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Brain-Derived Neurotrophic Factor's Neuroprotective Effects in Entorhinal Cortex

A thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

Yaswanraj Yuvaraj

Committee in charge:

Professor Mark H. Tuszynski, Chair.
Professor Yimin Zou, Co-Chair
Professor Andrew Chisholm

2016

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University of California, San Diego

2016

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Abstract of the Thesis

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by

Yaswanraj Yuvaraj

Master of Science in Biology

University of California, San Diego, 2016

Mark H. Tuszynski, Chair

Yimin Zou, Co-Chair

Entorhinal cortex (EC), primary source of input into the hippocampus, is important for memory and learning. Alzheimer's patients experience memory loss at early stages of the disease. The memory loss is associated with neuronal atrophy and death, which starts in EC. Previous evidence indicates that brain-derived neurotrophic factor (BDNF) could prevent cell loss and atrophy. The goal of this study was to test lower dosages of adeno-associated virus (AAV) 2-BDNF (1e11vp/ml, 3e11vp/ml and 3e11vp/ml x2 volume) and AAV9-BDNF (3e11vp/ml). Our hypothesis was the BDNF gene delivery into the EC will produce a neuroprotective effect preventing cell lose and cell body atrophy. A perforant pathway lesion was performed in rats that severed the

projections from EC layer II neurons to dentate gyrus to induce cell death or atrophy in these neurons. Three days prior to the lesion, subject received treatments into the EC. Rats were euthanized two weeks after lesion, and the brains were analyzed using stereology to quantify the number of neurons and cell atrophy by measuring cell body size. Results showed that BDNF treatment produced a neuroprotective effect that ameliorated cell loss ($p<0.01 – 0.05$), while control groups had ~30% cell loss. Cell atrophy was decreased within the AAV9 treatment group ($p<0.05$). Quantification of pAKT cell numbers in ECLII showed no effect of BDNF treatment on pAKT activation after 2 weeks. BDNF does have the potential to prevent early cell lose and atrophy in AD. This might prove to prevent the early memory observed AD.

I. INTRODUCTION

The overall goal of the project was to study the therapeutic effect of BDNF gene therapy on cell survival in the entorhinal cortex layer II in a rat model of AD. The introduction will provide a review of AD, early pathology of AD in the entorhinal cortex, and BDNF and it's potential therapeutic role for the treatment of AD.

Alzheimer's Diseases: Demography

The incidence of AD onset increases with age [1]. A study showed that chance of acquiring AD increased passed the age of 65 [1]. This is important to consider, since the average lifespan of humans has increased and is increasing [2, 3]. With increased age comes a higher risk of people acquiring AD. This means in the future there will be more people affected by AD.

AD was concluded to have affected 5.3 million people in 2015 in the United States [4]. By 2050 it is predicted that there will be 10 million people with AD, which a two fold increase [4]. The significant increase in the amount of people that will have Alzheimer's disease needs to be addressed properly. A virus-vector administration of BDNF might solve this problem by providing a potential solution for the treatment of cell loss experienced by AD [5].

Categories of AD

Early-onset Alzheimer's disease (EOAD) seems to follow the same pathological trend as the patients with late-onset Alzheimer's disease (LOAD). It has been estimated that EOAD affects around 200,000 people, which is about 4% of all cases of AD and so EOAD is considered rare [4]. Anyone with AD prior the age of 65 has been considered to have EOAD [6]. Of the group with EOAD, it has been suggested some have early-onset of familial Alzheimer's disease (EOFAD). Different mutations in three genes have been

suggested to be responsible for EOFAD: amyloid precursor protein (APP) mutations, presenilin (PSEN) 1 and PSEN2 [7-13]. PSEN participates in the cleaving of APP by gamma-secretase [14]. The three mutations increase amyloid beta production and plaque formation.

Mutations have been linked with LOAD, which has an onset of AD after 65 years of age. Evidence supports that alleles apolipoprotein E (ApoE) ε4/ε4 has higher chance of causing AD compared to ApoE ε3/ε4 or ε3/ε3 [15]. Other genome wide association studies support ApoE susceptibility for LOAD, but other genes such as encoding for the apolipoprotein J gene, CLU gene and PICALM gene have been associated with LOAD [16-18]. Evidence supports ApoE ε4/ε4 role in stabilizing oligomers and increases plaques levels leading to cognitive decline [19, 20].

Cognitive Deficits

The preclinical phase of AD has been known to show little cognitive deficits [21, 22]. The early sign of AD is typically amnestic mild cognitive impairment (MCI), but not all patients with MCI progress to AD [23, 24]. During this stage one can expect to see a higher rate of memory decline than healthy individuals, but MCI patients will not experience the same level of cognitive decline seen in AD patients [24]. Evidence supports that ~64% of MCI progress on AD [25]. In the mild stage of AD, patients will exhibit cognitive decline such as impaired ability to carry out daily tasks, memory loss and mood changes [24, 26, 27]. The next stage is moderate AD, where patient's conditions worsen [26] with effects on speech, memory and sensory processing [27, 28]. This stage is usually accompanied by the loss of former memories and the inability to

form new memories. During the final stage of AD patients cannot care for themselves and completely rely on external care for all daily actives.

Pathology

To find a solution for AD, one must first understand the pathology of the disease. Individuals with AD have several different forms of pathology that include the loss of synaptic connections, neuron atrophy and death, neurofibrillary tangle, and amyloid plaque formation. This pathology is thought to start in the entorhinal region of the brain spreading to the rest of the brain, possibly through anatomical pathways. A major theory for the pathology of AD is the amyloid hypothesis based on the genetic evidence and findings that beta amyloid peptide is associated with the different forms of pathology.

Senile Plaques

Senile plaques are the landmark pathology in AD. The plaques are made up of the insoluble amyloid β peptide (Aβ), other proteins and metal ions [29, 30]. The Aβ is a 4kD protein derived from the amyloid precursor protein (APP) when APP is cut by beta-secretase then gamma-secretase [31]. Beta-secretase is a transmembrane aspartic protease that cleaves APP between residues 671 and 672 [32]. Gamma-secretase is an integral membrane protein that is an aspartyl protease, which completes the production of Aβ via intramembrane cleavage of APP [33]. Gamma-secretase is an inaccurate protease that can cleave APP at different sites to form Aβ31 to Aβ50, but Aβ40 is known to be the most abundant form occurring naturally [34]. It was discovered that in neurons Aβ40 was produced in the trans-golgi network while Aβ42 was produced in the endoplasmic reticulum [35]. Non-neuronal cells on the other hand produce Aβ40 and Aβ42 on the cell surface [35]. The aggregation Aβ forms senile plaques, which are insoluble and consist of

many other proteins [30]. Recently, it has been shown that the plaques are inert, but what causes toxicity is the amyloid-beta derived diffusible ligands and Aβ soluble oligomers [36-38]. Aβ42 has been shown to be the more toxic compared to Aβ40, since it formed oligomers much quicker and was more neurotoxic in vivo and vitro [39, 40].

Neurofibrillary Tangles (NFT)

Alzheimer's patients also have NTF that are caused by a problem inside the cell that occurs with the tau protein [41, 42]. In neurons the tau protein plays an important role in stabilizing microtubules [43-45]. However, within some neurons of Alzheimer patients the tau protein is modified differently, which alter its functions [43]. The tau protein is hyperphosphorylated, which prevents the protein from binding and stabilizing microtubules in neurons [46, 47]. The hyperphosphorylation causes the tau protein to self-assemble into NTFs [46, 48]. The loss of function of the tau protein has been connected with the neurodegeneration in AD [49, 50].

Cell Death

A major feature of AD is the loss of cells throughout the brain. In the later stages of AD there is widespread cell loss in the cortex. AD has been theorized to start ten to twenty years before diagnosis with the accumulation of Aß peptide that leads to synaptic dysfunction, NFTs, and eventually neuronal death [21, 22, 51]. The spread of Aß and NTF may produce the neurotoxicity that causes cell atrophy and cell death [52]. There is a sequence of cell death that is observed in the EC followed by death of cells in the hippocampus, which is then followed by the cell atrophy and cell death of the cerebral cortex [52, 53].

Synaptic Loss

One of the earliest pathology is the loss of synaptic connections. Neurons can have numerous pre and postsynaptic connections, and the loss of some synapses does not always lead to neuronal death but it does lead to other memory impairments in individual. The degeneration of synapses has been known to be the cause of cognitive decline in individuals with AD [54]. Individuals experiencing MCI or early AD usually have a lowered hippocampal synaptic density compared to healthy individual [55-57]. The decrease in synapses starts during the MCI period prior to AD, and so MCI has been viewed as a transitional period prior to AD [57]. The loss of synaptic connections has been shown to occur because of the altered function of the plaques and tau protein [58, 59]. It has also been shown that Aβ oligomers, found to be localized within the radius of plaques suggesting plaques act as reservoirs, cause decreased dendritic spine density, LDP and synaptic loss [60-63]. The altered tau protein prevents the proper transportation of organelles to different areas of the neuron leading to the degeneration of synapses [58, 64]. BDNF can prevent the toxicity on synapses via its role in inducing LTP [65, 66]. Other pathologies that AD patients experiences on top the synaptic loss includes inflammations, cerebral amyloid angiopathy, the deposits of Aβ in intracerebral vessels, and cerebrovascular problems such as hemorrhages and lack of blood to areas [67-69].

Amyloid Hypothesis

The amyloid hypothesis claims AD occurs due to the increase levels of Aß peptide and the major pathology in AD is the consequence of the buildup and inability to clear Aß peptide in the brain [70]. More recently, the hypothesis shifted towards the idea that soluble oligomers and Aβ-derived diffusible ligands, are neurotoxic and can drive the

degeneration seen AD. Aß is linked to tau hyperphosphorylation that leads to the formation of NFT [71, 72]. Soluble Aß oligomers can also lead to the loss of synaptic connections. It has been suggested that the plaques can act as a reservoir for the oligomers and Aβ-derived diffusible ligands, which would supports the claim of the hypothesis that plaques can lead to degeneration in AD [63].

Progression of AD

A key characteristic of the pathology of AD is the progressive spread of certain pathology in the brain. The spread of NTF's seems to a follow a different pattern from the amyloid plaques in AD. The spread of NTF has been separated into six different stages. The first two stages occur during the preclinical phase of AD and are called the transentorhinal stages, since NTF's form in the superficial layers of the transentorhinal cortex and spread inward and medially towards the entorhinal cortex [42]. The second two stages occur during the early stages of AD and is called the limbic stage, since NTF's spread to the entorhinal cortex and CA1 of the hippocampus[42]. The final two stages are called the isocortical stage, since NTF spreads into the hippocampal formation and the primary neocortex causing dementia in AD patients [42]. The amyloid beta deposits begin in the isocortex and spread to the entorhinal and hippocampal region, and from there it continues to spread to the basal forebrain and eventually to the midbrain and then brainstem and then cerebellum [42, 73].

One possible mechanism for the spread of the pathology is the spread of tau pathology. Mutated tau proteins are able to enter neurons and assemble wild-type tau into aggregates and spread the tau-pathology to connected neurons [50, 74-76]. The entorhinal cortex, first area to express tau-pathology, projects out to the molecular layer of the

dentate gyrus via the perforant pathway [77] [78]. This is the primary source of input into the hippocampus [77, 78]. Termination of this pathway via pathology of AD can causes memory impairments [78]. The spread of NTF's via intraneuronal connections shows the progression of AD, since connected areas show distribution of NTF first [43, 48, 74, 75, 78]. Earlier stages of AD experience NTF formation in the lateral EC and outer molecular layers of dentate gyrus, which are connected via perforant pathway [42, 75]. The next stage is the spread of NTF from lateral to medial EC layer II [42]. This spread is accompanied by the spread of NTF to middle molecular layer of dentate gyrus as well, since the medial EC layer II is connected to the middle molecular layer of the dentate gyrus via perforant pathway [42, 75]. Studying and preventing the spread of tau-pathology might prevent cognitive decline in AD patients and this might also prevent the disease from reaching its critical later stages.

Beta amyloid may play a role in the spread of the pathology. The different soluble oligomer isoforms of Aβ can be internalized by neurons and transferred from neuron-to-neuron allowing for the spread of Aβ and formation of amyloid plaques [79, 80]. The exact neurotoxic role of Aβ is still unclear, but it has been shown that Aβ oligomer accumulation causes cytotoxicity after accumulating in the endoplasmic reticulum, endosome/lysosome and mitochondria [80, 81].

The spread of tau and Aβ pathology can originate form their synergistic properties. In mice Aβ can increase the spread of tau pathology and also promote the spread from the limbic cortices to the neocortex [82, 83]. It has also been suggested that tau is able to increase the plaque load in mice compared to wild-type mice [82, 84].

Role of EC in AD

The transentorhinal cortex is the first site of NTF formation and is known to be the first site of cell loss as the disease progresses [42]. It has been suggested that the pathology of AD spread via cell-to-cell connections; therefore, the synaptic loss and cell loss can progress from the transentorhinal region to EC and then into the dentate gyrus causing memory impairment seen in AD patients[42, 85-87]. On top of this, the loss of volume in the entorhinal cortex in AD patients has been correlated with loss of cells[52, 86, 87]. Cell loss can cause cognitive decline. Therefore, a proper treatment preventing the cell loss seen in EC can be an important therapy in preventing cognitive decline in AD patients.

Atrophy of the cells inside the EC layer II may lead to problems in memory processing [86]. This is mainly because EC Layer II provides the hippocampus with inputs from sensory-specific, multimodal association cortices and limbic cortices [78]. Evidence supports that atrophy of cells in the EC layer II could lead to cognitive impairment [52, 86]. For instance, in a study with participants with no cognitive impairment had an average of ~650,000 neurons in layer II of EC, while participants with mild dementia had ~30% ECLII cell loss and this progressed to ~90% cell loss in server cases AD [87]. Clearly, there is a large decrease in neurons of participants with mild cognitive impairment. This form of cell loss and cognitive impairment is seen to start as early as during the MCI stage prior AD [42, 52, 86]. On top of layer II loss, the other layers such as ECLIII has ~40% cell loss, ECLIV has ~50% cell loss and ECLV/ECLVI together have ~40% cell loss [87]. BDNF treatment at early stages of cell loss in MCI or AD can potentially prevent cell loss and atrophy.

The EC, specifically layer II, provides the primary source of neuronal inputs from sensory-specific, multimodal association cortices and limbic cortices to the hippocampus [78]. The layer II of EC consists of two different cell types called the stellate and pyramidal-like cells [88]. The stellate cells within the layer II of EC project to the molecular layer of dentate gyrus, via perforant pathway, where they synapse on the granular cells in the dentate gyrus [89]. This projection is the start of the trisynaptic circuit of the hippocampus that also includes the mossy fiber pathway to CA3 pyramidal cells [90] and the CA3 pyramidal cells project to the CA1 pyramidal cells via Schaffer collaterals [91, 92]. The perforant pathway also consists of the projections from EC layer III, which synapse on CA1 [93]. Finally, the cells in the CA1 project back to layer V of the EC. Therefore, EC is a key brain structure in hippocampal circuitry and the role in the formation of long-term memory.

Current and Proposed Treatment for AD

Currently there is no proposed cure for AD. The treatments that are available only delay and manage the symptoms of AD. The U.S. Food and Drug Administration have approved four drugs to treat AD: donepezil, rivastigmine, galantamine, and memantine. Donepezil, rivastigmine, and galantamine are cholinesterase inhibitors to increase the levels of acetylcholine. Memantine is an NDMA antagonist, which reduces glutamate toxicity.

Current clinical trials are focusing on anti-amyloid treatments, acetylcholinesterase inhibitor treatments, and tau pathology treatments. The anti-amyloid treatments so far have not been successful. For example, drug called bapineuzumab and solanezumab, Aβ antibodies that increase Aβ clearance, both failed in phase 3 clinical

trials and showed no cognitive improvements in AD patients [94, 95]. There is no Aβ treatment available on the market, since most do not pass phase 3, which might mean that non-amyloid therapy might not suffice for AD treatments and a focus should be placed on neurotrophin therapies such as BDNF gene delivery, since BDNF neuroprotective effects could potentially revert the cognitive decline in AD.

Currently, there are no effective treatments for AD. BDNF has the potential to treat some of the underlying problem in AD via early administration. The neuroprotective effects can prevent the cell loss in early AD in the EC, in addition to promote synaptic connectivity and improve learning and memory in animal models [5, 96]. Thus BDNF could be a potential treatment for AD.

Brain-derived Neurotrophic Factor (BDNF) Background

BDNF was first discovered in 1982 when it was isolated from the pig brain [97]. It 1982, the isolated factor from the pig brain was used by the Barde and colleagues to promote the survival of cultured embryonic chick sensory neurons [97]. This was the first time BDNF's neuroprotective effects were demonstrated in experiments. It was also confirmed the sequence of BDNF was closely related to nerve growth factor (NGF) [98]. BDNF was the second member of the neurotrophin family to ever be discovered [99].

The neurotrophin family is crucial for the survival, differentiation, and maintenance of neurons. The family consists of NGF, neurotrophin (NT)-3, NT-4/5, NT-6 and NT-7 [100]. BDNF is a secretory protein, which is able to help neuronal survival and growth [99]. The genes coding BDNF are expressed during the development stage and in adults [99]. It is expressed in the central nervous system (CNS) and the peripheral nervous system (PNS) [99, 101]. Specifically, BDNF is highly expressed in the

hippocampal formation and cerebral cortex, but found in a number of other structures [99, 102]. The genes for BDNF are expressed by neurons and controlled by neurotransmitters [99].

The neurotrophins are very important for the development of the central and peripheral nervous system [101, 103]. BDNF is highly conserved in structure and function throughout evolution [104]. In rats it has been shown that there is an increase in neurotrophins such as BDNF, NGF and NT-3 levels increase with embryogenesis occurring [105]. There seems to be a correlation between the expression of neurotrophins and neuronal growth and development. BDNF -/- mice usually die upon birth and a few live up to a month old [106]. These mice have an underdeveloped PNS and lack coordination [107]. Since BDNF promotes growth and development of neurons, it could potentially be used to treat neurodegenerative diseases such as AD patients. BDNF might be able to reverse the neuronal deaths that occur in AD patients.

