev Immunol **7**(2): 161-167. doi: 10.1038/nri2015. PMC: 17220915.

Petersen, R. C., R. Doody, A. Kurz, R. C. Mohs, J. C. Morris, P. V. Rabins, K. Ritchie, M. Rossor, L. Thal and B. Winblad (2001). "Current concepts in mild cognitive impairment." Arch Neurol **58**(12): 1985-1992. PMC: 11735772.

Petersen, R. C., R. O. Roberts, D. S. Knopman, B. F. Boeve, Y. E. Geda, R. J. Ivnik, G. E. Smith and C. R. Jack, Jr. (2009). "Mild cognitive impairment:

ten years later." Arch Neurol **66**(12): 1447-1455. doi: 10.1001/archneurol.2009.266. PMC: 20008648. PMCID: PMC3081688.

Petersen, R. C., G. E. Smith, R. J. Ivnik, E. G. Tangalos, D. J. Schaid, S. N. Thibodeau, E. Kokmen, S. C. Waring and L. T. Kurland (1995). "Apolipoprotein E status as a predictor of the development of Alzheimer's disease in memory-impaired individuals." Jama **273**(16): 1274-1278.

Petzold, A., L. Psotta, T. Brigadski, T. Endres and V. Lessmann (2015). "Chronic BDNF deficiency leads to an age-dependent impairment in spatial learning." Neurobiol Learn Mem **120**: 52-60. doi: 10.1016/j.nlm.2015.02.009. PMC: 25724412.

Phillips, C., M. A. Baktir, M. Srivatsan and A. Salehi (2014). "Neuroprotective effects of physical activity on the brain: a closer look at trophic factor signaling." Front Cell Neurosci **8**: 170. doi: 10.3389/fncel.2014.00170. PMC: 24999318. PMCID: PMC4064707.

Piepmeier, A. T. and J. L. Etnier (2015). "Brain-derived neurotrophic factor (BDNF) as a potential mechanism of the effects of acute exercise on cognitive performance." Journal of Sport and Health Science **4**(1): 14-23. doi: 10.1016/j.jshs.2014.11.001.

Poduslo, J. F. and G. L. Curran (1996). "Permeability at the blood-brain and blood-nerve barriers of the neurotrophic factors: NGF, CNTF, NT-3, BDNF." Brain Res Mol Brain Res **36**(2): 280-286. PMC: 8965648.

Polacchini, A., G. Metelli, R. Francavilla, G. Baj, M. Florean, L. G. Mascaretti and E. Tongiorgi (2015). "A method for reproducible measurements of serum BDNF: comparison of the performance of six commercial assays." Sci Rep **5**: 17989. doi: 10.1038/srep17989. PMC: 26656852. PMCID: PMC4675070.

Polyakova, M., H. Schlogl, J. Sacher, M. Schmidt-Kassow, J. Kaiser, M. Stumvoll, J. Kratzsch and M. L. Schroeter (2017). "Stability of BDNF in Human Samples Stored Up to 6 Months and Correlations of Serum and EDTA-Plasma Concentrations." Int J Mol Sci **18**(6). doi: 10.3390/ijms18061189. PMC: 28587193. PMCID: PMC5486012.

Preissner, C. M., W. M. Reilly, R. C. Cyr, D. J. O'Kane, R. J. Singh and S. K. Grebe (2004). "Plastic versus glass tubes: effects on analytical performance of selected serum and plasma hormone assays." Clin Chem **50**(7): 1245-1247. doi: 10.1373/clinchem.2004.034108. PMC: 15229156.

Prince, M. J., F. Wu, Y. Guo, L. M. Gutierrez Robledo, M. O'Donnell, R. Sullivan and S. Yusuf (2015). "The burden of disease in older people and

implications for health policy and practice." The Lancet **385**(9967): 549-562. doi: 10.1016/s0140-6736(14)61347-7.

Pugh, C. R., K. Kumagawa, M. Fleshner, L. R. Watkins, S. F. Maier and J. W. Rudy (1998). "Selective effects of peripheral lipopolysaccharide administration on contextual and auditory-cue fear conditioning." Brain Behav Immun **12**(3): 212-229. doi: 10.1006/brbi.1998.0524. PMC: 9769157.

Pugh, C. R., K. T. Nguyen, J. L. Gonyea, M. Fleshner, L. R. Wakins, S. F. Maier and J. W. Rudy (1999). "Role of interleukin-1 beta in impairment of contextual fear conditioning caused by social isolation." Behav Brain Res **106**(1-2): 109-118. PMC: 10595426.

