UNIVERSITY OF CALIFORNIA SAN DIEGO

Disease Modifying Effects of Stress and CRF Receptor 1 antagonism on Alzheimer's Disease Pathology

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Biology

by

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The Thesis of Jasmine Patanapirom is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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LIST OF ABBREVIATIONS

Aβ- Amyloid Beta

AD- Alzheimer's Disease

APP- Amyloid Precursor Protein

CRF- Corticotropin Releasing Factor

CRFR1- Corticotropin Releasing Factor Receptor 1

HPA Axis- Hypothalamic Pituitary Adrenal Cortex Axis

PSAPP- Transgenic AD mouse model used in this study

WT- Wild type Mice

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ABSTRACT OF THE THESIS

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Alzheimer's disease (AD) is a debilitating form of dementia associated with cognitive disabilities. The underlying mechanisms that cause AD are still relatively unknown but stress and corticotropin releasing factor (CRF) has been shown to contribute to disease progression. CRF receptors have become a target for therapeutic potential in AD as it is part of the stress-signaling pathway. CRF antagonist (R121919) has shown to decrease amyloid beta plaques in animals early in AD development. Since R121919 is most affective in early stage disease development, we tested if this antagonist could produce the same results in transgenic AD mice (PSAPP) that

were in late stage disease progression and when chronically stressed. We observed that R121919 is most affective in decreasing AD pathology and phenotypic changes in early stage AD mice when stressed; AD pathology was diminished in late stage AD progression as well. These findings indicated that drug administration with CRF antagonist slows down AD progression when stress is present in early stage AD development and has the potential to do the same in late stage AD progression. Our data demonstrate that CRFR1 receptors are a potential preventative therapeutic target for AD and that targeting them may be particularly beneficial for individuals who are prone to experiencing traumatic event.

Chapter 1: Introduction

Alzheimer's Disease

Alzheimer's Disease (AD) is a neurodegenerative disorder associated with progressive memory loss and cognitive impairment. Clinically diagnosed AD patients have difficulties in performing and concentrating on daily tasks (Alzheimer's Association, 2009). This impairment can cause patients to become depressed, confused, and can change a patient's personality (Alzheimer's Association, 2009). An AD patient's quality of life is impacted, as well as that of the patient caregiver, due to increased behavioral disturbances, increased dependency on the caregiver, and lack of resources to reduce symptoms (Masters et al., 2004). Current therapies for AD focus on improving the cognitive impairment but current therapies have done little to significantly decrease AD development and improve cognitive disabilities permanently (Mendiola et al., 2016). Current treatments for AD focus on treating AD once symptoms arise whereas there are no current treatments for preventative therapeutics. Research into preventative therapies and pathological treatments are therefore indispensable to improve quality of life for those who may be diagnosed as well as for their caregivers.

AD is typically diagnosed in patients between the ages of 49-80 as one of two forms, sporadic or familial (Selkoe, 2001). Sporadic cases, also known as non-familial forms of AD, are the most common with a 95% incidence (Masters et al., 2004; Selkoe, 2001). Sporadic cases, considered late-onset, have a mean age beginning at 80 years of age; familial forms vary from 5-10% of cases with a mean of onset at the age of 45 (Masters et al., 2004; Selkoe, 2001). In both AD forms, pathology and cognitive symptoms are equivalent and often indistinguishable but the main difference is the age of onset. Improper clearance of pathological substances, namely amyloid peptide, is thought to be the main reason that accounts for AD progression (Masters et

al., 2004; Kametan & Hasegawa, 2018). Mutations accounting for sporadic and familial forms are commonly gene missense mutations in genes such as APP, Presenilin 1, Presenilin 2, and APOE; these mutations phenotypically cause an increase in production of amyloid beta peptides as well as development of AD (Masters et al., 2004; Kametan & Hasegawa, 2018). Mutations seen in both forms are commonly used in mouse models for research.