BDNF Receptors

BDNF is located in the olfactory bulb, cortex, hippocampus, basal forebrain, basal ganglia, amygdala, thalamus, hypothalamus, epithalamus, brainstem, and spinal cord [108]. In these areas BDNF binds to tropomyosin-related kinase receptor type B (TrkB) in mammalian brains [109]. NGF is able to bind TrkA receptors [110]. NT-3 binds trkC receptor, but has been shown to activate trkA as well [111]. Nt4/5 has been shown to bind trkB receptors [112]. The binding of BDNF to TrkB receptors promotes neuronal survival and plasticity [109]. Upon binding of BDNF, many intracellular pathways activated such as the mitogen activated protein kinase (MAPK) pathways, phospholipase C gamma 1 (PLCg) pathway, and phosphoinositide 3-kinase (PI3K) pathway [109]. These pathways

are described later. These pathways are critical for BDNF's role in promoting neuronal survival and plasticity, which may prove beneficial for preventing neuronal loss.

TrkB can occur spliced in different isoforms that lack the catalytic domain [113] [114]. Two forms of truncated trkB have been suggested to exist: trkB.T1 and trkB.T2. The trkB and trkB.T1 receptors are conserved in-between humans and mammals, but trkB.T2 has only been found in rats [114-116]. The trkB.T1 receptor plays an important role in cell survival. For example, knockout mice of all truncated trkB receptors had sensory neuronal loss [117]. Heterodimers of trkB and trkB.T1 can be activated upon BDNF binding and have been known to participate in the development of the monkey prefrontal cortex, suggesting trkB.T1 role in participating in potential neuronal maintenance and development [118].

BDNF is also able to bind to the p75 receptors with very low affinity compared to trkB receptors [109]. The binding of BDNF to p75 is able to promote neuronal survival and plasticity [109]. Also, p75 has been widely called the death receptor, since it able to activate apoptotic pathways and cause cell death [119]. It has been suggested that p75 can heterodimerize with trkA, trkB and trkC receptors leading to change in affinity of receptor and corresponding ligand binding [120, 121]. In addition p75 receptors can act independent of ligands and co-expressed with truncated splice variants of trkB to modulate plasticity [122, 123].

BDNF's Role in Adult Brain

BDNF is able to improve learning and memory via the pathways that promote neuronal survival and plasticity [124-126]. Previous studies have shown BDNF improving learning and memory via cognitive tests on animal models[127, 128]. The

reduction in BDNF protein and mRNA expression via BDNF antagonist caused memory impairments such impaired spatial memory, and working memory in rats or mice in the Morris water maze and radial arm maze test [129-132]. The reduction in BDNF affected the retention of memory, since rats trained and then depleted of BDNF resulted in lower impaired working memory.

Long-term potentiation (LTP) is known as the increase in synaptic strength due to the reoccurring firing of the neuron. It was shown in hippocampal slices of BDNF knockout mice there was a reduction LTP. This was reversed by providing the hippocampal slices of the knockout mice with recombinant BDNF [126]. On top of this it was shown that BDNF could promote dendritic spine density[133, 134]. BDNF is able to modulate plasticity via dendritic spine density and induction of LTP. This potential effect of BDNF might be able to promote learning and memory, which may prove helpful in treating memory loss.

BDNF has been known to increase neurite growth and LTP via regulation of glutamate release [135-138]. BDNF stimulates the release of glutamate via the trkB and PLC-gamma1 pathway, a pathway known to be crucial for LTP. The increased release of glutamate and the simultaneous increased probability of NMDA opening have been suggested to participate in increasing learning and memory [136, 139]. Glutamate has also been shown to increase cell differentiation and proliferation [140, 141]. The potential role of glutamate in learning and memory can help prevent cognitive decline in AD patients administered BDNF.

BDNF is important to the maintenance of the nervous system. Many studies show BDNF's relationship to appetite, addiction/dependency and depression. BDNF and TrkB

are expressed in the hypothalamus, a region of the brain important for appetite control [142]. It was shown that BDNF +/- mutant mice experienced aggression, anxiety, obesity and abnormal eating behavior, which were reversed upon treatment with exogenous BDNF [142, 143]. Also it was shown that the Val66Met variant causes eating disorders and substance-related disorders [144]. Also, generally humans with major depressive disorder tend to experience a lower BDNF plasma level [145]. Administration of BDNF might prove beneficial to many other disorders other than just AD.

Cell Survival

BDNF is able to promote cell survival via the binding of its trkB by activating intracellular pathways that can cause synaptogenesis, cell survival and cell maintenance, and thus be a treatment for the synaptic loss and cell loss in AD patients. BDNF has been shown to prevent excitotoxicity, reduce damage from axotomy and prevent Aβ toxicity.

BDNF, Ischemia and Excitotoxicity

Previous studies have shown that cerebrovascular diseases can increase the risk of developing AD [146]. It is clear that with age people will experience an increased chance of cerebrovascular problems. With age also increases the chances of stroke, which has also been suggested in increasing the chances of developing AD [146]. Cerebrovascular problems might be the underlying reason for the increased chance of AD with age. Stroke and cerebrovascular diseases can cause cerebral ischemia [146]. This lack of blood and nutrition to the brain might decrease the rate metabolites are cleared causing a higher chance to experience AD.

Upon cerebral ischemia, one can expect inflammation to occur. Cytokines and chemokines are released upon inflammation to increase the amount of microglia. Studies

have shown that certain anti-inflammatory cytokines are better for ischemia. For example, it has been shown that TNF-alpha could worsen ischemia [147]. On the other hand interleukin-10 seems to produce a neuroprotective effect during ischemia caused by stroke. BDNF plays a role in regulating these cytokines. Studies have shown that BDNF increases microglia proliferation [148, 149]. TNF-alpha levels were decreased with its mRNA expression due to BDNF, while IL-10 levels were increased with its mRNA expression due to BDNF [148]. BDNF is able to reduce the damage caused by ischemic inflammation by regulating the inflammatory pathway. This neuroprotective effect of BDNF could be useful if administered properly to the elderly population who has increased chances of ischemia caused AD.

The neuroprotective effects of BDNF, such as its ability to activate anti-apoptotic pathways, can prevent the damages from hypoxic-ischemia related injuries. The deprivation of nutrients can cause apoptosis and build up harmful chemicals/metabolites leading to an imbalance. BDNF has been known to prevent cell death during ischemic injuries in animal models [150]. The damage from ischemic injuries is irreversible, but BDNF has been shown to promote neuronal regeneration and prevent neurological deficits in vivo and vitro [151, 152].

Previous studies have shown ischemia can cause excitotoxicity via increased glutamate levels [153]. Following ischemia there is a large influx in $Ca^{2+,}$ which has been linked to the activation of NMDA receptors by increased levels of glutamate [154]. The study suggests that BDNF is able to regulate Ca^{2+} levels via regulation of NMDA receptors to prevent neuronal death via excitotoxicity [154]. BDNF's neuroprotective effect can prevent exocitoxicity damage via administration BDNF prior onset of damage.

In a similar method to this study, BDNF gene delivery via virus vectors prior damage in vivo and vitro shows activation of PI3-K/p-AKT and MAPK pathways, which prevent exocitoxcity damage [155-158]. The neuroprotective effects of BDNF are relevant for treating cell atrophy and cell death in AD.

BDNF and Axotomy

Axotomy, the severing of axons, can occur via spinal cord injuries. Another neuroprotective effect BDNF is the prevention of cell death caused by axotomy. Previous studies have show that BDNF promotes axonal growth and reduced cells death occurring via axotomy in primate models [159]. Administration of BDNF prior to axotomy produces a neuroprotective effect, which is able to reduce cell loss and cell atrophy [160-164]. BDNF's prevention and recovery in axotomy potential could revert damages caused to axons in the perforant path lesion model. This could potentially reduce the cell death and cell atrophy that occurs in ECLII.

BDNF and Beta-Amyloid

Prior the stage of cell death and cell shrinkage in AD patients, there will the build up of amyloid plaques. In rat that were injected with Aβ in hippocampus displayed down-regulated BDNF mRNA and protein levels [165]. BDNF is important for the survival and maintenance of neurons [5]. Therefore, the prevention of BDNF down-regulation might be able to reverse the symptoms experienced by AD. Previous studies have shown increased BDNF levels prevent Aβ1-42 neurotoxicity in SH-SY5Y cell line [5, 166].

BDNF Signaling Pathways

BDNF binds to TrkB throughout the brain. The binding of TrkB causes self-phosphorylation and opens a docking spot of PLC gamma. Self-phosphorylation activates

the PLC gamma pathway, which is able to promote the regulation of neurons in the cortex [167]. PLC gamma activation causes the formation of inositol 1,4,5 triphosphate (IP3) and diacylgylcerol (DAG). IP3 increases intracellular Ca^{2+} by releasing Ca^{2+} from intracellular storage. DAG regulates protein kinases C (PKC) [168]. Studies have shown that inhibition of PLC gamma pathway inhibits long-term potentiation (LTP). The decrease in Ca^{2+} or DAG could be the underlying reason behind the decrease in LTP. The promotion of LTP could be important for maintenance of synaptic connections and thus prevent the loss of synapses seen in AD patients, potentially decreasing the cognitive impairment. On top of this, DAG regulates PKC and PKC has been known to regulate MAPK/ERK pathway conditionally [169].

The activation of MAPK/ERK has been suggested to promote cell survival and promote plasticity [133, 170, 171]. It has been suggested the activation of MAPK/ERK is able to inactivate cell death pathway and promote cell survival genes[170]. The activation of MAPK/ERK via BDNF and TrkB is able to increase neuronal survival in mammalians, which may be able to prevent neuronal death in ECLII by inactivating the cell death pathways. This potential effect of BDNF might reduce the amount of neuronal loss experienced by AD patients if administered before onset of large neuronal loss.

BDNF is able to activate PI3K, which phosphorylates other downstream proteins to activate AKT Kinases [172-174]. The activation of PI3K and AKT Kinases is able to prevent death of neurons [172]. The activation of AKT is able to control the Forkhead Transcription Factor FKHRL1 [175]. FKHRL1 controls apoptosis by activating the genes that carry out cell death [175]. AKT is able to phosphorylate FKHRL1, which causes the

transcription factor to move the cytoplasm where it cannot control the transcription of apoptotic genes [175, 176].

On top of this, the PI3K-Akt pathway is also able to control the transcriptional activity of p53 [177]. p53 is a known transcription factor, which promotes the production pro-apoptotic proteins [177, 178]. The AKT pathway is able to reduce the activity of p53, which reduces the expression of apoptotic genes in the hippocampal neurons [177]. Via BDNF, TrkB, and AKT apoptotic pathways can be shutdown and neuronal death can be avoided. This effect of BDNF can potentially prevent the loss of neurons in ECLII and potentially serve as a neuroprotective agent when administered prior to neuronal loss as a treatment to neurodegenerative diseases.

BDNF Treatments in Models of AD

The administration of BDNF shows a positive outlook for the treatment of AD. BDNF levels were discovered to be lower than normal in the temporal cortex and blood for AD and MCI patients [179-182]. This reduced BDNF levels might be contributing to the symptoms seen in AD patients, since BDNF is required for the maintenance of neurons. Thus, increasing BDNF with gene therapy in the cortex might prove to be a possible treatment.

BDNF's administration has the ability to prevent toxic effects of Aβ [5, 66, 183]. The loss of neuronal stability, from Aβ or tau toxicity, could cause neurons to die leading the cell loss seen in AD patients. However, BDNF might be able turn off the apoptotic pathways via pAKT or pERK preventing Aβ or NTF affected neurons from dying and this could reduce the amount of cells lost in AD patients [109, 170, 172, 177].

Our laboratory showed BDNF treatment prevented cell death and atrophy in different models of AD: APP transgenic mice, aged or lesion rats and aged or lesion primates [5, 184]. APP mice delivered BDNF gene therapy in EC performed better on the Morris water maze tests, which is a spatial memory test. Also, early gene delivery treatment of BDNF was able to prevent cell loss and atrophy in APP mice. APP mice treated also expressed higher levels of pERK. Learning and memory was tested in aged, cognitively-impaired rats using Morris water maze and BDNF protein infusion into medial entorhinal cortex of aged rats performed better than the control (aged rates without BDNF) on the Morris water maze [5].. BDNF treatment was able to increase pERK levels aged rats to match young rats. Next, rats were subjected to a perforant path lesion and lentiviral BDNF gene delivery treatment was able to reduce cell death and atrophy, while increasing pAKT, a cell survival pathway. The articles test learning and memory in aged monkeys. The aged monkeys were tested via visuospatial discrimination task and were shown be cognitively impaired. However, upon BDNF infusion into entorhinal cortex the aged monkeys showed an improvement in learning and memory via their improvement in their visuospatial discrimination task and hypertrophic effects were observed. Lentiviral BDNF gene delivery treatment of perforant path monkey models also showed reduced cell loss and atrophy. BDNF has the potential to increase learning and memory, which can potentially reduce the cognitive decline seen in AD patients.

The projections from EC, perforant pathway, are primary source of inputs into hippocampus and degeneration of EC, first areas of atrophy in AD, can cause memory impairments. AD [86]. The perforant path lesion will cause a 30% reduction in cells on the ispilateral ECLII of the lesion, [185]. which could be prevented via administration

BDNF gene therapy prior lesion. Since, MCI and AD have a ~37% and ~54% reduction in ECLII neurons respectively, BDNF gene therapy might prove as a potential answer in preventing cell loss and cognitive decline. ECLII also has ~25% cell atrophy caused by AD [86].

The present study examined the effects of BDNF gene delivery on perforant path lesion in rats. Previous studies in our laboratory showed the effect of lentiviral BDNF gene delivery in this model prevented cell death and atrophy [5]. An unpublished findings also showed the effects of a high titer (1e12 vp/ml) of AAV2-BDNF was able to prevent cell death and atrophy. The goal of this study was to test different virus vectors (AAV2 or AAV9) on lower dosages. Our hypothesis was that these forms of BDNF treatment would produce a neuroprotective effect and prevents cell loss and atrophy.
To properly address these problems, a perforant path rat model was used to cause ECLII cell death and atrophy. BDNF was injected into EC three days prior lesion to produce a neuroprotective effect. The neuroprotective effect was measured via quantifying number of cell, cell body size and pAKT levels in ECLII.

II. METHOD

Subjects

The subjects used in this study were male Fischer rats that were ~3 months old and weight ~250-300 grams. Rats had complete access to food and water, while housed in the cage at the vivarium of the Veterans Administration San Diego facility. The room had a 12-hour light-dark cycle with a controlled temperature. The Veterans Administration San Diego Healthcare System Institutional Animal Care and Use Committee (IACUC) provided approval for this study.

Surgical Procedure

The rats were anesthetized with one of two anesthesia mixtures: a triple cocktail mixture (100 mg/ml ketamine, 100 mg/ml xylazine, 10 mg/ml acepromazine and saline) administered at 0.4 – 0.5 ml/150g or a reversal anesthesia mixture (100 mg/ml ketamine and 0.5 mg/ml dexdormitor) administered at 0.1 – 0.2 ml/150g.

The rat underwent two surgical procedures. The first procedure was the infusion of the viral vectors: Control GFP-AAV2, BDNF-AAV2 (1e11 vector particles/milliliter (vp/ml) – 5 ul) or AAV2 (3e11 vp/ml – 5 µl) or AAV2 (3e11 vp/ml – 10 µl) and BDNF-AAV9 (3e11 vp/ml – 5 µl). First, the rats were anesthetized with an interperitoneal injection. After the rat was under anesthesia, the animal was placed in a stereotaxic with an ear bar placed inside both ears to hold the head still and centered. After a nose bar will be placed under the rat's teeth at -3.3 mm to keep the head from moving up and down. Puralube ointment was placed on the rat's eyes and the eyes would be covered with foil to limit light shining on the eyes. A scalpel was used to cut skin above skull. Saline was added to top of skull and the thin layer of tissue above skull was delicately spread apart. . Four hemostats are used to hold the tissues and skin back to reveal the skull. After, cotton

tip applicators are used to clean the excess blood. Depending on the rat, some bone wax was added to the top of the skull near area of bleeding. A bone drill will be centered at the bregma. After, coordinates formed from a rat atlas will be used to determine the areas of injection of the GFP-AAV2, BDNF-AAV2 or BDNF-AAV9 vectors. The coordinates will be used to drill three holes on top of the skull. After 27 gauge needle will be used to ensure the hole is complete. A 10 µl Hamilton syringe with a 30-gauge needle was loaded with one of the viral vectors and was attached to the Chymex syringe pump. The viral vectors were infused at three coordinate sites relative to bregma (AP -8.7 mm, ML -4.2 mm, DV -5.1 mm; AP -8.9 mm, ML -4.7 mm, DV -6.5 mm; AP -8.7 mm, ML -5.4 mm, DV -5.6 mm) at a rate of 1 µl per minute; after the infusion was complete, the needle remain in place for an additional 2-minute to allow spread of the viral vector. After the syringe was slowly raised out of the brain, the holes on top of the skull will be covered with bone wax. The nose and ear bars are removed and a layer of saline is added to the top of the skull. Wound clips are used to close the incision. Following the surgery, the rats given reversible cocktail received reversing agent (antisedan 5 mg/ml and saline) with an intramuscular injection. If the rats were given a triple cocktail, they woke up on their own after some time. After surgery all rats received a subcutaneous injection of lactated ringers containing banamine to reduce post-operative pain and ampicillin as an antibiotic. The vector was infused three days before the lesion, since it takes about 3 days to observe the expression of the BDNF in the brain. This procedure may allow BDNF to act as a more protective role rather than be a treatment.