Qin, L., X. Wu, M. L. Block, Y. Liu, G. R. Breese, J. S. Hong, D. J. Knapp and F. T. Crews (2007). "Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration." Glia **55**(5): 453-462. doi: 10.1002/glia.20467. PMC: 17203472. PMCID: PMC2871685.

Radka, S. F., P. A. Holst, M. Fritsche and C. A. Altar (1996). "Presence of brain-derived neurotrophic factor in brain and human and rat but not mouse serum detected by a sensitive and specific immunoassay." Brain Res **709**(1): 122-301. PMC: 8869564.

Rage, F., M. Silhol, F. Biname, S. Arancibia and L. Tapia-Arancibia (2007). "Effect of aging on the expression of BDNF and TrkB isoforms in rat pituitary." Neurobiol Aging **28**(7): 1088-1098. doi: 10.1016/j.neurobiolaging.2006.05.013. PMC: 16769156.

Raina, P. (2008). "Effectiveness of cholinesterase inhibitors and memantine for treating dementia: evidence review for a clinical practice guideline." PMC: 18316756.

Rasmussen, P., P. Brassard, H. Adser, M. V. Pedersen, L. Leick, E. Hart, N. H. Secher, B. K. Pedersen and H. Pilegaard (2009). "Evidence for a release of brain-derived neurotrophic factor from the brain during exercise." Exp Physiol **94**(10): 1062-1069. doi: 10.1113/expphysiol.2009.048512. PMC: 19666694.

Rengo, G., G. Pagano, D. F. Vitale, R. Formisano, K. Komici, L. Petraglia, V. Parisi, G. D. Femminella, C. de Lucia, S. Paolillo, A. Cannavo, E. Attena, T. Pellegrino, S. Dellegrottaglie, A. Memmi, B. Trimarco, A. Cuocolo, P. P. Filardi, D. Leosco and N. Ferrara (2016). "Impact of aging on cardiac sympathetic innervation measured by (123)I-mIBG imaging in patients with systolic heart failure." Eur J Nucl Med Mol Imaging **43**(13): 2392-2400. doi: 10.1007/s00259-016-3432-3. PMC: 27287990.

Rogers, J. L. and R. P. Kesner (2004). "Cholinergic modulation of the hippocampus during encoding and retrieval of tone/shock-induced fear conditioning." Learn Mem **11**(1): 102-107. doi: 10.1101/lm.64604. PMC: 14747523. PMCID: PMC321320.

Roggero, E., A. R. Perez, N. Pollachini, S. R. Villar, J. Wildmann, H. Besedovsky and A. Del Rey (2016). "The sympathetic nervous system affects the susceptibility and course of Trypanosoma cruzi infection." Brain Behav Immun **58**: 228-236. doi: 10.1016/j.bbi.2016.07.163. PMC: 27485039.

Rosa, E. and M. Fahnestock (2014). Amyloid-Beta, BDNF, and the Mechanism of Neurodegeneration in Alzheimer's Disease. Handbook of Neurotoxicity**:** 1597-1620.

Ross, J. A., P. McGonigle and E. J. Van Bockstaele (2015). "Locus Coeruleus, norepinephrine and Abeta peptides in Alzheimer's disease." Neurobiol Stress **2**: 73-84. doi: 10.1016/j.ynstr.2015.09.002. PMC: 26618188. PMCID: PMC4657149.

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. doi: 10.1007/s12640-014-9482-z. PMC: 24964753.

Rylett, R. J., M. J. Ball and E. H. Colhoun (1983). "Evidence for high affinity choline transport in synaptosomes prepared from hippocampus and neocortex of patients with Alzheimer's disease." Brain Research **289**(1-2): 169-175. doi: 10.1016/0006-8993(83)90017-3.

Sara, S. J. (2009). "The locus coeruleus and noradrenergic modulation of cognition." Nat Rev Neurosci **10**(3): 211-223. doi: 10.1038/nrn2573. PMC: 19190638.

Sartorius, A., R. Hellweg, J. Litzke, M. Vogt, C. Dormann, B. Vollmayr, H. Danker-Hopfe and P. Gass (2009). "Correlations and discrepancies between serum and brain tissue levels of neurotrophins after electroconvulsive treatment in rats." Pharmacopsychiatry **42**(6): 270-276. doi: 10.1055/s-0029-1224162. PMC: 19924587.

Schaaf, M. J., J. O. Workel, H. M. Lesscher, E. Vreugdenhil, M. S. Oitzl and E. R. de Kloet (2001). "Correlation between hippocampal BDNF mRNA expression and memory performance in senescent rats." Brain Res **915**(2): 227-233. PMC: 11595212.