Amyloid Processing

The two pathological hallmarks of AD are neurofibrillary tangles and amyloid beta plaques (Aβ) (Selkoe, 2001). Neurofibrillary tangles consist of distorted hyperphosphorylated tau (Selkoe, 2001). Tau is a microtubule protein that accounts for the stability and structural integrity of tubulin and microtubules (Masters et al., 2004; Selkoe, 2001). Aβ is a protein byproduct of amyloid precursor protein (APP) (Masters Et al., 2004; Selkoe 2001). There are two forms of Aβ: soluble (nonfibrillar) and insoluble (fibrillar) (Kirkwood et al., 2013). Insoluble Aβ is composed of Aβ and soluble are oligomeric and monomeric free-floating species (Masters et al., 2004). According to the amyloid cascade hypothesis, aggregated Aβ causes hyperphosphorylated tau to form neurofibrillary tangles in neurons (Hardy and Selkoe, 2002). The amyloid cascade ultimately causes synaptic dysfunction between neurons, which correlates with cognitive dysfunction in AD patients.

APP is a large transmembrane protein with two possible processing pathways, the non-amyloidgenic pathway and amyloidgenic pathway (Tan and Gleeson, 2019). In the non-amyloidgenic pathway, APP is cleaved by α-secretase to produce a membrane bound fragment (α-CTF/C83) that is subsequently cleaved by γ- secretase. The cleaved APP byproducts such as sAPPα and APP intracellular domain (AICD) are thought to have neuroprotective properties involving signal transduction and neuron outgrowth (Kametani & Hasegawa, 2018; Tan &

Gleeson, 2019). In the amyloidgenic pathway, β-secretase cleaves APP and leaves a βCTF/C99 fragment in the membrane; γ- secretase sequentially cleaves the membrane bound fragment into Aβ peptides. γ- secretase cleavage can occur on many sites in the amyloidgenic pathway producing different forms of Aβ peptides, namely $A\beta_{40}$ and $A\beta_{42}$ (Tan and Gleeson, 2019). Improper clearance of Aβ peptides causes accumulation of Aβ in AD. $A\beta_{42}$ specifically is neurotoxic and prone to aggregation than other forms of Aβ, thus causing Aβ plaques to form (Wang et al., 2017).

Alzheimer's and Stress

The negative impact of stress seems to play an integral role in many forms of diseases. There is an overlap of comorbidity between many forms of dementias and psychological disorders. Stress may play a significant role in the development of AD and has been implicated in psychological disorders such as depression and posttraumatic stress disorder (PTSD) (Justice 2018; Justice et al., 2015). Stress increases risk of AD through excessive release of corticotropin releasing factor (CRF) (Hauger et. al., 2009). The key pathway in the stress paradigm involves CRF release from the hypothalamic pituitary adrenal axis (HPA). CRF is released and activates CRF receptors on the anterior pituitary, which then releases adrenocorticotropic hormone (ACTH). The release of ACTH prompts a release of glucocorticoids by the adrenal cortex that provokes a physiological response in reaction to stress (Carmen, 2013; Hauger et al., 2006). The mechanisms by which stress, CRF, and the HPA axis exacerbate AD pathology are still unknown, but in animal models as well as clinical observations, there is a clear connection amongst these factors. Animal models with human AD expression has shown an increased in the amount of Aβ plaques when subjected to different forms of stress (Rissman, 2007; Baglietto-Vargas, 2015; Dong & Csernansky ,2018; Justice, 2018). Elderly individuals who are prone to

high levels of stress are 2.7 times more likely to develop AD than those who are not as prone to stress (Wilson et al., as cited by Dong & Csernansky, 2018).

Cognitive functioning is also heavily influenced by stress. Stress causes the HPA axis to release a range of hormones that affect different brain circuit and signaling pathways that ultimately affect the brain's information processing; various types of memory, attention, and perception are affected by stress (Carmen, 2013). The same forms of cognitive decline are seen in AD patients. Chronic anxiety after stress exposure has been shown to engage biological mechanisms predisposing those with PTSD to be twice as likely to develop an AD diagnosis (Adamec et al., 2010). PTSD is diagnosed in patients who have lived through life threatening situations resulting in extreme emotional and physical trauma (American Psychiatric Association, as cited by Qureshi et. al., 2010). Veterans who have served in combat are particularly vulnerable to PTSD diagnosis. Veterans with PTSD are thought to have an altered stress response, specifically caused by the over activation of the hypothalamic pituitary adrenal axis (Justice et al., 2015). PTSD increases the probability of dementia by two times in Veterans in comparison to those without PTSD (Qureshi et. al., 2010). Thus, Veterans are amongst the largest group at risk for AD and would benefit from preventative therapies that focus on their stress responses.