After three days the rats underwent surgery for the perforant path lesion. Using the procedure described above, a hole was drilled into the skull at coordinates from a rat

atlas for the perforant path lesion (AP -7.8 mm, ML -4.0 mm from Bregma). A Model 120 Adjustable wire knife (Kopf Instruments) was lowered into the brain to a depth -6 mm below the bregma. The wire knife was then extended out -2.5 mm in the medial direction. The wire knife was raised -3.5 mm below bregma (a distance of 2.5 mm in dorsal direction) and then back down to -6 mm below the bregma. Next, the wire was retracted and the wire knife was pulled out of the brain. Wound clips were used to close the incision above the skull. The rats were provided with a Lactate Ringer's Solution. The rats were put back into the cage and woke up after some time.

There was a survival period of two weeks. The rats were checked daily till euthanization to ensure they were healthy and well. After two weeks, the animals are euthanized and their brains were removed. On the morning of the procedure, a paraformaldehyde (PFA) with phosphate-buffered saline (PBS) solutions was made. First, a 4% PFA solution was created. Then a 0.4% parabenzoquinone (PBQ) solution was created separated. The solutions are then mixed freshly for each rat right before procedure to produce a final mixture of 2% PFA and 0.2% PBQ. In addition, a PBS solution was made that contained 50:50 of 0.2 PBS and H_2O. The solutions were stored on ice to keep it cold for the perfusion.

The rats were given 0.8 ml of triple anesthesia cocktail. They were checked for level of consciousness with a pinch to the legs and tail. No muscle contractions indicated the rats were unconscious and ready for the procedure. Once, the rats were unconscious an incision was made under the sternum. The ribs were cut to reveal the heart. Once the heart was visible, hemostats were used to hold the skin back. A needle connected to the Masterflex Console Drive perfusion pump was inserted into the right ventricle. The

needle is pushed up into the aorta of the heart and the needle is clipped shut to prevent backflow. A cut is made on the left atrium to release the pressure produced during the perfusion. First, the rats are perfused with 300 ml of 0.1% PBS. Next, the rats are perfused with 250mL of a 1:1 PBQ-PFA solution. Then the brains are carefully removed and stored in a 1:1 PBQ-PFA solution for two hours in the 4°C fridge. Some animals were not perfused well, so the brains were left in the PBQ-PFA solution for four hours. Then the brains are transferred to a sucrose solution and placed on a shaker in a 4°C cold room. The brains are left in the sucrose solution till the brains sink to the bottom of the solution. This took about three days. After the brains were taken off the shaker and kept in a 4°C refrigerator until sectioned.

The brains were cut on a JUNG SM200 Microtome. Tissue-Tek Optimal Cutting Temperature (O.C.T.) compound was spread evenly on top of the freezing stage and the brain will be place on top. The O.C.T. Compound acted a cutting medium to mount tissue onto stage. The brain was horizontally cut with a 40-micron thickness. The left side of the brain was marked with a puncture hole. Each section was placed into a twenty-four well plate with tissue cryoprotectant solution (TCS), a cryoprotectant containing phosphate buffer, ethylene glycol, sodium azide and sucrose. The sections are stored in the -20 °C freezer.

The sections were checked for BDNF expression via immunohistochemistry. Using 1 in every 12 sections checked the BDNF expression. The sections were first rinsed in TBS three times for 5 min each to remove the TCS cryoprotectant. Next, the tissue was place in tubes with 5-10 mL 0.01 Tris-HCl (pH 9) preheated at 80°C hot water bath for 20 min. The tubes are removed form the hot water bath and placed in room

temperature to cool for 30 min. The tissues are then post-fixed in a 2% PFA/0.2% PBQ for 5 min. The sections were then washed in TBS three times for ten minutes each. The incubation in Tris-HCL and post-fixation was part of an antigen retrieval process. Next, the tissues were incubated in 0.6% H_2O_2 in TBS for 30 min to prevent non-specific staining. Then, the sections are blocked in a 0.25% Triton X-100 + 5% heat inactivated normal horse serum in TBS for 60 minutes. The primary antibody incubation was performed in 1mLwith rabbit anti-BDNF (#091207) with a 1:5000 dilutions. It was produced by Chicago Proteintech for our laboratory. The tissues were incubated at 4^oC for four overnights.

After the primary antibody incubation, the tissues are once again washed in TBS three times for 5 min each. The tissue was placed in secondary antibody solution with Donkey anti Rabbit biotin conjugated (1:400) in 0.25% Triton X-100, 5% heat inactivated normal horse serum with TBS for two hours. Next the tissues was washed in TBS three times for ten minutes each and then placed in Vectastain Elite ABC solution (Standard) for two hours at room temperature. After rinsing in TBS, the tissue was incubated in 3,3-diamino-benzidine-HCl (DAB) solution freshly made with $NiCl_2$, H_2O_2. The sections are incubated in the DAB solution for 4 min. Then, the sections are washed in TBS twice for 10 min each. The sections are mounted onto a gelatin covered microscope slide. The sections are placed in the hood for three to four days to let dehydrate.

Nissl Staining

Nissl staining was performed on 1 in every 6 sections. After sections were mounted onto a microscope slide with gelatin to adhere the sections to the slide. Each

slide held either three to four sections. After the tissue had dried after at least two to three days, slides were placed in a microscope slide holder, which would be used to submerge the slides into different solutions for the nissl staining procedure. The slides were submerged in a 1:1 solution of Chloroform:Ethanol for 30 min. The solution will remove the lipid in the tissues. This reduces the non-specific binding of the dye to the lipids. Next, the slides are moved through a series of ethanol (ETOH) that included 100% ETOH for 2 min. 95% ETOH for 2 min, 70% ETOH for 2 min, and 50% ETOH for 2 min. The dye we are using is in a water-based solution; therefore, the tissues need to be rehydrated before they are placed in the solution. If tissues are not rehydrated they can crack. A graded ethanol is used to remove the alcohol and slowly hydrate the tissues. Finally the slides are placed in H_2O for 1 min. The slides are then submerged in 0.25% Thionin for 20 sec. The 0.25% Thionin was a mixture to 36 mL 1M NaOH, 200 mL 1M Acetic acid and 764 mL double-distilled H_2O heated to boil and then add 2.5 g of thionin acetate followed by boiling for 45 min, filtered and store at 37°C. Next, the slides were rinsed in water twice to remove the excess thionin and then rinsed in H_2O with 20 drops of glacial acetic acid that help remove the excess thionin on the tissues. The slides are usually checked after this step. If staining is too dark, then differentiate longer with the acetic acid and H_2O solution. If staining is too light, then repeat the thionin-staining step. Next, the excess thionin is washed off and the tissues are dehydrated. To do this was in: 50% ETOH for 2 min, 70% ETOH for 2 min, 80% ETOH for 2 min, 95% ETOH for 2 min and three times in Isopropanol for 2 min. Once again, a graded ethanol procedure is used to avoid cracks when dehydrating. By the last step, the tissues should be completely dehydrated. Next, the alcohol can be removed by washing it in: Citrisolv for 2 min,

Citrisolv for 3 min and Citrisolv for 5 min. Next, the slides are cover slipped with Distyrene, plasticizer, and xylene (DPX) from VWR Scientific. DPX is a resin, which hardens over some time. The DPX is added to the microscope slide and then it is covered with a Brain Research Laboratories division of Cambridge intelligent systems, Inc. 24x60 mm cover glass no.1 thickness. The slides are allowed to dry in fume hood overnight.

Stereology

The Nissl stained sections were used for quantification of cell number and cell body size using stereology. One in every twelve sections was counted for both the lesions and intact. A counting frame of 30 by 30 microns was used within a sampling frame of 120 by 120 microns. This meant that only 1/16th of the ECLII area was counted. The dissector height was 6 to 7 microns depending on the section. The dissector height ensured that no overlap was to occur between sections and all cells will only be counted once. The analysis program would provide an estimate of cells inside one sections counted and then this number would be multiplied by 12 to account for only counting one in every twelve sections. This provided the raw data for the number of cells on the intact and lesion side. During the counting process the cell would only be counted if the nucleus was focused within the dissector height and if it was within in the area outlined to be ECLII and if it was within in the counting frame. Cell bodies touching the top and right side of the counting frame were counted, while cell bodies touching the left and bottom of the sampling frame were excluded. The cell size was determined using the nucleator function, which outlined the cell and provided a cell size.

Immunofluorescence

Immunofluorescence was used to check was activation of downstream markers of BDNF. Before running all animals activation of specific downstream markers were tested on three animals. The markers tested were: phosphorylated cAMP response element-binding protein (CREB), cFos, phospho-AKT, and phosphor-ERK. The first trial ran took one in twenty-fours sections and sections were stained for BDNF, c-FOS and p-ERK. The tissue was washed in TBS for 10 minutes 3 times. The sections were blocked with 0.25% Triton-X-100 with 5% heat inactivated normal horse serum in TBS and 0.1% NaN3 for 60 min. A solution with the primary antibody was created according to 1 ml/well. The antibodies used were: rabbit anti-BDNF (#091207, Chicago Proteintech) 1:500 dilution, goat anti-cFOS (#sc-52-G, Santa Cruz Biotechnology) 1:200 dilution and mouse anti-p-ERK (#9106, Cell Signaling) 1:200 dilution. The diluent used was 5% heat inactivated normal horse serum in TBS and 0.1% NaN3. The first trial was incubated in the primary antibody solution for four overnights on a shaker in 4°C cold-room. In the second trial different antibodies were used. The antibodies were: rabbit anti-pAKT (#4060, Cell Signaling) 1:500 dilution and mouse anti-NeuN (MAB377, Millipore) 1:200 dilution in 5% heat inactivated normal horse serum in TBS and 0.1% NaN3. The second trial was incubated for 1 overnight in the 4°C cold-room. The third trial antibodies were: rabbit anti-pCREB (#9191, Cell Signaling) 1:200 dilution and mouse anti-synaptophysin (#SIG-3260, Covance) 1:1000 dilution in 5% heat inactivated normal horse serum in TBS and 0.1% NaN3. The third trial was incubated 2 overnights in 4°C cold-room.

After primary antibody incubation, the sections were now incubated in the secondary antibodies for two hours at room temperature consisting of appropriate

secondary antibodies at Alexa 488, Alexa 594, an Alexa 647 (1:500) and DAPI (1:1000) in 5% heat inactivated normal horse serum in TBS and 0.1% NaN3. The sections then were washed in TBS for 10 minutes 3 times. Next, the sections were air-dried, mounted and cover slipped with Flouromount-G. The sections were analyzed and the pAKT antibody showed great specificity and staining. It was chosen as the antibody that will be used to stain all animals to check for neuron viability and functionality.

For the pAKT, one in twelve sections were labeled with rabbit anti p-AKT(#4060, Cell Signaling) with 1:500 dilution, mouse anti NeuN (MAB377, Millipore) with 1:200 dilution, and chicken anti GFAP (#GFAP 1010, Aves). The secondary antibodies used were donkey anti chicken Alexa 488 with dilution 1:500, donkey anti rabbit Alexa 594 dilution 1:500, donkey anti mouse Alexa 647 dilution 1:250 and DAPI with 1:1000 dilution in 5% heat inactivated normal horse serum in TBS and 0.1% NaN3. The sections were mounted, dried and cover slipped with Flouromount-G.

pAKT Cells Quantification

The sections were then scanned onto the computer with 10x objective using the Keyence Microscope BZ-X710. The images were scanned in different channels: 488 nm, 594 nm and 647 nm. Using the program BZ-X Analyzer the images were stitched together. Using photoshop, the scanned and stitched images of the NeuN staining (channel 647) and pAKT staining (channel 594) were loaded in as stack with the NeuN section on top of the pAKT section. Then the bleed through from the 647 channel was subtracted from the 594 channel via using the subtract option in photo. The opacity was set to 25% to ensure cells that were false positive were not as brightly stained and will not be quantified. Next, the pAKT cells were counted via a rating scale of 1 – 3 with

brightest cells being rated as a 1. However, after the initial analyses, no difference was observed across the different rated cell counts and just the total number of counted cells were analyzed. One dorsal section and one ventral section were counted on both the intact and lesion sides.

III. Results

The Nissl stained sections were able to show that most animals had a correct perforant path lesion. As shown in Figure 1, the perforant path (arrows) lesions extended from the medial to the lateral between the EC and the hippocampus. The lesion had appeared as either a line (Figure 1A) or a cavity (Figure 1B) on the Nissl sections. Some animals did not have complete lesion due the placement too medially or too laterally that, resulted in an incomplete lesion. A few subjects had lesions too close to ECLII causing direct damage in layer II. Both misplacement of the lesions were excluded from analysis.

Next, using BDNF immunohistochemistry BDNF expression was examined, as shown in Figure 2. The lesion control (Fig. 2A) also showed very low amounts of BDNF expression in ECLII. The AAV2-BDNF at 1e11 vp/ml (Fig. 2B) resulted in localized expression of BDNF, but some BDNF fibers could be seen in ECLII. The AAV2-BDNF at 3e11 vp/ml (Fig. 2C) produced a higher levels of BDNF compared to AAV2 1e11vp/ml BDNF. The AAV2-BDNF at 3e11 vp/ml x 2 volume (Fig. 2D) had even wider expression of BDNF . Finally, the AAV9 3e11 vp/ml BDNF (Fig. 2E) show the greatest spread and most BDNF expression. Interestingly, BDNF expression was visible on the intact side of EC in the AAV9-BDNF group EC (Fig. 2G) that was much higher than the intact side of the control group (Fig. 2F). In summary, the AAV9 vector produced the largest spread and largest BDNF expression, and as expected the AAV2 group would have greater spread with the higher titer and volume infused.

The main goal of the present study was to examine the effects of different BDNF viral vectors (AAV2 vs. AAV9) and concentrations of AAV2 on cell survival of EC II neurons following a perforant path lesion. To determine these effects stereology was performed to quantify the number of cells and cell body size on the intact and lesion side

of the medial ECLII. Also, the area counted was measured, which is presented in Table 1 and a 2-way mixed (Treatment x Lesion/Intact) ANOVA for the area measured showed that there no significant difference in the area sampled between treatment groups ($F(4,24)=0.95$, $p=0.45$) or between lesion and intact sides ($F(1,24)=1.41$ $p=0.24$); no interaction effect (Treatment x Lesion/Intact) was observed ($F(4,24)=0.81$, $p=.53$). Therefore, the areas sampled were not statistically different between the treatment groups.

Next, the data from the stereology on neuron count from Nissl sections were analyzed. As shown in Figure 3, the control group showed a decrease in the number of cells and cell size on lesion side relative to the intact side (3A-B). In the BDNF groups, the Nissl stain generally showed (3C-F) a higher cell density and cell size when compared to control-lesion (3B). Figure 3A displays the control-intact, which looks more similar to the treatment lesion sides showing treatment was able to recover

Table 2 displays the mean value and standard errors of number of cells for each group for the intact and lesion sides. A 2-way mixed (Treatment x Lesion/Intact) ANOVA for the number of cells counted was ran and showed that there no significant difference between treatment groups ($F(4,24)=0.98$, $p=0.43$), there was a significant effects between lesion and intact sides ($F(1,24)=26.8$ $p=0.0001$) across the groups. In addition a significant interaction effect (Treatment x Lesion/Intact) was observed ($F(4,24)=3.79$, $p=0.0158$). The interaction effect led to post-hoc analyses to determine the source of interaction via comparing the intact and lesion side for each group. The 1-way repeated measure data is presented in Table 2. Here, only the control ($p = 0.0005$) and AAV2-3e11vp/ml ($p = 0.0418$*) group shows significant difference between he intact and

lesion side. There is about 25% decrease in number of cells in control treatment, but the BDNF groups showed a lower cell loss that ranged from 2% to 13% (Table 2).

Next, to reduce variability we measured the percent change from intact verse lesion. We were able to calculate this by dividing the number of intact cells by the number of lesion cells (size was determined similarly). The animals were processed at different times leading to possible differences in the levels of fixations and tissue processing. On top of this, 4-6 sections were counted per animals leading to more variability. To reduce this variability, the percentage change was measured and displayed in Figure 4A. The control group had a 27% cell loss verses the treatment groups, which had much less cell loss (2-13% loss). A 1-way ANOVA was run on percent change of cells for the different treatment groups to determine if there was a statistically significance between all five groups and the ANOVA results was significant ($F(4,24)=3.05$, $p=0.037$) (Figure 4A). Then a post hoc Fisher LSD test was run to determine, which groups produced the statistically significant by comparing the control group verses the different BDNF treated groups individually. The AAV2 1e11vp/ml BDNF and AAV9 3e11vp/ml BDNF infused groups were significantly different from the control group at $p < 0.01$, while AAV2 3e11vp/ml BDNF and AAV2 3e11 vp/ml x 2 volume BDNF group had a $p < 0.05$ (Figure 4A). These findings support BDNF does produce a neuroprotective effect to reduce cell loss formed by the perforant path lesion.

Table 3 displays the mean value and standard errors of measured size of cell body for each group for the intact and lesion sides. A 2-way mixed ANOVA was run, which showed that there was a significant main effect of intact/lesion ($F(1,24)=21.1$, $p=0.0001$) and the treatment groups showed a strong trend but was not a significant effect

($F(4,24)=2.64$, $p=0.059$). There was a clear trend for the AAV9 3e11 vp/ml BDNF to have larger cells on the intact and lesion side compared to the control. The interaction effect was not significant ($F(4,24)=1.41$, $p=0.25$). There was a 20% decrease in cell size without treatment. Upon treatment cell size was decreased by 3-14% depending on the group.