Scharfman, H. E. and M. V. Chao (2013). "The entorhinal cortex and neurotrophin signaling in Alzheimer's disease and other disorders." Cogn Neurosci **4**(3-4): 123-135. doi: 10.1080/17588928.2013.826184. PMC: 24168199. PMCID: PMC3836904.

Schindelin, J., I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J. Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri, P. Tomancak and A. Cardona (2012). "Fiji: an open-source platform for biological-image analysis." Nat Methods **9**(7): 676-682. doi: 10.1038/nmeth.2019. PMC: 22743772. PMCID: PMC3855844.

Schmued, L., J. Raymick, W. Tolleson, S. Sarkar, Y. H. Zhang and A. Bell-Cohn (2012). "Introducing Amylo-Glo, a novel fluorescent amyloid specific histochemical tracer especially suited for multiple labeling and large scale quantification studies." J Neurosci Methods **209**(1): 120-126. doi: 10.1016/j.jneumeth.2012.05.019. PMC: 22705750.

Schreuder, L., B. J. Eggen, K. Biber, R. G. Schoemaker, J. D. Laman and S. E. de Rooij (2017). "Pathophysiological and behavioral effects of systemic inflammation in aged and diseased rodents with relevance to delirium: A systematic review." Brain Behav Immun **62**: 362-381. doi: 10.1016/j.bbi.2017.01.010. PMC: 28088641.

Shaw, K. N., S. Commins and S. M. O'Mara (2001). "Lipopolysaccharide causes deficits in spatial learning in the watermaze but not in BDNF expression in the rat dentate gyrus." Behav Brain Res **124**(1): 47-54. PMC: 11423165.

Shimada, H., H. Makizako, T. Doi, D. Yoshida, K. Tsutsumimoto, Y. Anan, K. Uemura, S. Lee, H. Park and T. Suzuki (2014). "A large, cross-sectional observational study of serum BDNF, cognitive function, and mild cognitive impairment in the elderly." Front Aging Neurosci **6**: 69. doi: 10.3389/fnagi.2014.00069. PMC: 24782766. PMCID: PMC3995061.

Simen, A. A., K. A. Bordner, M. P. Martin, L. A. Moy and L. C. Barry (2011). "Cognitive dysfunction with aging and the role of inflammation." Ther Adv Chronic Dis **2**(3): 175-195. doi: 10.1177/2040622311399145. PMC: 23251749. PMCID: PMC3513880.

Simic, G., I. Kostovic, B. Winblad and N. Bogdanovic (1997). "Volume and number of neurons of the human hippocampal formation in normal aging and Alzheimer's disease." J Comp Neurol **379**(4): 482-494. PMC: 9067838.

Sims, N. R., D. M. Bowen, S. J. Allen, C. C. T. Smith, D. Neary, D. J. Thomas and A. N. Davison (1983). "Presynaptic Cholinergic Dysfunction in Patients with Dementia." Journal of Neurochemistry **40**(2): 503-509. doi: 10.1111/j.1471-4159.1983.tb11311.x.

Smets, E. M., J. E. Dijkstra-Lagemaat and M. A. Blankenstein (2004). "Influence of blood collection in plastic vs. glass evacuated serum-separator tubes on

hormone and tumour marker levels." Clin Chem Lab Med **42**(4): 435-439. doi: 10.1515/CCLM.2004.076. PMC: 15147155.

Soderquist, R. G., E. D. Milligan, E. M. Sloane, J. A. Harrison, K. K. Douvas, J. M. Potter, T. S. Hughes, R. A. Chavez, K. Johnson, L. R. Watkins and M. J. Mahoney (2009). "PEGylation of brain-derived neurotrophic factor for preserved biological activity and enhanced spinal cord distribution." J Biomed Mater Res A **91**(3): 719-729. doi: 10.1002/jbm.a.32254. PMC: 19048635. PMCID: PMC3990442.

Sonali, N., M. Tripathi, R. Sagar and S. Vivekanandhan (2013). "Val66Met polymorphism and BDNF levels in Alzheimer's disease patients in North Indian population." Int J Neurosci **123**(6): 409-416. doi: 10.3109/00207454.2012.762515. PMC: 23270505.

Sondag, C. M., G. Dhawan and C. K. Combs (2009). "Beta amyloid oligomers and fibrils stimulate differential activation of primary microglia." J Neuroinflammation **6**: 1. doi: 10.1186/1742-2094-6-1. PMC: 19123954. PMCID: PMC2632990.