CRF, CRFR1 Receptor, and AD

CRF is a stress related neuropeptide that is released by the hypothalamic pituitary adrenal (HPA) axis (Hauger et al., 2009; Jutkiewicz et al., 2015). Amongst other stress hormones released downstream of the HPA axis, CRF is thought to be critical for psychological maintenance, which ultimately allow for adaptive responses for an individual (Carmen, 2013). CRF receptors are expressed widely throughout the brain and peripheral organs (Dautzenberg

and Hauger, 2002). There are two forms of CRF receptors found in vertebrates, CRFR1 and CRFR2. Both CRF receptors are subtypes of B class G-protein coupled receptors (Hollensteina et al., 2014). CRF receptors have a large N-terminus extracellular domain where CRF binds to (Hollensteina et al., 2014). CRFR1 may be specifically implicate the increases of Aβ peptides and phosphorylated tau (Rissman, 2007; Campbell Et al., 2015).

Although CRFR1's molecular mechanisms of action have yet to be defined in AD, CRFR1 is a likely target for AD progression. Rissman and colleagues (2007) saw decreases in hyperphosphorylated tau in CRFR1 knockout mice. With the addition of acute and chronic restraint stress, CRFR1 receptor knockout improved AD pathology in comparison to wildtype littermates. Campbell and colleagues (2015) found Aβ pathology decreased when CRFR1 was knocked out in mice overexpressing presenilin-1 and AβPP (PSAPP mice) in 12-month aged mice. The PSAPP mouse model, which accumulates Aβ pathology, has also been used to test a CRFR1 antagonist to decrease AD pathology without a CRFR1 knockout. Zang and colleagues (2016) used a CRFR1 antagonist, R121919, on PSAPP mice and revealed decreases in Aβ plaque load as well as decreases in cognitive deficits in 6-month old mice. These researchers established CRFR1 as a potential therapeutic target as well as the importance of stress in AD pathology and cognitive defects. The next steps are to test whether R121919 is able to decrease pathology in long term AD progression. For those who are at risk to developing AD due to experiencing traumatic stressful events, such as veterans with PTSD, positive results from these experiments will prove beneficial.

Stress, AD, and Synaptic Loss

Along with Aβ plaques and tau neurofibrillary tangles, AD is associated with diminished numbers of synapses and neuron loss (Ingelsson et al., 2004 as cited by Kirkwood et al., 2013). It

has also been reported that stress impairs synaptic connections (Baglietto-Vargas et al., 2015) The loss of synapses is one rationale to explain the cognitive impairments seen in AD. Long-term potentiation (LTP) is caused by high frequency synaptic activity between neurons. LTP between neurons causes stronger connections and associations between neurons, which is thought to be the cellular basis of learning and memory (Kirkwood et al., 2013; Baglietto-Vargas et al., 2015). Enlargement of dendritic spines, which harbor synaptic connections between neurons, require polymerization of different forms of a structural protein called actin (Okamoto et al., 2004). Subsequently, depolymerization of actin forms will cause synapse loss, decrease of dendritic spines and long-term depression (LTD) (Kirkwood et al., 2013).

In one study, a reduced amount of synaptic connections were found in neurons that were near Aβ plaques in a PSAPP transgenic mouse model for AD (Kirkwood et al., 2013). Spine density and size were also affected by the amount of circulating soluble Aβ implicating a connection between Aβ and synaptic loss in the brain. This study provides supports for the Aβ hypothesis that claims Aβ is the ultimate cause for neuron loss and ultimately cognitive deficits in AD.

With the addition of stress, some studies have shown an increased amount of soluble and insoluble Aβ as well as decreases in dendritic spines in the CA3 area of the hippocampus (Baglietto-Vargas et al., 2015). Another study conducted by Dong and colleagues (2018) explored the benefits of CRFR1 antagonist, R121919, on synaptic loss in AD. Mice were subjected to chronic isolation- restraint stress and tested for memory and learning and for anxiety like behaviors. Dong and colleagues found that mice that had undergone stress showed an increased amount of anxiety like behavior and an increased loss in dendritic spines. However, mice that were treated with R121919 had reversed effects in spine density as well as blocked

anxiety like behavior. These studies suggest a connection between stress and neuronal synaptic connection. Stress and AD have overlapping features in more areas than one. For those who are at risk of developing AD or prone to anxiety and stress, there is an even greater risk to cognitive dysfunction.