Not all animals were stained at the same time, so difference during the processing of the tissue (e.g., perfusion, fixation, staining) could contribute to difference in cell size in individual subjects. Therefore, to reduce these differences we compared the percentage change in cell body size (intact/lesion) (Figure 4B). A 1-way ANOVA was run to compare all different groups on percentage change in cell body size and found not to be, significance ($F(4,24)=1.92$, $p=0.14$). However, there was trend that showed that the BDNF groups to show less cell atrophy (3-14%) than the control group (20%). Therefore, we did individual t-test to examine specific differences between each BDNF group and the control group. The AAV9-BDNF was significantly different with this analyses ($t(9)=6.60$, $p=0.033$).

To determine the pathway BDNF activated to promote cell survival we analyzed pAKT activation. The sections were stained for pAKT and the cells quantified and the raw data is represented in Table 4. For this analysis, the 1e11vp/ml AAV2-BDNF was not included. A 2-way mixed ANOVA was run (Treatment x Intact/Lesion), which showed no significance for the treatment ($F(3,18)=2$, $p=0.15$). The intact verse lesion comparison showed no significance ($F(1,18)=16.7$, $p=0.21$) and no interaction effect was found ($F(3,18)=0.64$, $p=0.59$).

Table 1: Entorhinal Cortex Layer II Area Sampled

The table displays the average area measured in each treatment group for both the intact and lesion side with the number of animals in each group. A 2-way mixed (Treatment x Intact/Lesion) ANOVA showed no treatment effect, no difference between lesion or intact side and no interaction effect.

Treatment	Number of Animals	Intact Area (± SEM) (μm^2)	Lesion Area (± SEM) (μm^2)
Control	5	1040671 ± 68574	1121185 ± 88255
1e11 BDNF	6	1151860 ± 62599	1188628 ± 80565
3e11 BDNF	8	1175494 ± 54212	1194882 ± 69772
3e11 x 2 BDNF	5	1059815 ± 68574	1044051 ± 88255
AAV9 BDNF	5	1227286 ± 68574	1216446 ± 88255

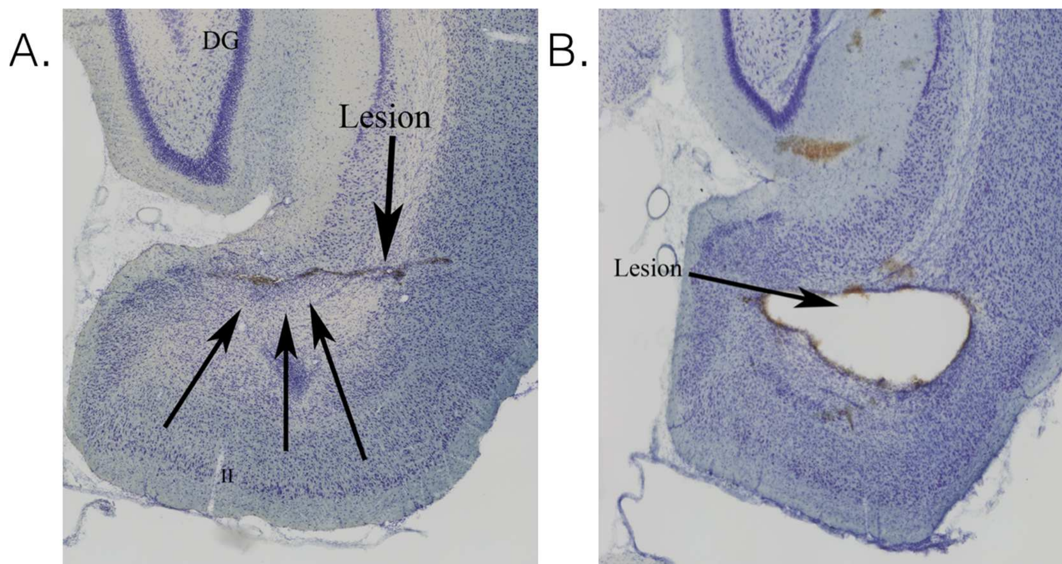

Figure 1: Entorhinal Cortex and Hippocampus Anatomy with Lesion
Entorhinal cortex layer II is labeled as II on Figure 1A. The three arrows extending from layer II represent the perforant pathway. The DG is the dentate gyrus. The lesion is labeled on both images.

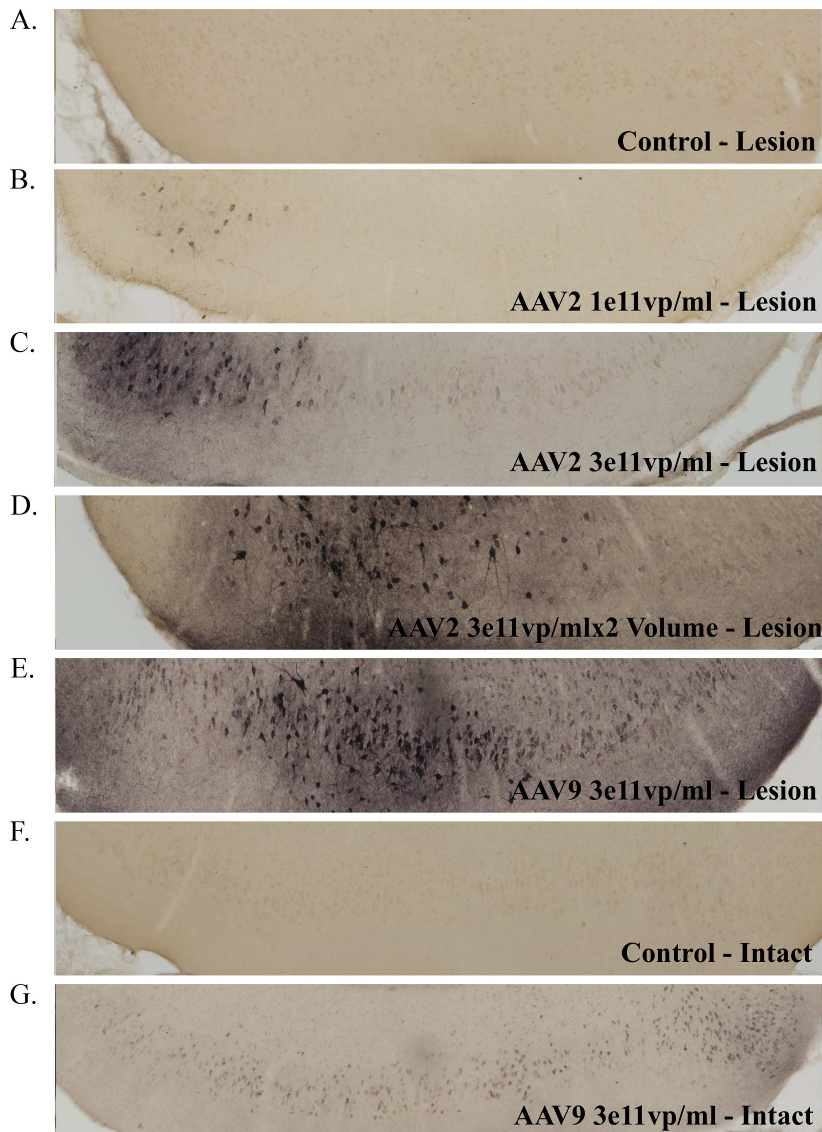

Figure 2: BDNF Expression

BDNF gene delivery into the EC on the lesion side resulted in elevated expression of BDNF in ECLII. (A) The control treatment (AAV2-GFP) showed very low levels of BDNF expression, while each of the AAV2-BDNF groups showed greater spread with amounts of AAV2-BDNF (B-D). AAV9-BDNF showed the highest expression and widespread (E). In the AAV9-BDNF group, BDNF expression was observed even on the intact EC (F), suggesting retrograde transport of the AAV9 to this side of the brain compared to intact controls (G).

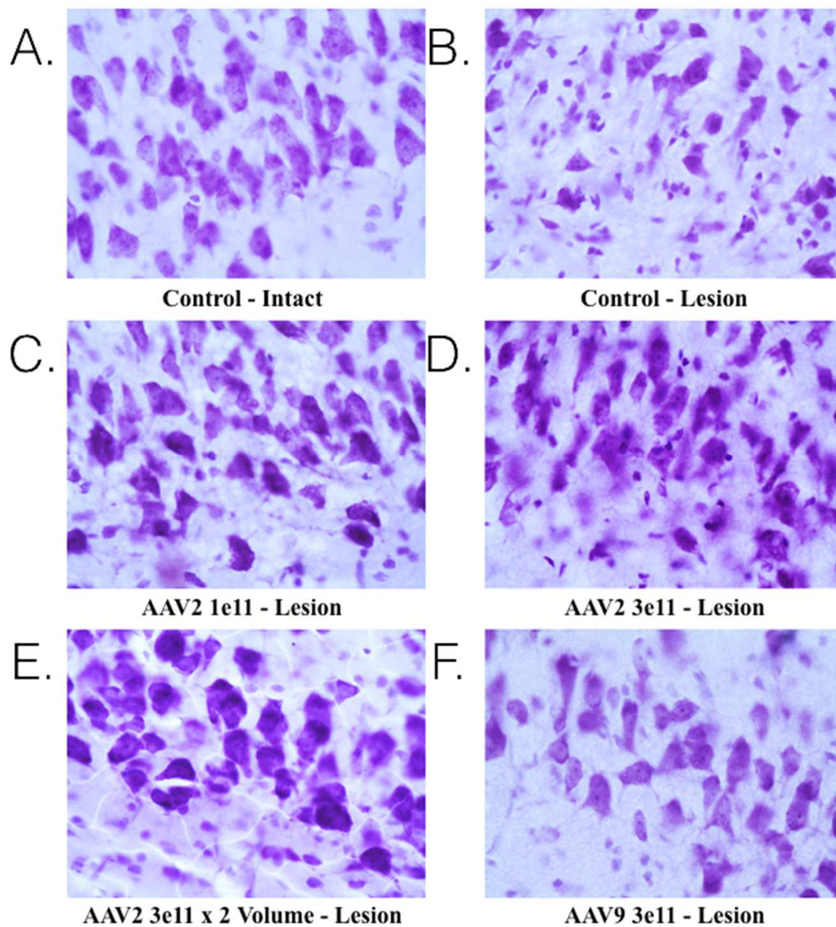

Figure 3: Nissl Staining in ECL II

Comparing 3A and 3B, there is a clear difference in cell density and cell size. Looking at 3C-3F, the treatment groups, compared to the 3B there is an increase in cell density and cell size. The image groups are labeled right under each image for A-F.

Table 2: Cell Count and Percentage

The data here displays the mean of cell count and the stand error of the mean (SEM) for the intact and lesion side. An interaction effect was observed and post-hoc analysis comparing lesion to intact side within groups with Fisher LSD p-values provided.

Treatment	N Animals	Intact Count (± SD)	Lesion Count (± SD)	Lesion/Intant (%)	P Value Intact vs. Lesion
Control	5	27,566 ± 5,160	20,436 ± 5,252	73	p = 0.0005*
1e11 BDNF	6	27,186 ± 5,021	26,420 ± 4,446	98	p = 0.4997
3e11 BDNF	8	25,763 ± 1,906	22,443 ± 3,700	87	p = 0.0418*
3e11 x 2 BDNF	5	24,112 ± 3,402	22,068 ± 2,108	92	p = 0.2483
AAV9 BDNF	5	25,472 ± 845	24,165 ± 2,263	94	p = 0.2983

Table 3: Cell Size and Percentage

The data here displays the mean of cell body size (μm^2) and the stand error of the mean (SEM) for the intact and lesion side. No significance in treatment group or interaction effect. Lesion verses intact showed a p = 0.00001. Post-hoc analysis comparing lesion to intact with Fisher LSD was run and p-values displayed.

Treatment	N Animals	Intact Cell Size (± SD)	Lesion Cell Size (± SD)	Lesion/Intact (%)	P Value Intact vs. Lesion
Control	5	163 ± 25	131 ± 26	80	p = 0.0246*
1e11 BDNF	6	156 ± 40	148 ± 45	93	p = 0.1928
3e11 BDNF	8	179 ± 29	161 ± 32	90	p = 0.0952
3e11 x 2 BDNF	5	171 ± 14	150 ± 22	86	p = 0.0149*
AAV9 BDNF	5	204 ± 33	197 ± 21	97	p = 0.5254

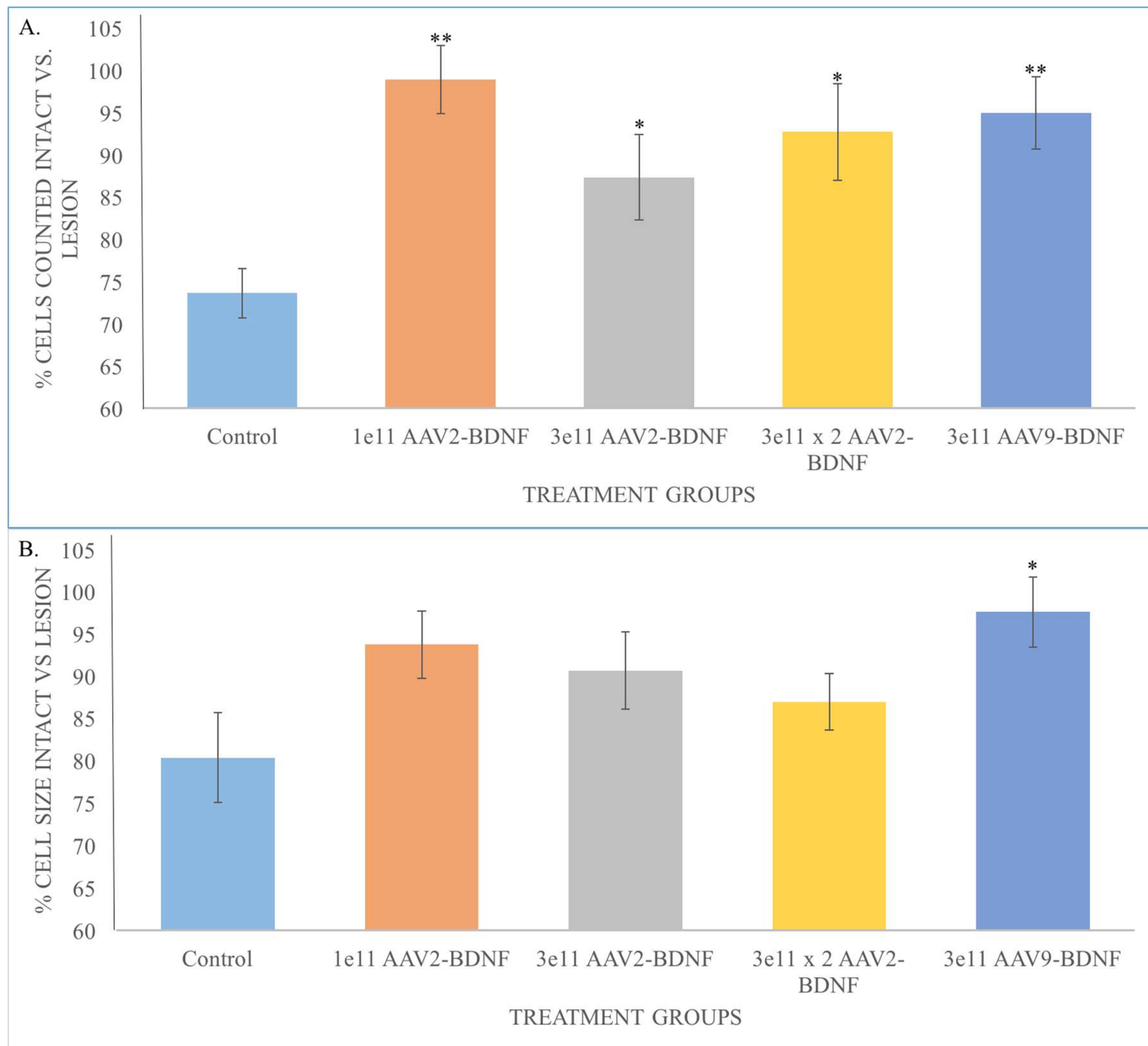

Figure 4: BDNF's Neuroprotective Effect

(**A**)Percentage of cell survival in ECLII (intact/lesion) showed that each of the BDNF treatment increased cell survival following perforant path lesion model. (*$p<0.05$ for ANOVA, post-hoc Fisher **=$p<0.01$ vs. Control, *=$p<0.05$ vs. control). (**B**) Percentage of cell size in ECLII (intact/lesion) showed a trend for less atrophy in the BDNF groups. Though the overall ANOVA not statistically significant, specific t-test comparisons between each treatment group vs. control showed that the AAV9-BDNF was significantly different.

Table 4: pAKT Cell Count

	MEAN		SEM	
Treatment	**Intact**	**Lesion**	**Intact**	**Lesion**
Control	17.3	17.5	2.7	4.0
3e11 AAV2-BDNF	22.4	28.3	2.7	5.8
3e11 x 2 AAV2-BDNF	12.4	14.6	2.4	3.4
1e11 AAV9-BDNF	21.8	22.6	2.7	4.0

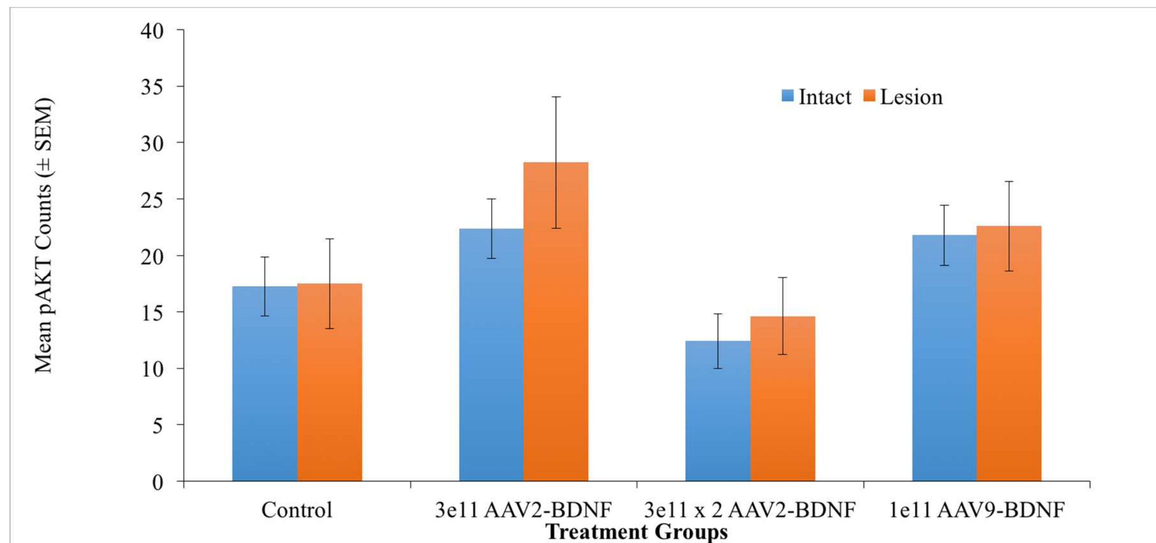

Figure 5: pAKT Cell Counts in ECLII.
Graph displays the average number of pAKT cells ± SEM in the control and 3 BDNF treatment groups. There were no significant differences between the treatment groups or a difference of intact vs. lesion counts of pAKT cell numbers. These findings showed that the number of pAKT cells was not altered by BDNF treatment at 2 weeks following treatment.