Song, J. H., J. T. Yu and L. Tan (2015). "Brain-Derived Neurotrophic Factor in Alzheimer's Disease: Risk, Mechanisms, and Therapy." Mol Neurobiol **52**(3): 1477-1493. doi: 10.1007/s12035-014-8958-4. PMC: 25354497.

Sotthibundhu, A., A. M. Sykes, B. Fox, C. K. Underwood, W. Thangnipon and E. J. Coulson (2008). "Beta-amyloid(1-42) induces neuronal death through the p75 neurotrophin receptor." J Neurosci **28**(15): 3941-3946. doi: 10.1523/JNEUROSCI.0350-08.2008. PMC: 18400893.

Sperling, R. A., C. R. Jack, Jr. and P. S. Aisen (2011). "Testing the right target and right drug at the right stage." Sci Transl Med **3**(111): 111cm133. doi: 10.1126/scitranslmed.3002609. PMC: 22133718. PMCID: PMC3752906.

Srinivasan, J. and W. J. Schmidt (2004). "Behavioral and neurochemical effects of noradrenergic depletions with N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine in 6-hydroxydopamine-induced rat model of Parkinson's disease." Behav Brain Res **151**(1-2): 191-199. doi: 10.1016/j.bbr.2003.08.016. PMC: 15084435.

Stevens, N. E., M. J. Chapman, C. K. Fraser, T. R. Kuchel, J. D. Hayball and K. R. Diener (2017). "Therapeutic targeting of HMGB1 during experimental sepsis modulates the inflammatory cytokine profile to one associated with improved clinical outcomes." Sci Rep **7**(1): 5850. doi: 10.1038/s41598-017-06205-z. PMC: 28724977. PMCID: PMC5517568.

Sun, J., S. Zhang, X. Zhang, X. Zhang, H. Dong and Y. Qian (2015). "IL-17A is implicated in lipopolysaccharide-induced neuroinflammation and cognitive

impairment in aged rats via microglial activation." J Neuroinflammation **12**: 165. doi: 10.1186/s12974-015-0394-5. PMC: 26373740. PMCID: PMC4572693.

Sun, Y., M. S. Lai, C. J. Lu and R. C. Chen (2008). "How long can patients with mild or moderate Alzheimer's dementia maintain both the cognition and the therapy of cholinesterase inhibitors: a national population-based study." Eur J Neurol **15**(3): 278-283. doi: 10.1111/j.1468-1331.2007.02049.x. PMC: 18290848.

Szuhany, K. L., M. Bugatti and M. W. Otto (2015). "A meta-analytic review of the effects of exercise on brain-derived neurotrophic factor." J Psychiatr Res **60**: 56-64. doi: 10.1016/j.jpsychires.2014.10.003. PMC: 25455510. PMCID: PMC4314337.

Tabesh, A., J. H. Jensen, B. A. Ardekani and J. A. Helpern (2011). "Estimation of tensors and tensor-derived measures in diffusional kurtosis imaging." Magn Reson Med **65**(3): 823-836. doi: 10.1002/mrm.22655. PMC: 21337412. PMCID: PMC3042509.

Tansey, M. G. and M. S. Goldberg (2010). "Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention." Neurobiol Dis **37**(3): 510-518. doi: 10.1016/j.nbd.2009.11.004. PMC: 19913097. PMCID: PMC2823829.

Tapia-Arancibia, L., E. Aliaga, M. Silhol and S. Arancibia (2008). "New insights into brain BDNF function in normal aging and Alzheimer disease." Brain Res Rev **59**(1): 201-220. doi: 10.1016/j.brainresrev.2008.07.007. PMC: 18708092.

Teng, H. K., K. K. Teng, R. Lee, S. Wright, S. Tevar, R. D. Almeida, P. Kermani, R. Torkin, Z. Y. Chen, F. S. Lee, R. T. Kraemer, A. Nykjaer and B. L. Hempstead (2005). "ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin." J Neurosci **25**(22): 5455-5463. doi: 10.1523/JNEUROSCI.5123-04.2005. PMC: 15930396.

Thal, D. R., U. Rub, M. Orantes and H. Braak (2002). "Phases of A beta-deposition in the human brain and its relevance for the development of AD." Neurology **58**(12): 1791-1800. PMC: 12084879.

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

Tong, L., R. Balazs, R. Soiampornkul, W. Thangnipon and C. W. Cotman (2008). "Interleukin-1 beta impairs brain derived neurotrophic factor-induced signal transduction." Neurobiol Aging **29**(9): 1380-1393. doi:

10.1016/j.neurobiolaging.2007.02.027. PMC: 17467122. PMCID: PMC4052889.