Experimental Aims

This study has tested the impact of CRFR1 antagonist, R121919, on amyloid pathology in the presence or absence of stress. R121919 is a highly lipophilic small molecule allowing it to pass through the blood brain barrier to reach the brain (Chen et al., 2004). This antagonist will bind to CRF receptors by allosteric inhibition of the C-terminus of the receptor (Hollensteina et al., 2014). PSAPP mice underwent CRFR1 antagonism and chronic restraint stress. 6 and 12-month aged mice were used to show two different time points of AD pathology and progression; both have been subjected to chronic restraint to induce stress. Because AD pathology is thought to progress and worsen over time, 6-month old mice represented early progression of AD while 12-month old mice represented late progression of AD (Masters Et al., 2004). 12-month old mice are expected to have worse cognitive abilities as well as more pathological Aβ plaques in comparison to 6- month mice. Our goal is to determine whether CRFR1 antagonist could be a future preventative therapy for those subjected to extreme stress as well as those who are inherently at risk of developing AD.

Chapter 2: Materials and Methods

PSAPP Mice

AD transgenic mice (JAX MMRRC Stock #34829) were used for this research study. This model, referred to as PSAPP mice, co-express mutant human APP gene and PS1 genes (Jankowsky et al., 2001). C57BL/6 wild-type mice (JAX Stock #000664) were bred with PSAPP mice to generate mice for each age cohort. PSAPP mice were maintained as heterozygous while wild-type littermates served as control. Mice were weaned at 21 days after birth. Mice were housed 2-5 per cage in a temperature-controlled room (22°C) with a 12-hour light –dark cycle. Female transgenic mice were used for this study.

R121919 Administration

R121919 is a small molecule selective antagonist to the CRFR1 receptor (Zhang et al., 2016). Drug was donated by Dr. *Kenner C. Rice from NIDA/ NIAAA/ NIH*). R121919 was dissolved in a solution of 0.01M tartaric acid and 5% castor oil. Vehicle solution consisted of tartaric acid and castor oil without R121919. R121919 solution was vigorously mixed until the drug was solubilized in tartaric acid before the addition of castor oil. Mice received subcutaneous injections of either drug or vehicle beginning at 2 months of age until sacrifice date at 6 months or 12 months of age. Mice were weighed weekly to determine dosage. 20mg/kg/ day were administered; 0.1 ml of drug or vehicle was kept constant. Mice were randomly assigned drug and vehicle administration.

Chronic Resistant Stress Treatment

Mice received injections an hour before chronic stress treatment. Mice cohorts were chronically stressed until two weeks before sacrificed date. 6- month mice cohorts were stressed from 2 months to 6 months of age. 12 –month mice cohorts were stressed from 6 months to 12

months of age. Paradigm of stress exposure consisted of 30 min restraint stress in 50 ml conical tubes. Conical tubes were constructed with multiple air holes to maintain temperature. Chronic restraint stress consisted of daily stress administration for 2 weeks followed by 2 weeks of rest.

Sample Collection

Isoflurane was used to anesthetize mice before decapitation. Trunk blood was collected and centrifuged at 3381 RCF for 6 minutes. Plasma was collected and frozen. Brain was harvested and one brain hemisphere was immediately sub -dissected for hippocampus and cortex and frozen. Frozen hippocampus and cortex were used for biochemical assays for future experiments. The other hemisphere was dropped fixed in 4% phosphate buffered paraformaldehyde for 48 hours.- Eyes and other organs were collected simultaneously and were frozen.

Immunohistochemical assays

Hemi-brains were cryoprotected in 30% sucrose for 24 hours and then cut, coronally, at 30 μm using Leica freezing sliding microtome. Brain sections were stored at -20° C in cryoprotectant containing 30% glycerol/ 30% ethylene glycol in PBS.

An N-terminal specific anti human Aβ monoclonal antibody (82E1) (IBL #10323) were used to assess diffuse and neuritic Aβ plaque (Zhang et al., 2016) Antigen retrieval for Aβ was done with 50% formic acid for 3 minutes. Tissue was permeabilized in 0.1% TritonX/ TBS, followed by blocking in 3% BSA in 0.1% TritonX/ TBS. 82E1 (0.1ug/ul) was diluted 1:500, and incubated overnight at room temperature. The next day, tissue was incubated in biotinylated mouse secondary antibody (VECTOR LABS #BA-2001). Then incubated ABC solution (VECTOR LABS #PK6100) before the DAB reaction (VECTOR LABS #SK4100).