IV. Discussion

The goal of this study was to determine the efficacy of BDNF gene therapy in a perforant pathway lesion model with different virus vectors (AAV2 vs. AAV9) and different dosages of AAV2-BDNF (1e11 vp/ml, 3e11 vp/ml and 3e11 vp/ml with twice the volume). Our laboratory has shown the effect of BDNF gene therapy using lentivirus vectors in rodent and primate models of AD [5, 184]. The data observed in this study indicate BDNF gene therapy was able to reduced cell death following perforant path lesion when with AAV2-BDNF at 1e11 vp/ml (5 µl), AAV2-BDNF at 3e11 vp/ml at 5 µl and AAV2-BDNF 3e11 vp/ml at 10 µl and AAV9 at 3e11 vp/ml at 5 µl. Also, BDNF gene therapy was able to prevent cell atrophy when delivered with AAV9.

Two virus vectors were used during this study: AAV2 and AAV9, which both produced different levels of BDNF expression in the EC. As expected, the amount of BDNF expression with the AAV2 was smaller with the AAV2-BDNF 1e11 vp/ml, higher followed by the AAV2-BDNF 3e11 vp/ml at 5 µl , and then slightly more wider with AAV2-BDNF 3e1ll vp/ml at 10 µl . Finally, the AAV9-BDNF 3e11 vp/ml produced the largest spread and had the most transfected cells (Figure 2). Evidence supports AAV9's capabilities in producing a widespread effect [186]. Following AAV9-BDNF, BDNF expression was also visible on the contralateral ECLII that was higher than intact-control (Figure 2). This increase in BDNF expression in the contralateral ECLII might be due to retrograde transport of AAV9, since these neurons project to the contralateral side to innervate the subicular area and dentate granule cells [187]. AAV9-BDNF spread on the ipsilateral (lesion) side included the subicular area and parts of the hippocampus. Retrograde transport of AAV9 can occur through axonal processes after uptake via ligand bound endocytosis and transport back to the cell body [188-190]. Evidence supports that

AAV2 does not experience a widespread effects unlike other AAVs [191, 192]; the reduce spread of AAV2 is likely due to AAV2 entering cells via heparan sulfate proteoglycan and the high amount of this protein found in the brain. [193-197].

In our study, the perforant path lesion successfully induced cell loss with about 27% decrease in neuron number. This finding is consistent with previous studies that found a 30% decrease [185] and about 20% decrease in perforant path rat lesion model and [5]. Also on top of this, there was a 45.9% decrease in ECLII in a non-human primate lesion model (Nagahara et al., 2009). The perforant path lesion models allowed us to determine the neuroprotective effects of BDNF delivered via AAV2 or AAV9 with different dosages.

A major finding of this study was that all groups treated with BDNF gene delivery showed a statistically significant reduction in the percent cell loss (lesion/intact) following the perforant path lesion. While the control group showed a 27% decrease in cell number, the AAV2-BDNF (1e11vp/ml, 3e11 vp/ml and 3e11 vp/ml x 2 volume) and the AAV9-BDNF (3e11 vp/ml) showed only a 2-13% cell loss when compared to the intact side. All virus vectors and concentration produced a neuroprotective effect on ECLII. Previous studies in our laboratory showed that lentiviral delivery of BDNF produced a neuroprotective effect on ECLII and treated rodents had almost no loss of ECLII cells, while non-human primates experienced a 15% decrease in ECLII cells. [184]. Also, in an unpublished finding in our laboratory showed that AAV2-BDNF at a high dosage of 1e12 vp/ml could also prevented cell loss in ECLII. ECLII, primary source of input into hippocampus, is one of the first areas of cell loss in AD [42, 85-87].

BDNF gene therapy in AD could potentially prevent cell loss of ECLII neurons and reduce memory impairment.

These findings support that BDNF does produce a neuroprotective effects and promotes cell survival. As noted in the introduction, BDNF possesses neuroprotective effects that reduce neuron death from ischemia, excitotoxicity and axotomy. Our findings further support the notion that BDNF does increase cell survival. In addition, BDNF also reduces the neurotoxicity effects of Aß in cell cultures of primary neurons [5, 183]. On top of this, BDNF was able to prevent cell death in APP mouse, an AD model, with early treatment by reducing cell loss from 22% to 11% with BDNF treatment [184]. Together, these finding suggest BDNF could act as a potential treatment for the prevention of cell loss in AD.

BDNF's neuroprotective effects have been shown to prevent cell atrophy and prevent the shrinkage of cells. In this study, there was a trend for the different BDNF group to produce less atrophy than the control group control 20% reduction vs. BDNF 2-14% reduction. However, only the AAV9 showed a significant reduction in the cell atrophy. Lentiviral delivery of BDNF has been known to reduce cell atrophy. Previous papers with monkey perforant path lesion models, BDNF gene delivery via lentivirus was able to prevent cell atrophy. The monkeys treated with lentiviral-BDNF had cell size decreased to 92%, while untreated monkeys had cell size decreased to 84% [5]. Previous evidence supports BDNF's role in hypertrophy [184, 198, 199]. Interestingly, AAV9 delivery of BDNF had the highest cell body size on the intact (204 μm^2) and lesion (197 μm^2) side. The intact control size had a cell size that averaged about to 163 μm^2, which was much lower than the AAV9. This might have occurred due to elevated BDNF

expression in ECLII on the intact side from retrograde transport of the AAV9-BDNF, described earlier. The difference in cell size on the intact side between the control and AAV9-BDNF might arise from BDNF's hypertrophic effects.

BDNF's neuroprotective effects arise from the pathways it is able to activate. One such pathway would be the activation of the pAKT, which is a pro-survival pathway as stated in the introduction. BDNF should activate the PI3K, which is able to activate AKT via intermediate proteins. pAKT has been suggested to turn off apoptotic genes and prevent cell death [170, 176, 177]. Therefore, we hypothesized that BDNF gene delivery would activate the AKT pathway and mediate cell survival effects. However, there were no statistically significance effects of BDNF treatment on the number of pAKT cells in ECLII. The quantification of the pAKT occurred two weeks after lesion. It is possible that BDNF effects on pAKT expression were more prominent at an early time point after the lesion. Another possible explanation behind the cell survival properties of BDNF is that BDNF might have activation pERK pathways. However, pERK immunohistochemistry was attempted with two different pERK antibodies, but they did not show good labeling and therefore, were not used.

To summarize our findings, BDNF treatments at these lower concentrations of AAV2 and the AAV9 were able to produce a neuroprotective effect in rats to decrease loss of entorhinal neurons in layer II. Previous studies with BDNF lentivirus and a high dosage of AAV2-BDNF produced similar effects. In addition, AAV9-BDNF group was shown to significantly reduce cell atrophy, while the other BDNF groups displayed a trend of decreasing cell atrophy compared to the control. These finding support AAV2-

BDNF treatment at lower concentrations of AAV2 has the potential to prevent cell atrophy in AD

Future Directions

BDNF has been shown to be a critical neurotrophin in the brain and future studies examining its effects in preventing cell survival and atrophy is required to get a better understanding of the pathways it plays a role in and better delivery methods. For example, future studies can look at the pERK or PLCgamma pathway to show an in vivo connection between preventing cell death and atrophy. In addition, future studies could look at different time points after the lesion to capture the possible effects of BDNF treatment on the different pathways. AAV2-BDNF at lower dosages and with AAV9-BDNF were both successful and future studies can test there effects on other AD models such as APP mice, aged rats, or lesion monkeys. Further investigating BDNF's neuroprotective effect against Aβ and tau neurotoxicity will be useful in understanding the potential use of BDNF at a treatment for AD. Preliminary experiment in our lab showed that BDNF was able to reduce tau phosphorylation in primary cultured cortical cells. Future studies could expand this evidence and test lower dosages of AAV2 and AAV9 in transgenic mice overexpressing tau.

Conclusions

Overall, BDNF does produce a neuroprotective effect in ECLII. This is important, since the first areas of cell loss occur in the EC in AD, which inevitably leads to the cognitive impairment symptoms. For instance, mild to moderate AD there was ~60% decrease in ECLII cells [86, 87]. Previous studies have also shown cell atrophy in AD, where there was a 25.1% reduction in neuronal volume of cell body [86]. The treatment

of ECLII via BDNF gene therapy can potentially prevent the cell loss and atrophy in ECLII, which will hopefully prevent cognitive decline AD

References

1. Evans, D.A., L.A. Smith, P.A. Scherr, M.S. Albert, H.H. Funkenstein, and L.E. Hebert, *Risk of death from Alzheimer's disease in a community population of older persons.* Am J Epidemiol, 1991. **134**(4): p. 403-12.

2. Wilmoth, J.R., L.J. Deegan, H. Lundstrom, and S. Horiuchi, *Increase of maximum life-span in Sweden, 1861-1999.* Science, 2000. **289**(5488): p. 2366-8.

3. Vaupel, J.W., *Biodemography of human ageing.* Nature, 2010. **464**(7288): p. 536-42.

4. Association, A.s., *2015 Alzheimer's disease facts and figures.* Alzheimers Dement, 2015. **11**(3): p. 332-84.

5. Nagahara, A.H., D.A. Merrill, G. Coppola, S. Tsukada, B.E. Schroeder, G.M. Shaked, L. Wang, A. Blesch, A. Kim, J.M. Conner, E. Rockenstein, M.V. Chao, E.H. Koo, D. Geschwind, E. Masliah, A.A. Chiba, and M.H. Tuszynski, *Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease.* Nat Med, 2009. **15**(3): p. 331-7.

6. Campion, D., C. Dumanchin, D. Hannequin, B. Dubois, S. Belliard, M. Puel, C. Thomas-Anterion, A. Michon, C. Martin, F. Charbonnier, G. Raux, A. Camuzat, C. Penet, V. Mesnage, M. Martinez, F. Clerget-Darpoux, A. Brice, and T. Frebourg, *Early-onset autosomal dominant Alzheimer disease: prevalence, genetic heterogeneity, and mutation spectrum.* Am J Hum Genet, 1999. **65**(3): p. 664-70.

7. Goate, A., M.C. Chartier-Harlin, M. Mullan, J. Brown, F. Crawford, L. Fidani, L. Giuffra, A. Haynes, N. Irving, and L. James, *Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease.* Nature, 1991. **349**(6311): p. 704-6.

8. Goate, A.M., A.R. Haynes, M.J. Owen, M. Farrall, L.A. James, L.Y. Lai, M.J. Mullan, P. Roques, M.N. Rossor, and R. Williamson, *Predisposing locus for Alzheimer's disease on chromosome 21.* Lancet, 1989. **1**(8634): p. 352-5

9. Theuns, J., E. Marjaux, M. Vandenbulcke, K. Van Laere, S. Kumar-Singh, G. Bormans, N. Brouwers, M. Van den Broeck, K. Vennekens, E. Corsmit, M. Cruts De Strooper, C. Van Broeckhoven, and R. Vandenberghe, *Alzheimer dementia caused by a novel mutation located in the APP C-terminal intracytosolic fragment.* Hum Mutat, 2006. **27**(9): p. 888-96.

10. Mullan, M., H. Houlden, M. Windelspecht, L. Fidani, C. Lombardi, P. Diaz, M. Rossor, R. Crook, J. Hardy, and K. Duff, *A locus for familial early-onset Alzheimer's disease on the long arm of chromosome 14, proximal to the alpha 1-antichymotrypsin gene.* Nat Genet, 1992. **2**(4): p. 340-2.

11. Hutton, M., F. Busfield, M. Wragg, R. Crook, J. Perez-Tur, R.F. Clark, G. Prihar, C. Talbot, H. Phillips, K. Wright, M. Baker, C. Lendon, K. Duff, A. Martinez, H. Houlden, A. Nichols, E. Karran, G. Roberts, P. Roques, M. Rossor, J.C. Venter, M.D. Adams, R.T. Cline, C.A. Phillips, and A. Goate, *Complete analysis of the presenilin 1 gene in early onset Alzheimer's disease.* Neuroreport, 1996. **7**(3): p. 801-5.

12. Rogaev, E.I., R. Sherrington, E.A. Rogaeva, G. Levesque, M. Ikeda, Y. Liang, H. Chi, C. Lin, K. Holman, and T. Tsuda, *Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene.* Nature, 1995. **376**(6543): p. 775-8.

13. Cruts, M., C.M. van Duijn, H. Backhovens, M. Van den Broeck, A. Wehnert, S. Serneels, R. Sherrington, M. Hutton, J. Hardy, P.H. St George-Hyslop, A. Hofman, and C. Van Broeckhoven, *Estimation of the genetic contribution of presenilin-1 and -2 mutations in a population-based study of presenile Alzheimer disease.* Hum Mol Genet, 1998. **7**(1): p. 43-51.

14. Sobhanifar, S., B. Schneider, F. Lohr, D. Gottstein, T. Ikeya, K. Mlynarczyk, W. Pulawski, U. Ghoshdastider, M. Kolinski, S. Filipek, P. Guntert, F. Bernhard, and V. Dotsch, *Structural investigation of the C-terminal catalytic fragment of presenilin 1.* Proc Natl Acad Sci U S A, 2010. **107**(21): p. 9644-9.

15. Myers, R.H., E.J. Schaefer, P.W. Wilson, R. D'Agostino, J.M. Ordovas, A. Espino, R. Au, R.F. White, J.E. Knoefel, J.L. Cobb, K.A. McNulty, A. Beiser, and P.A. Wolf, *Apolipoprotein E epsilon4 association with dementia in a population-based study: The Framingham study.* Neurology, 1996. **46**(3): p. 673-7.

16. Harold, D., R. Abraham, P. Hollingworth, R. Sims, A. Gerrish, M.L. Hamshere, J.S. Pahwa, V. Moskvina, K. Dowzell, A. Williams, N. Jones, C. Thomas, A. Stretton, A.R. Morgan, S. Lovestone, J. Powell, P. Proitsi, M.K. Lupton, C. Brayne, D.C. Rubinsztein, M. Gill, B. Lawlor, A. Lynch, K. Morgan, K.S. Brown, P.A. Passmore, D. Craig, B. McGuinness, S. Todd, C. Holmes, D. Mann, A.D. Smith, S. Love, P.G. Kehoe, J. Hardy, S. Mead, N. Fox, M. Rossor, J. Collinge, W. Maier, F. Jessen, B. Schurmann, R. Heun, H. van den Bussche, I. Heuser, J. Kornhuber, J. Wiltfang, M. Dichgans, L. Frolich, H. Hampel, M. Hull, D. Rujescu, A.M. Goate, J.S. Kauwe, C. Cruchaga, P. Nowotny, J.C. Morris, K. Mayo, K. Sleegers, K. Bettens, S. Engelborghs, P.P. De Deyn, C. Van Broeckhoven, G. Livingston, N.J. Bass, H. Gurling, A. McQuillin, R. Gwilliam, P. Deloukas, A. Al-Chalabi, C.E. Shaw, M. Tsolaki, A.B. Singleton, R. Guerreiro, T.W. Muhleisen, M.M. Nothen, S. Moebus, K.H. Jockel, N. Klopp, H.E. Wichmann, M.M. Carrasquillo, V.S. Pankratz, S.G. Younkin, P.A. Holmans, M. O'Donovan, M.J. Owen, and J. Williams, *Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease.* Nat Genet, 2009. **41**(10): p. 1088-93.

17. Lambert, J.C., S. Heath, G. Even, D. Campion, K. Sleegers, M. Hiltunen, O. Combarros, D. Zelenika, M.J. Bullido, B. Tavernier, L. Letenneur, K. Bettens, C. Berr, F. Pasquier, N. Fievet, P. Barberger-Gateau, S. Engelborghs, P. De Deyn, I. Mateo, A. Franck, S. Helisalmi, E. Porcellini, O. Hanon, I. European Alzheimer's Disease Initiative, M.M. de Pancorbo, C. Lendon, C. Dufouil, C. Jaillard, T. Leveillard, V. Alvarez, P. Bosco, M. Mancuso, F. Panza, B. Nacmias, P. Bossu, P. Piccardi, G. Annoni, D. Seripa, D. Galimberti, D. Hannequin, F. Licastro, H. Soininen, K. Ritchie, H. Blanche, J.F. Dartigues, C. Tzourio, I. Gut, C. Van Broeckhoven, A. Alperovitch, M. Lathrop, and P. Amouyel, *Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease.* Nat Genet, 2009. **41**(10): p. 1094-9.