Tong, L., G. A. Prieto, E. A. Kramar, E. D. Smith, D. H. Cribbs, G. Lynch and C. W. Cotman (2012). "Brain-derived neurotrophic factor-dependent synaptic plasticity is suppressed by interleukin-1beta via p38 mitogen-activated protein kinase." J Neurosci **32**(49): 17714-17724. doi: 10.1523/JNEUROSCI.1253-12.2012. PMC: 23223292. PMCID: PMC3687587.

Tost, H., T. Alam, M. Geramita, C. Rebsch, B. Kolachana, D. Dickinson, B. A. Verchinski, H. Lemaitre, A. S. Barnett, J. W. Trampush, D. R. Weinberger and S. Marenco (2013). "Effects of the BDNF Val66Met polymorphism on white matter microstructure in healthy adults." Neuropsychopharmacology **38**(3): 525-532. doi: 10.1038/npp.2012.214. PMC: 23132269. PMCID: PMC3547204.

Trager, U. and S. J. Tabrizi (2013). "Peripheral inflammation in neurodegeneration." J Mol Med (Berl) **91**(6): 673-681. doi: 10.1007/s00109-013-1026-0. PMC: 23546523.

Trajkovska, V., A. B. Marcussen, M. Vinberg, P. Hartvig, S. Aznar and G. M. Knudsen (2007). "Measurements of brain-derived neurotrophic factor: methodological aspects and demographical data." Brain Res Bull **73**(1-3): 143-149. doi: 10.1016/j.brainresbull.2007.03.009. PMC: 17499648.

Tsuchimine, S., N. Sugawara, M. Ishioka and N. Yasui-Furukori (2014). "Preanalysis storage conditions influence the measurement of brain-derived neurotrophic factor levels in peripheral blood." Neuropsychobiology **69**(2): 83-88. doi: 10.1159/000358061. PMC: 24577046.

Tuszynski, M. H. (2017). A Clinical Trial of BDNF Gene Therapy in Alzheimer's Disease. University of California, San Diego Part the Cloud: Translational Research Funding for Alzheimer's Disease.

Tuszynski, M. H., K. Bankiewicz, J. Bringas, I. Kovacs, A. Nagahara, J. Koffler and K. Kadoya (2017). BDNF Gene Delivery for Alzheimer's Disease. Annals of Neurology, Wiley.

Ubhi, K., E. Rockenstein, E. Doppler, M. Mante, A. Adame, C. Patrick, M. Trejo, L. Crews, A. Paulino, H. Moessler and E. Masliah (2009). "Neurofibrillary and neurodegenerative pathology in APP-transgenic mice injected with AAV2-mutant TAU: neuroprotective effects of Cerebrolysin." Acta Neuropathol **117**(6): 699-712. doi: 10.1007/s00401-009-0505-4. PMC: 19252918. PMCID: PMC3049872.

Uematsu, A., B. Z. Tan and J. P. Johansen (2015). "Projection specificity in heterogeneous locus coeruleus cell populations: implications for learning and memory." Learn Mem **22**(9): 444-451. doi: 10.1101/lm.037283.114. PMC: 26330494. PMCID: PMC4561410.

van Maurik, I. S., M. D. Zwan, B. M. Tijms, F. H. Bouwman, C. E. Teunissen, P. Scheltens, M. P. Wattjes, F. Barkhof, J. Berkhof, W. M. van der Flier and I. Alzheimer's Disease Neuroimaging (2017). "Interpreting Biomarker Results in Individual Patients With Mild Cognitive Impairment in the Alzheimer's Biomarkers in Daily Practice (ABIDE) Project." JAMA Neurol **74**(12): 1481-1491. doi: 10.1001/jamaneurol.2017.2712. PMC: 29049480. PMCID: PMC5822193.

Ventriglia, M., R. Zanardini, C. Bonomini, O. Zanetti, D. Volpe, P. Pasqualetti, M. Gennarelli and L. Bocchio-Chiavetto (2013). "Serum brain-derived neurotrophic factor levels in different neurological diseases." Biomed Res Int **2013**: 901082. doi: 10.1155/2013/901082. PMC: 24024214. PMCID: PMC3760208.

Vepsalainen, S., E. Castren, S. Helisalmi, S. Iivonen, A. Mannermaa, M. Lehtovirta, T. Hanninen, H. Soininen and M. Hiltunen (2005). "Genetic analysis of BDNF and TrkB gene polymorphisms in Alzheimer's disease." J Neurol **252**(4): 423-428. doi: 10.1007/s00415-005-0667-5. PMC: 15726264.