Double labeling of microtubule-associated protein (MAP2) (Millipore # MAB378) and synaptophysin (Millipore # MAB528-I) was used to assess cell changes in synaptic density. MAP2 (1:100) and anti-synaptophysin (1:2000), and secondary antibodies, FITC (VECTOR LABS #FL2000) and tyramide red (PERKIN ELMER #NEL 70200), were used respectively.

Aβ Quantification

For identification of Aβ plaques, images were taken of 82E1 stains using a Leica confocal microscope. Quantifications of percent plaque load were used through Image J software to collect several measurements including plaque size and amount of area Aβ plaque covered (percent plaque load). T test and ANOVA test using GraphPad Prism software for statistical analysis. All statistical data is expressed +/- standard error of the mean.

Synaptophysin and MAP2 Quantifications

For identification of synaptic loss, images were taken of synaptophysin and MAP2 double labeled stains. Quantification of percent area of neuropil synaptophysin and MAP2 covered was done through Image J software. T-test and ANOVA testing used GraphPad Prismsoftware for statistical analysis. All statistical data is expressed +/- standard error of the mean.

Chapter 3: Results

To investigate the effects of R121919 during early stage AD development, 6-month old mice groups were studied. 6-month-old PSAPP mice and wild type (WT) cohorts were subjected to vehicle or drug injections. Stress was conducted until 2 weeks before sacrifice. Immunohistochemistry assays were used to determine percent area Aβ occupied in brain regions of the hippocampus and cortex. A nonspecific N-terminus anti-human Aβ antibody, 82E1, was used to identify Aβ plaques. WT cohorts were not quantified when testing for Aβ plaque load because human APP gene was not present. Therefore, human Aβ plaques would not accumulate. In the 6 -month non-stressed PSAPP cohorts, both vehicle and drug treated mice showed accumulation of Aβ plaques, but no significance was determined in brain areas of the hippocampus or the cortex when mice were treated with drug or vehicle (Figure 4). When 6-month mice were exposed to chronic stress, significant reductions in plaque load was exhibited. Specifically, the outer dentate of the hippocampus and the retrosplenial (RS) in the cortex had showed significant reductions of plaque load in mice when treated with drug compared to vehicle treated mice (Outer dentate $p \leq 0.05$; RS $p \leq 0.05$; Figure 5C and Figure 5E). See Figure 2 for quantified regions of Aβ plaques. In conclusion, R121919 presented the most significant reductions in Aβ plaque load for 6-month mice in the presence of stress.

The accumulation of Aβ may play a role in decreasing synapses and dendritic density (Kirkwood et al., 2013). A subsequent immunohistochemistry staining was used to determine percent area axon terminals (Synaptophysin) and dendrites (MAP2) remained in the hippocampus and the cortex. CA1 and CA3 of the hippocampus as well as cortex regions directly adjacent to hippocampal regions were quantified (Figure 3). 6- month non- stressed PSAPP mice displayed an increase amount of dendrites density in the CA3 cortex region ($p \leq 0.05$) in

comparison to vehicle treated PSAPP mice (Figure 6I). With the introduction of stress, 6 month stressed mice displayed an increase in dendritic density in the CA1 ($p \leq 0.01$; Figure 7C), CA1 cortex ($p \leq 0.05$; Figure 7E) and CA3 ($p \leq 0.01$; Figure 7G) when treated with drug in comparison to vehicle treated mice. In the presence of stress, the administration of R121919 may promote dendritic formation in AD. The administration of R121919 presented consistent trends in reducing AD progression in 6-month mice cohort. Drug was most effective in reducing Aβ plaque load and AD phenotypes in the presence of stress for early stage AD development.