18. Shi, H., O. Belbin, C. Medway, K. Brown, N. Kalsheker, M. Carrasquillo, P. Proitsi, J. Powell, S. Lovestone, A. Goate, S. Younkin, P. Passmore, Genetic, C. Environmental Risk for Alzheimer's Disease, K. Morgan, and U.K.C. Alzheimer's Research, *Genetic variants influencing human aging from late-onset Alzheimer's disease (LOAD) genome-wide association studies (GWAS).* Neurobiol Aging, 2012. **33**(8): p. 1849 e5-18.

19. Cerf, E., A. Gustot, E. Goormaghtigh, J.M. Ruysschaert, and V. Raussens, *High ability of apolipoprotein E4 to stabilize amyloid-beta peptide oligomers, the pathological entities responsible for Alzheimer's disease.* FASEB J, 2011. **25**(5): p. 1585-95.

20. Drzezga, A., T. Grimmer, G. Henriksen, M. Muhlau, R. Perneczky, I. Miederer, C. Praus, C. Sorg, A. Wohlschlager, M. Riemenschneider, H.J. Wester, H. Foerstl, M. Schwaiger, and A. Kurz, *Effect of APOE genotype on amyloid plaque load and gray matter volume in Alzheimer disease.* Neurology, 2009. **72**(17): p. 1487-94.

21. Price, J.L. and J.C. Morris, *Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease.* Ann Neurol, 1999. **45**(3): p. 358-68.

22. Vos, S.J., C. Xiong, P.J. Visser, M.S. Jasielec, J. Hassenstab, E.A. Grant, N.J. Cairns, J.C. Morris, D.M. Holtzman, and A.M. Fagan, *Preclinical Alzheimer's disease and its outcome: a longitudinal cohort study.* Lancet Neurol, 2013. **12**(10): p. 957-65.

23. Apostolova, L.G., R.A. Dutton, I.D. Dinov, K.M. Hayashi, A.W. Toga, J.L. Cummings, and P.M. Thompson, *Conversion of mild cognitive impairment to Alzheimer disease predicted by hippocampal atrophy maps.* Arch Neurol, 2006. **63**(5): p. 693-9.

24. Petersen, R.C., G.E. Smith, S.C. Waring, R.J. Ivnik, E.G. Tangalos, and E. Kokmen, *Mild cognitive impairment: clinical characterization and outcome.* Arch Neurol, 1999. **56**(3): p. 303-8.

25. Mauri, M., E. Sinforiani, C. Zucchella, M.G. Cuzzoni, and G. Bono, *Progression to dementia in a population with amnestic mild cognitive impairment: clinical variables associated with conversion.* Funct Neurol, 2012. **27**(1): p. 49-54.

26. Ringman, J.M., L.J. Liang, Y. Zhou, S. Vangala, E. Teng, S. Kremen, D. Wharton, A. Goate, D.S. Marcus, M. Farlow, B. Ghetti, E. McDade, C.L. Masters, R.P. Mayeux, M. Rossor, S. Salloway, P.R. Schofield, J.L. Cummings, V. Buckles, R. Bateman, J.C. Morris, and N. Dominantly Inherited Alzheimer, *Early behavioural changes in familial Alzheimer's disease in the Dominantly Inherited Alzheimer Network.* Brain, 2015. **138**(Pt 4): p. 1036-45.

27. Silagi, M.L., P.H. Bertolucci, and K.Z. Ortiz, *Naming ability in patients with mild to moderate Alzheimer's disease: what changes occur with the evolution of the disease?* Clinics (Sao Paulo), 2015. **70**(6): p. 423-8.

28. Jensen-Dahm, C., M.U. Werner, J.B. Dahl, T.S. Jensen, M. Ballegaard, A.M. Hejl, and G. Waldemar, *Quantitative sensory testing and pain tolerance in patients with mild to moderate Alzheimer disease compared to healthy control subjects.* Pain, 2014. **155**(8): p. 1439-45.

29. Atwood, C.S., R.N. Martins, M.A. Smith, and G. Perry, *Senile plaque composition and posttranslational modification of amyloid-beta peptide and associated proteins.* Peptides, 2002. **23**(7): p. 1343-50.

30. Cruz, L., B. Urbanc, S.V. Buldyrev, R. Christie, T. Gomez-Isla, S. Havlin, M. McNamara, H.E. Stanley, and B.T. Hyman, *Aggregation and disaggregation of senile plaques in Alzheimer disease.* Proc Natl Acad Sci U S A, 1997. **94**(14): p. 7612-6.

31. Esch, F.S., P.S. Keim, E.C. Beattie, R.W. Blacher, A.R. Culwell, T. Oltersdorf, D. McClure, and P.J. Ward, *Cleavage of amyloid beta peptide during constitutive processing of its precursor.* Science, 1990. **248**(4959): p. 1122-4.

32. Sinha, S., J.P. Anderson, R. Barbour, G.S. Basi, R. Caccavello, D. Davis, M. Doan, H.F. Dovey, N. Frigon, J. Hong, K. Jacobson-Croak, N. Jewett, P. Keim, J. Knops, I. Lieberburg, M. Power, H. Tan, G. Tatsuno, J. Tung, D. Schenk, P. Seubert, S.M. Suomensaari, S. Wang, D. Walker, J. Zhao, L. McConlogue, and V. John, *Purification and cloning of amyloid precursor protein beta-secretase from human brain.* Nature, 1999. **402**(6761): p. 537-40.

33. Osenkowski, P., H. Li, W. Ye, D. Li, L. Aeschbach, P.C. Fraering, M.S. Wolfe, D.J. Selkoe, and H. Li, *Cryoelectron microscopy structure of purified gamma-secretase at 12 A resolution.* J Mol Biol, 2009. **385**(2): p. 642-52.

34. Olsson, F., S. Schmidt, V. Althoff, L.M. Munter, S. Jin, S. Rosqvist, U. Lendahl, G. Multhaup, and J. Lundkvist, *Characterization of intermediate steps in amyloid beta (Abeta) production under near-native conditions.* J Biol Chem, 2014. **289**(3): p. 1540-50.

35. Hartmann, T., S.C. Bieger, B. Bruhl, P.J. Tienari, N. Ida, D. Allsop, G.W. Roberts, C.L. Masters, C.G. Dotti, K. Unsicker, and K. Beyreuther, *Distinct sites of intracellular production for Alzheimer's disease A beta40/42 amyloid peptides.* Nat Med, 1997. **3**(9): p. 1016-20.

36. Martins, I.C., I. Kuperstein, H. Wilkinson, E. Maes, M. Vanbrabant, W. Jonckheere, P. Van Gelder, D. Hartmann, R. D'Hooge, B. De Strooper, J. Schymkowitz, and F. Rousseau, *Lipids revert inert Abeta amyloid fibrils to neurotoxic protofibrils that affect learning in mice.* EMBO J, 2008. **27**(1): p. 224-33.

37. Klein, W.L., G.A. Krafft, and C.E. Finch, *Targeting small Abeta oligomers: the solution to an Alzheimer's disease conundrum?* Trends Neurosci, 2001. **24**(4): p. 219-24.

38. Lambert, M.P., A.K. Barlow, B.A. Chromy, C. Edwards, R. Freed, M. Liosatos, T.E. Morgan, I. Rozovsky, B. Trommer, K.L. Viola, P. Wals, C. Zhang, C.E. Finch, G.A. Krafft, and W.L. Klein, *Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins.* Proc Natl Acad Sci U S A, 1998. **95**(11): p. 6448-53.

39. El-Agnaf, O.M., D.S. Mahil, B.P. Patel, and B.M. Austen, *Oligomerization and toxicity of beta-amyloid-42 implicated in Alzheimer's disease.* Biochem Biophys Res Commun, 2000. **273**(3): p. 1003-7.

40. Klein, A.M., N.W. Kowall, and R.J. Ferrante, *Neurotoxicity and oxidative damage of beta amyloid 1-42 versus beta amyloid 1-40 in the mouse cerebral cortex.* Ann N Y Acad Sci, 1999. **893**: p. 314-20.

41. Wood, J.G., S.S. Mirra, N.J. Pollock, and L.I. Binder, *Neurofibrillary tangles of Alzheimer disease share antigenic determinants with the axonal microtubule-associated protein tau (tau).* Proc Natl Acad Sci U S A, 1986. **83**(11): p. 4040-3.

42. Braak, H. and E. Braak, *Neuropathological stageing of Alzheimer-related changes.* Acta Neuropathol, 1991. **82**(4): p. 239-59.

43. Crowther, R.A., O.F. Olesen, M.J. Smith, R. Jakes, and M. Goedert, *Assembly of Alzheimer-like filaments from full-length tau protein.* FEBS Lett, 1994. **337**(2): p. 135-8.

44. Weingarten, M.D., A.H. Lockwood, S.Y. Hwo, and M.W. Kirschner, *A protein factor essential for microtubule assembly.* Proc Natl Acad Sci U S A, 1975. **72**(5): p. 1858-62.

45. Harada, A., K. Oguchi, S. Okabe, J. Kuno, S. Terada, T. Ohshima, R. Sato-Yoshitake, Y. Takei, T. Noda, and N. Hirokawa, *Altered microtubule organization in small-calibre axons of mice lacking tau protein.* Nature, 1994. **369**(6480): p. 488-91.

46. Goedert, M., *Tau protein and the neurofibrillary pathology of Alzheimer's disease.* Trends Neurosci, 1993. **16**(11): p. 460-5.

47. Lindwall, G. and R.D. Cole, *Phosphorylation affects the ability of tau protein to promote microtubule assembly.* J Biol Chem, 1984. **259**(8): p. 5301-5.

48. Alonso, A.C., I. Grundke-Iqbal, and K. Iqbal, *Alzheimer's disease hyperphosphorylated tau sequesters normal tau into tangles of filaments and disassembles microtubules.* Nat Med, 1996. **2**(7): p. 783-7.

49. Lewis, J., E. McGowan, J. Rockwood, H. Melrose, P. Nacharaju, M. Van Slegtenhorst, K. Gwinn-Hardy, M. Paul Murphy, M. Baker, X. Yu, K. Duff, J. Hardy, A. Corral, W.L. Lin, S.H. Yen, D.W. Dickson, P. Davies, and M. Hutton, *Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein.* Nat Genet, 2000. **25**(4): p. 402-5.

50. Guo, J.L. and V.M. Lee, *Seeding of normal Tau by pathological Tau conformers drives pathogenesis of Alzheimer-like tangles.* J Biol Chem, 2011. **286**(17): p. 15317-31.

51. Sperling, R.A., P.S. Aisen, L.A. Beckett, D.A. Bennett, S. Craft, A.M. Fagan, T. Iwatsubo, C.R. Jack, Jr., J. Kaye, T.J. Montine, D.C. Park, E.M. Reiman, C.C. Rowe, E. Siemers, Y. Stern, K. Yaffe, M.C. Carrillo, B. Thies, M. Morrison-Bogorad, M.V. Wagster, and C.H. Phelps, *Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease.* Alzheimers Dement, 2011. **7**(3): p. 280-92.

52. Pennanen, C., M. Kivipelto, S. Tuomainen, P. Hartikainen, T. Hanninen, M.P. Laakso, M. Hallikainen, M. Vanhanen, A. Nissinen, E.L. Helkala, P. Vainio, R. Vanninen, K. Partanen, and H. Soininen, *Hippocampus and entorhinal cortex in mild cognitive impairment and early AD.* Neurobiol Aging, 2004. **25**(3): p. 303-10.

53. Mouton, P.R., L.J. Martin, M.E. Calhoun, G. Dal Forno, and D.L. Price, *Cognitive decline strongly correlates with cortical atrophy in Alzheimer's dementia.* Neurobiol Aging, 1998. **19**(5): p. 371-7.

54. Scheff, S.W., D.A. Price, F.A. Schmitt, M.A. Scheff, and E.J. Mufson, *Synaptic loss in the inferior temporal gyrus in mild cognitive impairment and Alzheimer's disease.* J Alzheimers Dis, 2011. **24**(3): p. 547-57.

55. Scheff, S.W., D.A. Price, F.A. Schmitt, and E.J. Mufson, *Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment.* Neurobiol Aging, 2006. **27**(10): p. 1372-84.

56. Mueller, S.G., N. Schuff, K. Yaffe, C. Madison, B. Miller, and M.W. Weiner, *Hippocampal atrophy patterns in mild cognitive impairment and Alzheimer's disease.* Hum Brain Mapp, 2010. **31**(9): p. 1339-47.

57. Scheff, S.W., D.A. Price, F.A. Schmitt, S.T. DeKosky, and E.J. Mufson, *Synaptic alterations in CA1 in mild Alzheimer disease and mild cognitive impairment.* Neurology, 2007. **68**(18): p. 1501-8.

58. Hall, G.F., B. Chu, G. Lee, and J. Yao, *Human tau filaments induce microtubule and synapse loss in an in vivo model of neurofibrillary degenerative disease.* J Cell Sci, 2000. **113 (Pt 8)**: p. 1373-87.

59. Meyer-Luehmann, M., T.L. Spires-Jones, C. Prada, M. Garcia-Alloza, A. de Calignon, A. Rozkalne, J. Koenigsknecht-Talboo, D.M. Holtzman, B.J. Bacskai, and B.T. Hyman, *Rapid appearance and local toxicity of amyloid-beta plaques in a mouse model of Alzheimer's disease.* Nature, 2008. **451**(7179): p. 720-4.

60. Shankar, G.M., S. Li, T.H. Mehta, A. Garcia-Munoz, N.E. Shepardson, I. Smith, F.M. Brett, M.A. Farrell, M.J. Rowan, C.A. Lemere, C.M. Regan, D.M. Walsh, B.L. Sabatini, and D.J. Selkoe, *Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory.* Nat Med, 2008. **14**(8): p. 837-42.

61. Li, S., S. Hong, N.E. Shepardson, D.M. Walsh, G.M. Shankar, and D. Selkoe, *Soluble oligomers of amyloid Beta protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake.* Neuron, 2009. **62**(6): p. 788-801.

62. Shankar, G.M., B.L. Bloodgood, M. Townsend, D.M. Walsh, D.J. Selkoe, and B.L. Sabatini, *Natural oligomers of the Alzheimer amyloid-beta protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway.* J Neurosci, 2007. **27**(11): p. 2866-75.

63. Koffie, R.M., M. Meyer-Luehmann, T. Hashimoto, K.W. Adams, M.L. Mielke, M. Garcia-Alloza, K.D. Micheva, S.J. Smith, M.L. Kim, V.M. Lee, B.T. Hyman, and T.L. Spires-Jones, *Oligomeric amyloid beta associates with postsynaptic densities and correlates with excitatory synapse loss near senile plaques.* Proc Natl Acad Sci U S A, 2009. **106**(10): p. 4012-7.

64. Ebneth, A., R. Godemann, K. Stamer, S. Illenberger, B. Trinczek, and E. Mandelkow, *Overexpression of tau protein inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum: implications for Alzheimer's disease.* J Cell Biol, 1998. **143**(3): p. 777-94.

65. Kuipers, S.D., A. Trentani, A. Tiron, X. Mao, D. Kuhl, and C.R. Bramham, *BDNF-induced LTP is associated with rapid Arc/Arg3.1-dependent enhancement in adult hippocampal neurogenesis.* Sci Rep, 2016. **6**: p. 21222.

66. Criscuolo, C., C. Fabiani, C. Bonadonna, N. Origlia, and L. Domenici, *BDNF prevents amyloid-dependent impairment of LTP in the entorhinal cortex by attenuating p38 MAPK phosphorylation.* Neurobiol Aging, 2015. **36**(3): p. 1303-9.

67. Kovari, E., F.R. Herrmann, P.R. Hof, and C. Bouras, *The relationship between cerebral amyloid angiopathy and cortical microinfarcts in brain ageing and Alzheimer's disease.* Neuropathol Appl Neurobiol, 2013. **39**(5): p. 498-509.

68. Akiyama, H., S. Barger, S. Barnum, B. Bradt, J. Bauer, G.M. Cole, N.R. Cooper, P. Eikelenboom, M. Emmerling, B.L. Fiebich, C.E. Finch, S. Frautschy, W.S. Griffin, H. Hampel, M. Hull, G. Landreth, L. Lue, R. Mrak, I.R. Mackenzie, P.L. McGeer, M.K. O'Banion, J. Pachter, G. Pasinetti, C. Plata-Salaman, J. Rogers, R. Rydel, Y. Shen, W. Streit, R. Strohmeyer, I. Tooyoma, F.L. Van Muiswinkel, R. Veerhuis, D. Walker, S. Webster, B. Wegrzyniak, G. Wenk, and T. Wyss-Coray, *Inflammation and Alzheimer's disease.* Neurobiol Aging, 2000. **21**(3): p. 383-421.

69. Jellinger, K.A., *Prevalence and impact of cerebrovascular lesions in Alzheimer and lewy body diseases.* Neurodegener Dis, 2010. **7**(1-3): p. 112-5.

70. Hardy, J. and D.J. Selkoe, *The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics.* Science, 2002. **297**(5580): p. 353-6.

71. Lloret, A., M.C. Badia, E. Giraldo, G. Ermak, M.D. Alonso, F.V. Pallardo, K.J. Davies, and J. Vina, *Amyloid-beta toxicity and tau hyperphosphorylation are linked via RCAN1 in Alzheimer's disease.* J Alzheimers Dis, 2011. **27**(4): p. 701-9.

72. Zheng, W.H., S. Bastianetto, F. Mennicken, W. Ma, and S. Kar, *Amyloid beta peptide induces tau phosphorylation and loss of cholinergic neurons in rat primary septal cultures.* Neuroscience, 2002. **115**(1): p. 201-11.

73. Thal, D.R., U. Rub, M. Orantes, and H. Braak, *Phases of A beta-deposition in the human brain and its relevance for the development of AD.* Neurology, 2002. **58**(12): p. 1791-800.

74. Clavaguera, F., T. Bolmont, R.A. Crowther, D. Abramowski, S. Frank, A. Probst, G. Fraser, A.K. Stalder, M. Beibel, M. Staufenbiel, M. Jucker, M. Goedert, and M. Tolnay, *Transmission and spreading of tauopathy in transgenic mouse brain.* Nat Cell Biol, 2009. **11**(7): p. 909-13.