Veraart, J., D. S. Novikov, D. Christiaens, B. Ades-Aron, J. Sijbers and E. Fieremans (2016). "Denoising of diffusion MRI using random matrix theory." Neuroimage **142**: 394-406.

Vereker, E., V. Campbell, E. Roche, E. McEntee and M. A. Lynch (2000). "Lipopolysaccharide inhibits long term potentiation in the rat dentate gyrus by activating caspase-1." J Biol Chem **275**(34): 26252-26258. doi: 10.1074/jbc.M002226200. PMC: 10856294.

Verhagen, M., A. van der Meij, P. A. van Deurzen, J. G. Janzing, A. Arias-Vasquez, J. K. Buitelaar and B. Franke (2010). "Meta-analysis of the BDNF Val66Met polymorphism in major depressive disorder: effects of gender and ethnicity." Mol Psychiatry **15**(3): 260-271. doi: 10.1038/mp.2008.109. PMC: 18852698.

Vermeiren, Y. and P. P. De Deyn (2017). "Targeting the norepinephrinergic system in Parkinson's disease and related disorders: The locus coeruleus story." Neurochem Int **102**: 22-32. doi: 10.1016/j.neuint.2016.11.009. PMC: 27899296.

von Bohlen und Halbach, O. (2010). "Involvement of BDNF in age-dependent alterations in the hippocampus." Front Aging Neurosci **2**. doi: 10.3389/fnagi.2010.00036. PMC: 20941325. PMCID: PMC2952461.

Wakelkamp, I., O. Bakker, L. Baldeschi, W. Wiersinga and M. Prummel (2003). "TSH-R expression and cytokine profile in orbital tissue of active vs. inactive Graves' ophthalmopathy patients." Clinical endocrinology **58**(3): 280-287.

Walsh, D. M. and D. J. Selkoe (2004). "Deciphering the molecular basis of memory failure in Alzheimer's disease." Neuron **44**(1): 181-193. doi: 10.1016/j.neuron.2004.09.010. PMC: 15450169.

Wang, C., Y. Cui, J. Yang, J. Zhang, D. Yuan, Y. Wei, Y. Li, Y. Duo, S. Li, W. Zhu and L. Zheng (2015). "Combining serum and urine biomarkers in the early diagnosis of mild cognitive impairment that evolves into Alzheimer's disease in patients with the apolipoprotein E 4 genotype." Biomarkers **20**(1): 84-88. doi: 10.3109/1354750X.2014.994036. PMC: 25532446.

Wang, H.-Y., A. Stucky, C.-G. Hahn, R. Wilson, D. Bennett and S. Arnold (2011). "BDNF-trkB signaling in late life cognitive decline and Alzheimer's disease." Translational Neuroscience **2**(2). doi: 10.2478/s13380-011-0015-4.

Wang, X., M. Zhu, E. Hjorth, V. Cortes-Toro, H. Eyjolfsdottir, C. Graff, I. Nennesmo, J. Palmblad, M. Eriksdotter, K. Sambamurti, J. M. Fitzgerald, C. N. Serhan, A. C. Granholm and M. Schultzberg (2015). "Resolution of inflammation is altered in Alzheimer's disease." Alzheimers Dement **11**(1): 40-50 e41-42. doi: 10.1016/j.jalz.2013.12.024. PMC: 24530025. PMCID: PMC4275415.

Waterhouse, E. G. and B. Xu (2009). "New insights into the role of brain-derived neurotrophic factor in synaptic plasticity." Mol Cell Neurosci **42**(2): 81-89. doi: 10.1016/j.mcn.2009.06.009. PMC: 19577647. PMCID: PMC2748315.

Weinstein, G., A. S. Beiser, S. H. Choi, S. R. Preis, T. C. Chen, D. Vorgas, R. Au, A. Pikula, P. A. Wolf, A. L. DeStefano, R. S. Vasan and S. Seshadri (2014). "Serum brain-derived neurotrophic factor and the risk for dementia: the Framingham Heart Study." JAMA Neurol **71**(1): 55-61. doi: 10.1001/jamaneurol.2013.4781. PMC: 24276217. PMCID: PMC4056186.

Wharton, W., C. E. Gleason, K. R. Lorenze, T. S. Markgraf, M. L. Ries, C. M. Carlsson and S. Asthana (2009). "Potential role of estrogen in the pathobiology and prevention of Alzheimer's disease." Am J Transl Res **1**(2): 131-147. PMC: 19956426. PMCID: PMC2776312.

Whitehouse, P. J., D. L. Price, R. G. Struble, A. W. Clark, J. T. Coyle and M. R. Delon (1982). "Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain." Science **215**(4537): 1237-1239.