The presence of stress and R121919 administration was then examined in late stage AD. Mice were aged to 12 months to represent late stage AD progression. Before chronic stress was administrated, mice had 6 months to accumulate AD pathology. Age of mice and time period mice received chronic stress was to determine R121919's effect when AD pathology had already accumulated and when stress occurred later in life. Based on trends seen in 6-month mice group, R121919 was expected to decrease AD progression to some varying degree. 12- month old groups were quantified under the same conditions as 6-month old age groups. In the 12- month non-stressed group, whole hippocampus exhibited a significant decrease ($p \leq 0.05$; Figure 8B) in Aβ plaque load in drug administered PSAPP mice in comparison to vehicle treated mice. In the subdivisions of the hippocampus, the outer dentate ($p \leq 0.05$: Figure 8C) and the CA1 ($p \leq 0.05$; Figure 8C) demonstrated the most significant difference in the amount of plaque load. With the presence of chronic stress, PSAPP mice had reduced accumulation of Aβ in the whole cortex ($p \leq 0.05$; Figure 9D), specifically in the RS ($p \leq 0.05$; Figure 9E) and the ectorhinal/ perirhinal ($p \leq 0.05$; Figure 9E) sub-regions of the cortex. Unlike trends seen in 6-month mice group, when determining changes in synaptic connections and dendritic density no significance was exhibited for 12-month mice that were not stress (Figure 10). AD pathology may have accumulated to a

point in which R121919 could not stop disease progression. Similarly, 12-month mice that were stressed presented an unusual trend showing decreased in dendritic density in CA1 cortex in PSAPP drug treated mice (Figure 11E). WT cohorts also showed an increase amount of synaptic connections when treated with drug (Figure 11H). Stressed 12-month mice were expected to show some varying degree of AD phenotypic reduction with the administration of CRFR1 antagonism. 12-month mice showed reductions in Aβ plaque load regardless of stress, but dendritic reductions were not rescued.

T-test and 2-way ANOVA was used to determine significance for all reported results.

Chapter 4: Discussion

There have been many studies implicating stress and AD. Excessive levels of stress, both short term and chronic, have been seen to increase AD disease progression through the activation of the HPA axis; specifically, CRF on to the HPA axis (Dong et al., 2010; Justice et al., 2018). The exact mechanism that links CRF and AD pathology is largely unknown, but it is clear that stress plays an important role in accelerating disease progression. There are two primary papers this study built its foundation on. Both papers focused on the effects of CRFR1 activation on AD. Campbell and colleagues (2015) developed an AD transgenic mouse model that contained a knockout for CRFR1. Mice that contained CRFR1 knockout exhibited decreases in two forms of Aβ. There were also decreases of Aβ in specific regions including insular, retrosplenial, ectorhinal, and perirhinal cortices. Quantification of these regions gave rise to the specific areas quantified in this study. Campbell's data provided evidence that CRFR1 is linked to Aβ accumulation. Campbell also measured C terminal fragments of APP and reported decreased in the amount in knockout mice which was not done in this study. Another paper by Zhang and Colleges (2016) looked at the effects of R121919 on 6 month aged PSAPP mice and did a comprehensive study on synaptic deficits, memory and learning, and different Aβ species accumulation. Zhang reported a decrease in synaptic defiticits in PSAPP mice treated with R121919; memory and learning was retained and there was a decrease in Aβ formation. Unlike this study, Zhang tested on both male and female mice and saw reductions in pathology and retention in learning and memory in both groups. This study focused on female mice as females are more supseptible to stress than males (Dong et al., 2018). Zhang also measured for C terminal fragments similarly to Campbell. Zhang provided insight to the importance of CRF in

AD development, both behaviroally and pathologically. The next steps were to see the interaction of CRF when chronic stress was introduced.

CRFR1 antagonist R121919 is expected to decrease amyloid plaque load in brain regions associated with memory and learning (Zhang et al., 2015). R121919, as an allosteric inhibitor of CRFR1, should be able to inhibit activation of the HPA axis that ultimately leads to increased amyloidgenic processing. The goal of this study was to determine R121919's affect on AD pathology with the induction of chronic stress. As age is a factor that increases severity of AD, two different time points of AD progression was also tested in the AD transgenic mouse model.

6-month non-stressed mice was administered R121919 to replicate data as seen in previous data by Zhang and colleagues (2016). Previous data had reported that same aged PSAPP mice treated with R121919 displayed a decrease in plaque load in the entire hippocampus and cortex, but data generated in this study did not show the same results. In this study, 6-month non-stressed mice showed no significance. A likely explanation could be due to the age timepoint of AD progression. Mice at 6 months of age have low levels of Aβ plaque accumulation as disease is early in progression. This could also account for difficulties in achieving significance between drug and vehicle-treated mice. With the addition of chronic stress, 6-month mice exhibited a significant decrease in Aβ plaque accumulation in certain regions of the hippocampus and the cortex. When comparing the two 6-month groups, stress seems to be an important factor in contributing to the effectiveness of R121919.