75. de Calignon, A., M. Polydoro, M. Suarez-Calvet, C. William, D.H. Adamowicz, K.J. Kopeikina, R. Pitstick, N. Sahara, K.H. Ashe, G.A. Carlson, T.L. Spires-Jones, and B.T. Hyman, *Propagation of tau pathology in a model of early Alzheimer's disease.* Neuron, 2012. **73**(4): p. 685-97.

76. Frost, B., R.L. Jacks, and M.I. Diamond, *Propagation of tau misfolding from the outside to the inside of a cell.* J Biol Chem, 2009. **284**(19): p. 12845-52.

77. Witter, M.P., P.A. Naber, T. van Haeften, W.C. Machielsen, S.A. Rombouts, F. Barkhof, P. Scheltens, and F.H. Lopes da Silva, *Cortico-hippocampal communication by way of parallel parahippocampal-subicular pathways.* Hippocampus, 2000. **10**(4): p. 398-410.

78. Hyman, B.T., G.W. Van Hoesen, L.J. Kromer, and A.R. Damasio, *Perforant pathway changes and the memory impairment of Alzheimer's disease.* Ann Neurol, 1986. **20**(4): p. 472-81.

79. Domert, J., S.B. Rao, L. Agholme, A.C. Brorsson, J. Marcusson, M. Hallbeck, and S. Nath, *Spreading of amyloid-beta peptides via neuritic cell-to-cell transfer is dependent on insufficient cellular clearance.* Neurobiol Dis, 2014. **65**: p. 82-92.

80. Nath, S., L. Agholme, F.R. Kurudenkandy, B. Granseth, J. Marcusson, and M. Hallbeck, *Spreading of neurodegenerative pathology via neuron-to-neuron transmission of beta-amyloid.* J Neurosci, 2012. **32**(26): p. 8767-77.

81. Umeda, T., T. Tomiyama, N. Sakama, S. Tanaka, M.P. Lambert, W.L. Klein, and H. Mori, *Intraneuronal amyloid beta oligomers cause cell death via endoplasmic reticulum stress, endosomal/lysosomal leakage, and mitochondrial dysfunction in vivo.* J Neurosci Res, 2011. **89**(7): p. 1031-42.

82. Pooler, A.M., M. Polydoro, E.A. Maury, S.B. Nicholls, S.M. Reddy, S. Wegmann, C. William, L. Saqran, O. Cagsal-Getkin, R. Pitstick, D.R. Beier, G.A. Carlson, T.L. Spires-Jones, and B.T. Hyman, *Amyloid accelerates tau propagation and toxicity in a model of early Alzheimer's disease.* Acta Neuropathol Commun, 2015. **3**: p. 14.

83. Gotz, J., F. Chen, J. van Dorpe, and R.M. Nitsch, *Formation of neurofibrillary tangles in P301l tau transgenic mice induced by Abeta 42 fibrils.* Science, 2001. **293**(5534): p. 1491-5.

84. Ribe, E.M., M. Perez, B. Puig, I. Gich, F. Lim, M. Cuadrado, T. Sesma, S. Catena, B. Sanchez, M. Nieto, P. Gomez-Ramos, M.A. Moran, F. Cabodevilla, L. Samaranch, L. Ortiz, A. Perez, I. Ferrer, J. Avila, and T. Gomez-Isla, *Accelerated amyloid deposition, neurofibrillary degeneration and neuronal loss in double mutant APP/tau transgenic mice.* Neurobiol Dis, 2005. **20**(3): p. 814-22.

85. Di, J., L.S. Cohen, C.P. Corbo, G.R. Phillips, A. El Idrissi, and A.D. Alonso, *Abnormal tau induces cognitive impairment through two different mechanisms: synaptic dysfunction and neuronal loss.* Sci Rep, 2016. **6**: p. 20833.

86. Kordower, J.H., Y. Chu, G.T. Stebbins, S.T. DeKosky, E.J. Cochran, D. Bennett, and E.J. Mufson, *Loss and atrophy of layer II entorhinal cortex neurons in elderly people with mild cognitive impairment.* Ann Neurol, 2001. **49**(2): p. 202-13.

87. Gomez-Isla, T., J.L. Price, D.W. McKeel, Jr., J.C. Morris, J.H. Growdon, and B.T. Hyman, *Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease.* J Neurosci, 1996. **16**(14): p. 4491-500.

88. Alonso, A. and R. Klink, *Differential electroresponsiveness of stellate and pyramidal-like cells of medial entorhinal cortex layer II.* J Neurophysiol, 1993. **70**(1): p. 128-43.

89. Witter, M.P. and D.G. Amaral, *Entorhinal cortex of the monkey: V. Projections to the dentate gyrus, hippocampus, and subicular complex.* J Comp Neurol, 1991. **307**(3): p. 437-59.

90. Claiborne, B.J., D.G. Amaral, and W.M. Cowan, *A light and electron microscopic analysis of the mossy fibers of the rat dentate gyrus.* J Comp Neurol, 1986. **246**(4): p. 435-58.

91. Kajiwara, R., F.G. Wouterlood, A. Sah, A.J. Boekel, L.T. Baks-te Bulte, and M.P. Witter, *Convergence of entorhinal and CA3 inputs onto pyramidal neurons and interneurons in hippocampal area CA1--an anatomical study in the rat.* Hippocampus, 2008. **18**(3): p. 266-80.

92. Bittner, K.C., C. Grienberger, S.P. Vaidya, A.D. Milstein, J.J. Macklin, J. Suh, S. Tonegawa, and J.C. Magee, *Conjunctive input processing drives feature selectivity in hippocampal CA1 neurons.* Nat Neurosci, 2015. **18**(8): p. 1133-42.

93. Honda, Y., H. Sasaki, Y. Umitsu, and N. Ishizuka, *Zonal distribution of perforant path cells in layer III of the entorhinal area projecting to CA1 and subiculum in the rat.* Neurosci Res, 2012. **74**(3-4): p. 200-9.

94. Doody, R.S., R.G. Thomas, M. Farlow, T. Iwatsubo, B. Vellas, S. Joffe, K. Kieburtz, R. Raman, X. Sun, P.S. Aisen, E. Siemers, H. Liu-Seifert, R. Mohs, C. Alzheimer's Disease Cooperative Study Steering, and G. Solanezumab Study, *Phase 3 trials of solanezumab for mild-to-moderate Alzheimer's disease.* N Engl J Med, 2014. **370**(4): p. 311-21.

95. Salloway, S., R. Sperling, N.C. Fox, K. Blennow, W. Klunk, M. Raskind, M. Sabbagh, L.S. Honig, A.P. Porsteinsson, S. Ferris, M. Reichert, N. Ketter, B. Nejadnik, V. Guenzler, M. Miloslavsky, D. Wang, Y. Lu, J. Lull, I.C. Tudor, E. Liu, M. Grundman, E. Yuen, R. Black, H.R. Brashear, Bapineuzumab, and I.

Clinical Trial, *Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease.* N Engl J Med, 2014. **370**(4): p. 322-33.

96. Nagahara, A.H. and M.H. Tuszynski, *Potential therapeutic uses of BDNF in neurological and psychiatric disorders.* Nat Rev Drug Discov, 2011. **10**(3): p. 209-19.

97. Barde, Y.A., D. Edgar, and H. Thoenen, *Purification of a new neurotrophic factor from mammalian brain.* EMBO J, 1982. **1**(5): p. 549-53.

98. Leibrock, J., F. Lottspeich, A. Hohn, M. Hofer, B. Hengerer, P. Masiakowski, H. Thoenen, and Y.A. Barde, *Molecular cloning and expression of brain-derived neurotrophic factor.* Nature, 1989. **341**(6238): p. 149-52.

99. Barde, Y.A., *The nerve growth factor family.* Prog Growth Factor Res, 1990. **2**(4): p. 237-48.

100. Lai, K.O., W.Y. Fu, F.C. Ip, and N.Y. Ip, *Cloning and expression of a novel neurotrophin, NT-7, from carp.* Mol Cell Neurosci, 1998. **11**(1-2): p. 64-76.

101. Chan, J.R., J.M. Cosgaya, Y.J. Wu, and E.M. Shooter, *Neurotrophins are key mediators of the myelination program in the peripheral nervous system.* Proc Natl Acad Sci U S A, 2001. **98**(25): p. 14661-8.

102. Canals, J.M., N. Checa, S. Marco, P. Akerud, A. Michels, E. Perez-Navarro, E. Tolosa, E. Arenas, and J. Alberch, *Expression of brain-derived neurotrophic factor in cortical neurons is regulated by striatal target area.* J Neurosci, 2001. **21**(1): p. 117-24.

103. Mattson, M.P., S. Maudsley, and B. Martin, *BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders.* Trends Neurosci, 2004. **27**(10): p. 589-94.

104. Gotz, R., F. Raulf, and M. Schartl, *Brain-derived neurotrophic factor is more highly conserved in structure and function than nerve growth factor during vertebrate evolution.* J Neurochem, 1992. **59**(2): p. 432-42.

105. Maisonpierre, P.C., L. Belluscio, B. Friedman, R.F. Alderson, S.J. Wiegand, M.E. Furth, R.M. Lindsay, and G.D. Yancopoulos, *NT-3, BDNF, and NGF in the developing rat nervous system: parallel as well as reciprocal patterns of expression.* Neuron, 1990. **5**(4): p. 501-9.

106. Jones, K.R., I. Farinas, C. Backus, and L.F. Reichardt, *Targeted disruption of the BDNF gene perturbs brain and sensory neuron development but not motor neuron development.* Cell, 1994. **76**(6): p. 989-99.

107. Ernfors, P., K.F. Lee, and R. Jaenisch, *Mice lacking brain-derived neurotrophic factor develop with sensory deficits.* Nature, 1994. **368**(6467): p. 147-50.

108. Conner, J.M., J.C. Lauterborn, Q. Yan, C.M. Gall, and S. Varon, *Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport.* J Neurosci, 1997. **17**(7): p. 2295-313.

109. Numakawa, T., S. Suzuki, E. Kumamaru, N. Adachi, M. Richards, and H. Kunugi, *BDNF function and intracellular signaling in neurons.* Histol Histopathol, 2010. **25**(2): p. 237-58.

110. Perez, P., P.M. Coll, B.L. Hempstead, D. Martin-Zanca, and M.V. Chao, *NGF binding to the trk tyrosine kinase receptor requires the extracellular immunoglobulin-like domains.* Mol Cell Neurosci, 1995. **6**(2): p. 97-105.

111. Ivanisevic, L., W. Zheng, S.B. Woo, K.E. Neet, and H.U. Saragovi, *TrkA receptor "hot spots" for binding of NT-3 as a heterologous ligand.* J Biol Chem, 2007. **282**(23): p. 16754-63.

112. Gao, W.Q., J.L. Zheng, and M. Karihaloo, *Neurotrophin-4/5 (NT-4/5) and brain-derived neurotrophic factor (BDNF) act at later stages of cerebellar granule cell differentiation.* J Neurosci, 1995. **15**(4): p. 2656-67.

113. Eide, F.F., E.R. Vining, B.L. Eide, K. Zang, X.Y. Wang, and L.F. Reichardt, *Naturally occurring truncated trkB receptors have dominant inhibitory effects on brain-derived neurotrophic factor signaling.* J Neurosci, 1996. **16**(10): p. 3123-9.

114. Klein, R., D. Conway, L.F. Parada, and M. Barbacid, *The trkB tyrosine protein kinase gene codes for a second neurogenic receptor that lacks the catalytic kinase domain.* Cell, 1990. **61**(4): p. 647-56.

115. Shelton, D.L., J. Sutherland, J. Gripp, T. Camerato, M.P. Armanini, H.S. Phillips, K. Carroll, S.D. Spencer, and A.D. Levinson, *Human trks: molecular cloning, tissue distribution, and expression of extracellular domain immunoadhesins.* J Neurosci, 1995. **15**(1 Pt 2): p. 477-91.

116. Middlemas, D.S., R.A. Lindberg, and T. Hunter, *trkB, a neural receptor protein-tyrosine kinase: evidence for a full-length and two truncated receptors.* Mol Cell Biol, 1991. **11**(1): p. 143-53.

117. Luikart, B.W., S. Nef, T. Shipman, and L.F. Parada, *In vivo role of truncated trkb receptors during sensory ganglion neurogenesis.* Neuroscience, 2003. **117**(4): p. 847-58.

118. Ohira, K., K. Shimizu, and M. Hayashi, *TrkB dimerization during development of the prefrontal cortex of the macaque.* J Neurosci Res, 2001. **65**(5): p. 463-9.

119. Truzzi, F., A. Marconi, P. Atzei, M.C. Panza, R. Lotti, K. Dallaglio, R. Tiberio, E. Palazzo, C. Vaschieri, and C. Pincelli, *p75 neurotrophin receptor mediates apoptosis in transit-amplifying cells and its overexpression restores cell death in psoriatic keratinocytes.* Cell Death Differ, 2011. **18**(6): p. 948-58.

120. Hempstead, B.L., D. Martin-Zanca, D.R. Kaplan, L.F. Parada, and M.V. Chao, *High-affinity NGF binding requires coexpression of the trk proto-oncogene and the low-affinity NGF receptor.* Nature, 1991. **350**(6320): p. 678-83.

121. Bibel, M., E. Hoppe, and Y.A. Barde, *Biochemical and functional interactions between the neurotrophin receptors trk and p75NTR.* EMBO J, 1999. **18**(3): p. 616-22.

122. Michaelsen, K., M. Zagrebelsky, J. Berndt-Huch, M. Polack, A. Buschler, M. Sendtner, and M. Korte, *Neurotrophin receptors TrkB.T1 and p75NTR cooperate in modulating both functional and structural plasticity in mature hippocampal neurons.* Eur J Neurosci, 2010. **32**(11): p. 1854-65.

123. Hartmann, M., T. Brigadski, K.S. Erdmann, B. Holtmann, M. Sendtner, F. Narz, and V. Lessmann, *Truncated TrkB receptor-induced outgrowth of dendritic filopodia involves the p75 neurotrophin receptor.* J Cell Sci, 2004. **117**(Pt 24): p. 5803-14.

124. Ma, L., D.D. Wang, T.Y. Zhang, H. Yu, Y. Wang, S.H. Huang, F.S. Lee, and Z.Y. Chen, *Region-specific involvement of BDNF secretion and synthesis in conditioned taste aversion memory formation.* J Neurosci, 2011. **31**(6): p. 2079-90.

125. Ninan, I., K.G. Bath, K. Dagar, R. Perez-Castro, M.R. Plummer, F.S. Lee, and M.V. Chao, *The BDNF Val66Met polymorphism impairs NMDA receptor-dependent synaptic plasticity in the hippocampus.* J Neurosci, 2010. **30**(26): p. 8866-70.

126. Patterson, S.L., T. Abel, T.A. Deuel, K.C. Martin, J.C. Rose, and E.R. Kandel, *Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice.* Neuron, 1996. **16**(6): p. 1137-45.

127. Rantamaki, T., S. Kemppainen, H. Autio, S. Staven, H. Koivisto, M. Kojima, H. Antila, P.O. Miettinen, E. Karkkainen, N. Karpova, L. Vesa, L. Lindemann, M.C. Hoener, H. Tanila, and E. Castren, *The impact of Bdnf gene deficiency to the memory impairment and brain pathology of APPswe/PS1dE9 mouse model of Alzheimer's disease.* PLoS One, 2013. **8**(7): p. e68722.

128. Yamada, K., M. Mizuno, and T. Nabeshima, *Role for brain-derived neurotrophic factor in learning and memory.* Life Sci, 2002. **70**(7): p. 735-44.

129. Heldt, S.A., L. Stanek, J.P. Chhatwal, and K.J. Ressler, *Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories.* Mol Psychiatry, 2007. **12**(7): p. 656-70.

130. Mizuno, M., K. Yamada, A. Olariu, H. Nawa, and T. Nabeshima, *Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats.* J Neurosci, 2000. **20**(18): p. 7116-21.

131. Mu, J.S., W.P. Li, Z.B. Yao, and X.F. Zhou, *Deprivation of endogenous brain-derived neurotrophic factor results in impairment of spatial learning and memory in adult rats.* Brain Res, 1999. **835**(2): p. 259-65.

132. Jiang, Q.S., Z.L. Liang, M.J. Wu, L. Feng, L.L. Liu, and J.J. Zhang, *Reduced brain-derived neurotrophic factor expression in cortex and hippocampus involved in the learning and memory deficit in molarless SAMP8 mice.* Chin Med J (Engl), 2011. **124**(10): p. 1540-4.

133. Alonso, M., J.H. Medina, and L. Pozzo-Miller, *ERK1/2 activation is necessary for BDNF to increase dendritic spine density in hippocampal CA1 pyramidal neurons.* Learn Mem, 2004. **11**(2): p. 172-8.

134. Kellner, Y., N. Godecke, T. Dierkes, N. Thieme, M. Zagrebelsky, and M. Korte, *The BDNF effects on dendritic spines of mature hippocampal neurons depend on neuronal activity.* Front Synaptic Neurosci, 2014. **6**: p. 5.

135. Takei, N., T. Numakawa, S. Kozaki, N. Sakai, Y. Endo, M. Takahashi, and H. Hatanaka, *Brain-derived neurotrophic factor induces rapid and transient release of glutamate through the non-exocytotic pathway from cortical neurons.* J Biol Chem, 1998. **273**(42): p. 27620-4.

136. Levine, E.S., R.A. Crozier, I.B. Black, and M.R. Plummer, *Brain-derived neurotrophic factor modulates hippocampal synaptic transmission by increasing N-methyl-D-aspartic acid receptor activity.* Proc Natl Acad Sci U S A, 1998. **95**(17): p. 10235-9.