Willis, L., E. M. Quintero, M. Nelson and A. C. Granholm (2005). "Regulation of Trophic Factor Expression by Innervating Target Regions in Intraocular Double Transplants." Cell Transplant **14**(1): 21-29. doi: 10.3727/000000005783983313. PMC: 28863735.

Woerman, A. L., S. A. Kazmi, S. Patel, Y. Freyman, A. Oehler, A. Aoyagi, D. A. Mordes, G. M. Halliday, L. T. Middleton, S. M. Gentleman, S. H. Olson and S. B. Prusiner (2018). "MSA prions exhibit remarkable stability and resistance to inactivation." Acta Neuropathol **135**(1): 49-63. doi: 10.1007/s00401-017-1762-2. PMC: 28849371. PMCID: PMC5756500.

Wolburg, H. and A. Lippoldt (2002). "Tight junctions of the blood–brain barrier: development, composition and regulation." Vascular pharmacology **38**(6): 323-337.

Wolk, D. A. and B. C. Dickerson (2018). Clinical features and diagnosis of Alzheimer disease. UpToDate. S. T. DekKosky and J. L. Witterdink. UpToDate, Waltham, MA (Accessed on May 19, 2018), UpToDate.

Wyss-Coray, T., J. D. Loike, T. C. Brionne, E. Lu, R. Anankov, F. Yan, S. C. Silverstein and J. Husemann (2003). "Adult mouse astrocytes degrade amyloid-beta in vitro and in situ." Nat Med **9**(4): 453-457. doi: 10.1038/nm838. PMC: 12612547.

Xiao, J., A. W. Wong, M. M. Willingham, M. van den Buuse, T. J. Kilpatrick and S. S. Murray (2010). "Brain-derived neurotrophic factor promotes central nervous system myelination via a direct effect upon oligodendrocytes." Neurosignals **18**(3): 186-202. doi: 10.1159/000323170. PMC: 21242670.

Xu, D., D. Lian, J. Wu, Y. Liu, M. Zhu, J. Sun, D. He and L. Li (2017). "Brain-derived neurotrophic factor reduces inflammation and hippocampal apoptosis in experimental Streptococcus pneumoniae meningitis." J Neuroinflammation **14**(1): 156. doi: 10.1186/s12974-017-0930-6. PMC: 28778220. PMCID: PMC5545027.

Xu, X. J., Y. M. Tang, H. Song, S. L. Yang, W. Q. Xu, S. W. Shi, N. Zhao and C. Liao (2013). "A multiplex cytokine score for the prediction of disease severity in pediatric hematology/oncology patients with septic shock." Cytokine **64**(2): 590-596. doi: 10.1016/j.cyto.2013.08.012. PMC: 24051223.

Yamagishi, T., H. Takagishi, S. Fermin Ade, R. Kanai, Y. Li and Y. Matsumoto (2016). "Cortical thickness of the dorsolateral prefrontal cortex predicts

strategic choices in economic games." Proc Natl Acad Sci U S A **113**(20): 5582-5587. doi: 10.1073/pnas.1523940113. PMC: 27140622. PMCID: PMC4878491.

Yamazaki, Y. and T. Kanekiyo (2017). "Blood-Brain Barrier Dysfunction and the Pathogenesis of Alzheimer's Disease." Int J Mol Sci **18**(9). doi: 10.3390/ijms18091965. PMC: 28902142. PMCID: PMC5618614.

Yan, Q., M. J. Radeke, C. R. Matheson, J. Talvenheimo, A. A. Welcher and S. C. Feinstein (1997). "Immunocytochemical localization of TrkB in the central nervous system of the adult rat." J Comp Neurol **378**(1): 135-157. PMC: 9120052.

Yasutake, C., K. Kuroda, T. Yanagawa, T. Okamura and H. Yoneda (2006). "Serum BDNF, TNF-alpha and IL-1beta levels in dementia patients: comparison between Alzheimer's disease and vascular dementia." Eur Arch Psychiatry Clin Neurosci **256**(7): 402-406. doi: 10.1007/s00406-006-0652-8. PMC: 16783499.

Yoshida, T. (2012). "Serum Levels of Mature Brain-Derived Neurotrophic Factor (BDNF) and Its Precursor proBDNF in Healthy Subjects." The Open Clinical Chemistry Journal **5**(1): 7-12. doi: 10.2174/1874241601205010007.