12-month PSAPP mice are expected to have more plaque load in comparison to 6-month mice due to AD's characteristic to worsen over time (Masters et al., 2004). Daily injections of R121919 decreased Aβ plaques in the hippocampus of 12-month non-stressed PSAPP mice in the entire hippocampus and different regions of the hippocampus. With the introduction of stress,

12- month PSAPP mice treated with drug showed the most significance in the cortex and its sub regions; there was still a trend toward decreased plaque in the hippocampus. It is likely that the increasing the n number in the 12- month stressed mice could show a greater reduction of Aβ plaque in the hippocampus. If this were to be true, R121919 would show most effective in reducing AD pathology when mice are subjected to chronic stress regardless of age. What can be concluded is that R121919 is effective in reducing Aβ plaques in stressed mice during early AD development. Additionally, R121919 is effective in reducing Aβ plaques in late stage AD but further data is needed to substantiate.

Decreasing memory and cognitive abilities is a major symptom seen in AD. Synaptic strength between neurons and dendritic density is thought to be foundational to learning and memory. Changes in the synapses and dendritic areas of the neuron could account for these symptoms seen in AD (Kirkwood et al., 2013). Neuronal deficits have been seen to increase when chronic stress is present as well (Baglietto-Vargas et al., 2015). This study wanted to test if R121919 could save neuronal connection in AD in the presence of stress. It was predicted that R121919 could save neurons, as the drug would be able to block the HPA axis from producing more Aβ plaques and disrupt neuron signaling. Aβ plaques are thought to be detrimental to neuron signaling as it may cause a disruption in neuron connection between cells (Kirkwood et al., 2013). CA1 and CA3 regions of the hippocampus were quantified, as they are important regions in the hippocampus for learning and memory as well as the most easily defined. 6-month non-stressed mice showed an increase dendritic density in the cortex when PSAPP mice was treated with R121919. Treatment with R121919 exhibited neuron rescue as PSAPP mice treated with vehicle had a reduction of neurons. In the presence of stress, 6- month mice saw an increased neuronal density in both quantified hippocampal regions and one of the cortex regions.

Overall it can be concluded that PSAPP mice treated with R121919 had an increase in dendrites, possibly helping increase neuronal connections that might have been lost otherwise.

In late stage mice, 12-month non-stressed mice showed no clear trends or significance. Similarly when stressed, 12 month stressed mice showed unexpected trends with decreased amount of dendritic density in CA1 cortex of PSAPP mice and a significant increase in WT mice treated with drug. Unexpected results could be due to low n number in PSAPP mice treated with drug. It has been seen that in the presence of stress and the administration of drug, R121919 has been the most effective in reducing AD pathology and neuronal deficits. With the addition of more mice, data may show that with the administration of R121919 synapses and neuron density may be higher as seen by trends throughout the study.

By the trends exhibited through this study, it can be concluded that CRFR1 antagonists are most affective in decreasing AD progression in the presence of stress during early AD development. There is potential that R121919 can slow down disease progression in late stage AD, but continued testing is needed to determine a final conclusion. These findings show promise that CRFR1 antagonism can help reduce disease pathology and progression. Decreasing Aβ pathology is a possible mechanism in combatting cognitive impairment as less Aβ plaques are present to affect dendritic connections (Kirkwood et al., 2013).

Amyloid Beta Problem

This study, like many others, uses the quantitative measure of amyloid beta plaques as a representation of AD pathology; however, recent studies have questioned its reliability. Aβ plaques can be insoluble or soluble (Golde Et al., 2000). Insoluble Aβ results in the characteristic plaques seen in pathologic AD brains. Aβ plaques are not the sole indicator for memory impairment. Studies have shown the number of Aβ does not correlate consistently with the

severity of cognitive impairment in human cases, but rather soluble plaques are better correlative to disease severity (Kametani & Hasegawa, 2018; Selkoe et al., 2002). Soluble Aβ is not seen in histological quantifications in the brain but can be seen in cerebral spinal fluid or in plasma (Masters Et al., 2004; Rissman et al., 2007; Selkoe et al, 2002). Thus, both soluble and insoluble amyloid beta molecules are important in AD progression but quantifying the molecules to properly correlate AD progression requires additional testing. Future experiments can include looking at soluble Aβ levels in plasma to see how much Aβ is circulating and accumulating.