137. Katagiri, H., K. Tanaka, and T. Manabe, *Requirement of appropriate glutamate concentrations in the synaptic cleft for hippocampal LTP induction.* Eur J Neurosci, 2001. **14**(3): p. 547-53.

138. Finsterwald, C., H. Fiumelli, J.R. Cardinaux, and J.L. Martin, *Regulation of dendritic development by BDNF requires activation of CRTC1 by glutamate.* J Biol Chem, 2010. **285**(37): p. 28587-95.

139. Cao, X., Z. Cui, R. Feng, Y.P. Tang, Z. Qin, B. Mei, and J.Z. Tsien, *Maintenance of superior learning and memory function in NR2B transgenic mice during ageing.* Eur J Neurosci, 2007. **25**(6): p. 1815-22.

140. Brazel, C.Y., J.L. Nunez, Z. Yang, and S.W. Levison, *Glutamate enhances survival and proliferation of neural progenitors derived from the subventricular zone.* Neuroscience, 2005. **131**(1): p. 55-65.

141. Suzuki, M., A.D. Nelson, J.B. Eickstaedt, K. Wallace, L.S. Wright, and C.N. Svendsen, *Glutamate enhances proliferation and neurogenesis in human neural progenitor cell cultures derived from the fetal cortex.* Eur J Neurosci, 2006. **24**(3): p. 645-53.

142. Kernie, S.G., D.J. Liebl, and L.F. Parada, *BDNF regulates eating behavior and locomotor activity in mice.* EMBO J, 2000. **19**(6): p. 1290-300.

143. Ito, W., M. Chehab, S. Thakur, J. Li, and A. Morozov, *BDNF-restricted knockout mice as an animal model for aggression.* Genes Brain Behav, 2011. **10**(3): p. 365-74.

144. Gratacos, M., J.R. Gonzalez, J.M. Mercader, R. de Cid, M. Urretavizcaya, and X. Estivill, *Brain-derived neurotrophic factor Val66Met and psychiatric disorders: meta-analysis of case-control studies confirm association to substance-related disorders, eating disorders, and schizophrenia.* Biol Psychiatry, 2007. **61**(7): p. 911-22.

145. Lee, B.H., H. Kim, S.H. Park, and Y.K. Kim, *Decreased plasma BDNF level in depressive patients.* J Affect Disord, 2007. **101**(1-3): p. 239-44.

146. Kalaria, R.N., *The role of cerebral ischemia in Alzheimer's disease.* Neurobiol Aging, 2000. **21**(2): p. 321-30.

147. Liu, T., R.K. Clark, P.C. McDonnell, P.R. Young, R.F. White, F.C. Barone, and G.Z. Feuerstein, *Tumor necrosis factor-alpha expression in ischemic neurons.* Stroke, 1994. **25**(7): p. 1481-8.

148. Jiang, Y., N. Wei, T. Lu, J. Zhu, G. Xu, and X. Liu, *Intranasal brain-derived neurotrophic factor protects brain from ischemic insult via modulating local inflammation in rats.* Neuroscience, 2011. **172**: p. 398-405.

149. Zhang, J., C. Geula, C. Lu, H. Koziel, L.M. Hatcher, and F.J. Roisen, *Neurotrophins regulate proliferation and survival of two microglial cell lines in vitro.* Exp Neurol, 2003. **183**(2): p. 469-81.

150. Zhang, J., Z. Yu, Z. Yu, Z. Yang, H. Zhao, L. Liu, and J. Zhao, *rAAV-mediated delivery of brain-derived neurotrophic factor promotes neurite outgrowth and*

protects neurodegeneration in focal ischemic model. Int J Clin Exp Pathol, 2011. **4**(5): p. 496-504.

151. Kiprianova, I., J. Sandkuhler, S. Schwab, S. Hoyer, and M. Spranger, *Brain-derived neurotrophic factor improves long-term potentiation and cognitive functions after transient forebrain ischemia in the rat.* Exp Neurol, 1999. **159**(2): p. 511-9.

152. Zhu, J.M., Y.Y. Zhao, S.D. Chen, W.H. Zhang, L. Lou, and X. Jin, *Functional recovery after transplantation of neural stem cells modified by brain-derived neurotrophic factor in rats with cerebral ischaemia.* J Int Med Res, 2011. **39**(2): p. 488-98.

153. Gong, J., S. Gong, M. Zhang, L. Zhang, Y. Hu, Y. Liu, and W. Li, *Cerebral ischemic preconditioning reduces glutamate excitotoxicity by up-regulating the uptake activity of GLT-1 in rats.* Amino Acids, 2014. **46**(6): p. 1537-45.

154. Brandoli, C., A. Sanna, M.A. De Bernardi, P. Follesa, G. Brooker, and I. Mocchetti, *Brain-derived neurotrophic factor and basic fibroblast growth factor downregulate NMDA receptor function in cerebellar granule cells.* J Neurosci, 1998. **18**(19): p. 7953-61.

155. Almeida, R.D., B.J. Manadas, C.V. Melo, J.R. Gomes, C.S. Mendes, M.M. Graos, R.F. Carvalho, A.P. Carvalho, and C.B. Duarte, *Neuroprotection by BDNF against glutamate-induced apoptotic cell death is mediated by ERK and PI3-kinase pathways.* Cell Death Differ, 2005. **12**(10): p. 1329-43.

156. Bemelmans, A.P., I. Husson, M. Jaquet, J. Mallet, B.E. Kosofsky, and P. Gressens, *Lentiviral-mediated gene transfer of brain-derived neurotrophic factor is neuroprotective in a mouse model of neonatal excitotoxic challenge.* J Neurosci Res, 2006. **83**(1): p. 50-60.

157. Nakao, N., P. Brundin, K. Funa, O. Lindvall, and P. Odin, *Trophic and protective actions of brain-derived neurotrophic factor on striatal DARPP-32-containing neurons in vitro.* Brain Res Dev Brain Res, 1995. **90**(1-2): p. 92-101.

158. Perez-Navarro, E., N. Gavalda, E. Gratacos, and J. Alberch, *Brain-derived neurotrophic factor prevents changes in Bcl-2 family members and caspase-3*

activation induced by excitotoxicity in the striatum. J Neurochem, 2005. **92**(3): p. 678-91.

159. Brock, J.H., E.S. Rosenzweig, A. Blesch, R. Moseanko, L.A. Havton, V.R. Edgerton, and M.H. Tuszynski, *Local and remote growth factor effects after primate spinal cord injury.* J Neurosci, 2010. **30**(29): p. 9728-37.

160. Eriksson, N.P., R.M. Lindsay, and H. Aldskogius, *BDNF and NT-3 rescue sensory but not motoneurones following axotomy in the neonate.* Neuroreport, 1994. **5**(12): p. 1445-8.

161. Knusel, B., K.D. Beck, J.W. Winslow, A. Rosenthal, L.E. Burton, H.R. Widmer, K. Nikolics, and F. Hefti, *Brain-derived neurotrophic factor administration protects basal forebrain cholinergic but not nigral dopaminergic neurons from degenerative changes after axotomy in the adult rat brain.* J Neurosci, 1992. **12**(11): p. 4391-402.

162. Peinado-Ramon, P., M. Salvador, M.P. Villegas-Perez, and M. Vidal-Sanz, *Effects of axotomy and intraocular administration of NT-4, NT-3, and brain-derived neurotrophic factor on the survival of adult rat retinal ganglion cells. A quantitative in vivo study.* Invest Ophthalmol Vis Sci, 1996. **37**(4): p. 489-500.

163. Yan, Q., J. Elliott, and W.D. Snider, *Brain-derived neurotrophic factor rescues spinal motor neurons from axotomy-induced cell death.* Nature, 1992. **360**(6406): p. 753-5.

164. Widmer, H.R., B. Knusel, and F. Hefti, *BDNF protection of basal forebrain cholinergic neurons after axotomy: complete protection of p75NGFR-positive cells.* Neuroreport, 1993. **4**(4): p. 363-6.

165. Li, Q., J. Cui, C. Fang, X. Zhang, and L. Li, *S-adenosylmethionine Administration Attenuates Low Brain-Derived Neurotrophic Factor Expression Induced by Chronic Cerebrovascular Hypoperfusion or Beta Amyloid Treatment.* Neurosci Bull, 2016. **32**(2): p. 153-61.

166. Shin, M.K., H.G. Kim, and K.L. Kim, *A novel brain-derived neurotrophic factor-modulating peptide attenuates Abeta1-42-induced neurotoxicity in vitro.* Neurosci Lett, 2015. **595**: p. 63-8.

167. Zhang, Z., J. Fan, Y. Ren, W. Zhou, and G. Yin, *The release of glutamate from cortical neurons regulated by BDNF via the TrkB/Src/PLC-gamma1 pathway.* J Cell Biochem, 2013. **114**(1): p. 144-51.

168. Zirrgiebel, U., Y. Ohga, B. Carter, B. Berninger, N. Inagaki, H. Thoenen, and D. Lindholm, *Characterization of TrkB receptor-mediated signaling pathways in rat cerebellar granule neurons: involvement of protein kinase C in neuronal survival.* J Neurochem, 1995. **65**(5): p. 2241-50.

169. Ueda, Y., S. Hirai, S. Osada, A. Suzuki, K. Mizuno, and S. Ohno, *Protein kinase C activates the MEK-ERK pathway in a manner independent of Ras and dependent on Raf.* J Biol Chem, 1996. **271**(38): p. 23512-9.

170. Bonni, A., A. Brunet, A.E. West, S.R. Datta, M.A. Takasu, and M.E. Greenberg, *Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms.* Science, 1999. **286**(5443): p. 1358-62.

171. Lee, S., M. Yang, J. Kim, Y. Son, J. Kim, S. Kang, W. Ahn, S.H. Kim, J.C. Kim, T. Shin, H. Wang, and C. Moon, *Involvement of BDNF/ERK signaling in spontaneous recovery from trimethyltin-induced hippocampal neurotoxicity in mice.* Brain Res Bull, 2016. **121**: p. 48-58.

172. Philpott, K.L., M.J. McCarthy, A. Klippel, and L.L. Rubin, *Activated phosphatidylinositol 3-kinase and Akt kinase promote survival of superior cervical neurons.* J Cell Biol, 1997. **139**(3): p. 809-15.

173. Chen, A., L.J. Xiong, Y. Tong, and M. Mao, *Neuroprotective effect of brain-derived neurotrophic factor mediated by autophagy through the PI3K/Akt/mTOR pathway.* Mol Med Rep, 2013. **8**(4): p. 1011-6.

174. Jantas, D., M. Szymanska, B. Budziszewska, and W. Lason, *An involvement of BDNF and PI3-K/Akt in the anti-apoptotic effect of memantine on staurosporine-evoked cell death in primary cortical neurons.* Apoptosis, 2009. **14**(7): p. 900-12.

175. Brunet, A., A. Bonni, M.J. Zigmond, M.Z. Lin, P. Juo, L.S. Hu, M.J. Anderson, K.C. Arden, J. Blenis, and M.E. Greenberg, *Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor.* Cell, 1999. **96**(6): p. 857-68.

176. Uddin, S., S. Kottegoda, D. Stigger, L.C. Platanias, and A. Wickrema, *Activation of the Akt/FKHRL1 pathway mediates the antiapoptotic effects of erythropoietin in primary human erythroid progenitors.* Biochem Biophys Res Commun, 2000. **275**(1): p. 16-9.

177. Yamaguchi, A., M. Tamatani, H. Matsuzaki, K. Namikawa, H. Kiyama, M.P. Vitek, N. Mitsuda, and M. Tohyama, *Akt activation protects hippocampal neurons from apoptosis by inhibiting transcriptional activity of p53.* J Biol Chem, 2001. **276**(7): p. 5256-64.

178. Matsuzaki, H., M. Tamatani, N. Mitsuda, K. Namikawa, H. Kiyama, S. Miyake, and M. Tohyama, *Activation of Akt kinase inhibits apoptosis and changes in Bcl-2 and Bax expression induced by nitric oxide in primary hippocampal neurons.* J Neurochem, 1999. **73**(5): p. 2037-46.

179. Qin, X.Y., C. Cao, N.X. Cawley, T.T. Liu, J. Yuan, Y.P. Loh, and Y. Cheng, *Decreased peripheral brain-derived neurotrophic factor levels in Alzheimer's disease: a meta-analysis study (N=7277).* Mol Psychiatry, 2016.

180. Yu, H., Z. Zhang, Y. Shi, F. Bai, C. Xie, Y. Qian, Y. Yuan, and L. Deng, *Association study of the decreased serum BDNF concentrations in amnestic mild cognitive impairment and the Val66Met polymorphism in Chinese Han.* J Clin Psychiatry, 2008. **69**(7): p. 1104-11.

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

182. Peng, S., J. Wuu, E.J. Mufson, and M. Fahnestock, *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, 2005. **93**(6): p. 1412-21.

183. Arancibia, S., M. Silhol, F. Mouliere, J. Meffre, I. Hollinger, T. Maurice, and L. Tapia-Arancibia, *Protective effect of BDNF against beta-amyloid induced neurotoxicity in vitro and in vivo in rats.* Neurobiol Dis, 2008. **31**(3): p. 316-26.

184. Nagahara, A.H., M. Mateling, I. Kovacs, L. Wang, S. Eggert, E. Rockenstein, E.H. Koo, E. Masliah, and M.H. Tuszynski, *Early BDNF treatment ameliorates*

cell loss in the entorhinal cortex of APP transgenic mice. J Neurosci, 2013. **33**(39): p. 15596-602.

185. Peterson, D.A., C.A. Lucidi-Phillipi, K.L. Eagle, and F.H. Gage, *Perforant path damage results in progressive neuronal death and somal atrophy in layer II of entorhinal cortex and functional impairment with increasing postdamage age.* J Neurosci, 1994. **14**(11 Pt 2): p. 6872-85.

186. Cearley, C.N. and J.H. Wolfe, *A single injection of an adeno-associated virus vector into nuclei with divergent connections results in widespread vector distribution in the brain and global correction of a neurogenetic disease.* J Neurosci, 2007. **27**(37): p. 9928-40.

187. van Groen, T. and J.M. Wyss, *The connections of presubiculum and parasubiculum in the rat.* Brain Res, 1990. **518**(1-2): p. 227-43.

188. Castle, M.J., Z.T. Gershenson, A.R. Giles, E.L. Holzbaur, and J.H. Wolfe, *Adeno-associated virus serotypes 1, 8, and 9 share conserved mechanisms for anterograde and retrograde axonal transport.* Hum Gene Ther, 2014. **25**(8): p. 705-20.

189. Castle, M.J., E. Perlson, E.L. Holzbaur, and J.H. Wolfe, *Long-distance axonal transport of AAV9 is driven by dynein and kinesin-2 and is trafficked in a highly motile Rab7-positive compartment.* Mol Ther, 2014. **22**(3): p. 554-66.

190. Bartlett, J.S., R. Wilcher, and R.J. Samulski, *Infectious entry pathway of adeno-associated virus and adeno-associated virus vectors.* J Virol, 2000. **74**(6): p. 2777-85.

191. Taymans, J.M., L.H. Vandenberghe, C.V. Haute, I. Thiry, C.M. Deroose, L. Mortelmans, J.M. Wilson, Z. Debyser, and V. Baekelandt, *Comparative analysis of adeno-associated viral vector serotypes 1, 2, 5, 7, and 8 in mouse brain.* Hum Gene Ther, 2007. **18**(3): p. 195-206.

192. Zincarelli, C., S. Soltys, G. Rengo, and J.E. Rabinowitz, *Analysis of AAV serotypes 1-9 mediated gene expression and tropism in mice after systemic injection.* Mol Ther, 2008. **16**(6): p. 1073-80.

193. Ciesielska, A., G. Mittermeyer, P. Hadaczek, A.P. Kells, J. Forsayeth, and K.S. Bankiewicz, *Anterograde axonal transport of AAV2-GDNF in rat basal ganglia.* Mol Ther, 2011. **19**(5): p. 922-7.

194. Jara, J.H., S.R. Villa, N.A. Khan, M.C. Bohn, and P.H. Ozdinler, *AAV2 mediated retrograde transduction of corticospinal motor neurons reveals initial and selective apical dendrite degeneration in ALS.* Neurobiol Dis, 2012. **47**(2): p. 174-83.

195. Kern, A., K. Schmidt, C. Leder, O.J. Muller, C.E. Wobus, K. Bettinger, C.W. Von der Lieth, J.A. King, and J.A. Kleinschmidt, *Identification of a heparin-binding motif on adeno-associated virus type 2 capsids.* J Virol, 2003. **77**(20): p. 11072-81.

196. Litwack, E.D., J.K. Ivins, A. Kumbasar, S. Paine-Saunders, C.S. Stipp, and A.D. Lander, *Expression of the heparan sulfate proteoglycan glypican-1 in the developing rodent.* Dev Dyn, 1998. **211**(1): p. 72-87.

197. Pajusola, K., M. Gruchala, H. Joch, T.F. Luscher, S. Yla-Herttuala, and H. Bueler, *Cell-type-specific characteristics modulate the transduction efficiency of adeno-associated virus type 2 and restrain infection of endothelial cells.* J Virol, 2002. **76**(22): p. 11530-40.

198. Guilhem, D., P.A. Dreyfus, Y. Makiura, F. Suzuki, and B. Onteniente, *Short increase of BDNF messenger RNA triggers kainic acid-induced neuronal hypertrophy in adult mice.* Neuroscience, 1996. **72**(4): p. 923-31.

199. Aguado, F., M.A. Carmona, E. Pozas, A. Aguilo, F.J. Martinez-Guijarro, S. Alcantara, V. Borrell, R. Yuste, C.F. Ibanez, and E. Soriano, *BDNF regulates spontaneous correlated activity at early developmental stages by increasing synaptogenesis and expression of the K+/Cl- co-transporter KCC2.* Development, 2003. **130**(7): p. 1267-80.