Yoshida, T., M. Ishikawa, T. Niitsu, M. Nakazato, H. Watanabe, T. Shiraishi, A. Shiina, T. Hashimoto, N. Kanahara, T. Hasegawa, M. Enohara, A. Kimura, M. Iyo and K. Hashimoto (2012). "Decreased serum levels of mature brain-derived neurotrophic factor (BDNF), but not its precursor proBDNF, in patients with major depressive disorder." PLoS One **7**(8): e42676. doi: 10.1371/journal.pone.0042676. PMC: 22880079. PMCID: PMC3411809.

Yuan, P. and N. Raz (2014). "Prefrontal cortex and executive functions in healthy adults: a meta-analysis of structural neuroimaging studies." Neurosci Biobehav Rev **42**: 180-192. doi: 10.1016/j.neubiorev.2014.02.005. PMC: 24568942. PMCID: PMC4011981.

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. PMC: 12633144.

Zenaro, E., G. Piacentino and G. Constantin (2017). "The blood-brain barrier in Alzheimer's disease." Neurobiol Dis **107**: 41-56. doi: 10.1016/j.nbd.2016.07.007. PMC: 27425887. PMCID: PMC5600438.

Zeng, F., J. J. Lu, X. F. Zhou and Y. J. Wang (2011). "Roles of p75NTR in the pathogenesis of Alzheimer's disease: a novel therapeutic target." Biochem

Pharmacol **82**(10): 1500-1509. doi: 10.1016/j.bcp.2011.06.040. PMC: 21762680.

Zhang, F., Z. Kang, W. Li, Z. Xiao and X. Zhou (2012). "Roles of brain-derived neurotrophic factor/tropomyosin-related kinase B (BDNF/TrkB) signalling in Alzheimer's disease." J Clin Neurosci **19**(7): 946-949. doi: 10.1016/j.jocn.2011.12.022. PMC: 22613489.

Zhang, J., Z. Liu, Y. Pei, W. Yang, C. Xie and S. Long (2018). "MicroRNA-322 Cluster Promotes Tau Phosphorylation via Targeting Brain-Derived Neurotrophic Factor." Neurochem Res **43**(3): 736-744. doi: 10.1007/s11064-018-2475-1. PMC: 29464486.

Zhang, J., I. Sokal, E. R. Peskind, J. F. Quinn, J. Jankovic, C. Kenney, K. A. Chung, S. P. Millard, J. G. Nutt and T. J. Montine (2008). "CSF multianalyte profile distinguishes Alzheimer and Parkinson diseases." Am J Clin Pathol **129**(4): 526-529. doi: 10.1309/W01Y0B808EMEH12L. PMC: 18343778. PMCID: PMC2688655.

Zhu, B., Z. G. Wang, J. Ding, N. Liu, D. M. Wang, L. C. Ding and C. Yang (2014). "Chronic lipopolysaccharide exposure induces cognitive dysfunction without affecting BDNF expression in the rat hippocampus." Exp Ther Med **7**(3): 750-754. doi: 10.3892/etm.2014.1479. PMC: 24520281. PMCID: PMC3919865.

Zhu, Y., X. Chen, Z. Liu, Y. P. Peng and Y. H. Qiu (2015). "Interleukin-10 Protection against Lipopolysaccharide-Induced Neuro-Inflammation and Neurotoxicity in Ventral Mesencephalic Cultures." Int J Mol Sci **17**(1). doi: 10.3390/ijms17010025. PMC: 26729090. PMCID: PMC4730272.

Ziegenhorn, A. A., O. Schulte-Herbruggen, H. Danker-Hopfe, M. Malbranc, H. D. Hartung, D. Anders, U. E. Lang, E. Steinhagen-Thiessen, R. T. Schaub and R. Hellweg (2007). "Serum neurotrophins--a study on the time course and influencing factors in a large old age sample." Neurobiol Aging **28**(9): 1436-1445. doi: 10.1016/j.neurobiolaging.2006.06.011. PMC: 16879899.

Zotova, E., J. A. Nicoll, R. Kalaria, C. Holmes and D. Boche (2010). "Inflammation in Alzheimer's disease: relevance to pathogenesis and therapy." Alzheimers Res Ther **2**(1): 1. doi: 10.1186/alzrt24. PMC: 20122289. PMCID: PMC2874260.

Zuccato, C., M. Marullo, B. Vitali, A. Tarditi, C. Mariotti, M. Valenza, N. Lahiri, E. J. Wild, J. Sassone, A. Ciammola, A. C. Bachoud-Levi, S. J. Tabrizi, S. Di Donato and E. Cattaneo (2011). "Brain-derived neurotrophic factor in patients with Huntington's disease." PLoS One **6**(8): e22966. doi: 10.1371/journal.pone.0022966. PMC: 21857974. PMCID: PMC3155522.