On a greater scale Aβ may not be a good indicator of AD progression at all. There are problems with the amyloid cascade hypothesis in which certain AD cases show that the accumulation of Aβ plaque is inconsistent in correlating neuron cell death and hyperphosphorylated tau accumulation (Bryan et al., 2009 as cited by Kametani & Hasegawa, 2018). In some cases, older people may have brains riddled with amyloid beta but only showed a mild form of AD (Edison et al., 2007; Li et al., 2008 as cited by Kametani & Hasegawa, 2018). It is thought that increased levels of APP may be the reason for neurodegeneration rather than Aβ itself (Takahashi et al., 2015; Klevanski et al., 2015; Kametani & Hasegawa, 2018). Some APP products are seen to have neuroprotective and neuron functional effects. C-terminal fragments include AICD for signal transduction while N-terminal fragments include sAPPα for neuron outgrowth (Takahashi et al., 2015; Kametani & Hasegawa, 2018). APP impairment and overexpression has been seen to increase synaptic deficits, which triggers tau mislocalization (Kametani & Hasegawa, 2018). Tau mislocalization causes seeding and aggregation. Seeding then causes synaptic damage to nearby neurons, ultimately leading to neuronal cell death in AD (Bejanin et al., 2017; Kametani & Hasegawa, 2018). Tau pathology may also be a better biomarker to test for as it is seen as having a direct cause in neuron dysfunction. The spreading

of hyperphosphorylated tau has also been correlative to increased cognitive and clinical symptoms (Braak & Braak, 1991 as cited by Takahashi et al., 2015; Bejanin et al., 2017). In either case, to accurately quantify AD severity and progression it could be useful to measure many different forms of Aβ, to measure tau pathology, and other by products of APP.

Stress and Clinical significance of CRFR1 antagonism

Although R121919 is now only used for preclinical work, this drug gives insight to the potentials of CRFR1 antagonism for future therapeutics. R121919 is an allosteric inhibitor on the C-terminal side of the receptor. Instead of completely blocking CRF from binding to its receptor, CRF may still be present on the receptor. Thus, there is still a chance for activation of the HPA axis depending on when the drug was administered. Further studies can focus on targeting N-terminal region of CRFR1 to completely block binding of CRF.

Finding targets for therapeutics concerning stress is not only important for AD but for other stress related diagnosis such as depression, anxiety, and PTSD. Many of these psychological diseases are comorbid with neurodegenerative diseases. AD patients with depression have increased amount of Aβ plaques and tau tangles compared to AD patients without depression (Kim et al., 2016). PTSD diagnosed Veterans also have a higher chance of AD development and show symptoms of depression and anxiety (Qureshi et, al., 2010; Justice, 2018). Therefore, future developments toward combatting chronic stress and AD are important for many different forms of neurodegenerative and neuropsychological diseases.

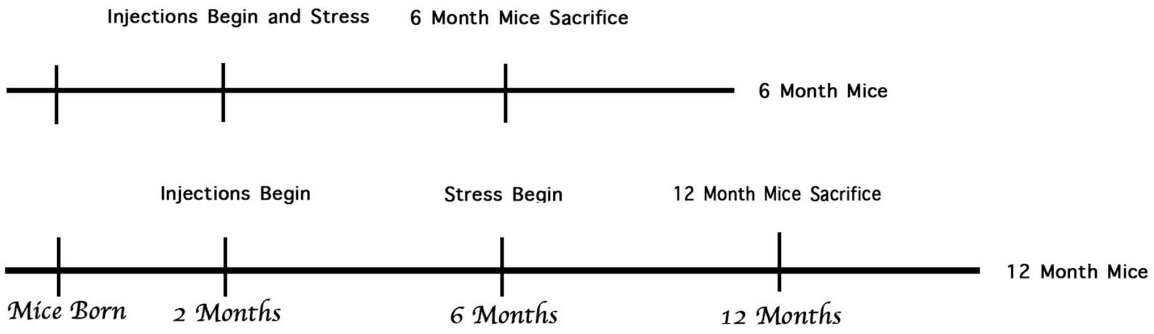


Figure 1. Injection and Stress Diagram. Schematic outline of injections and stress for 6 month and 12-month age mice groups. Injections of R121919 or vehicle began after 2 months of age for both groups. Injections and stress diagram was conducted for both PSAPP and WT mice.

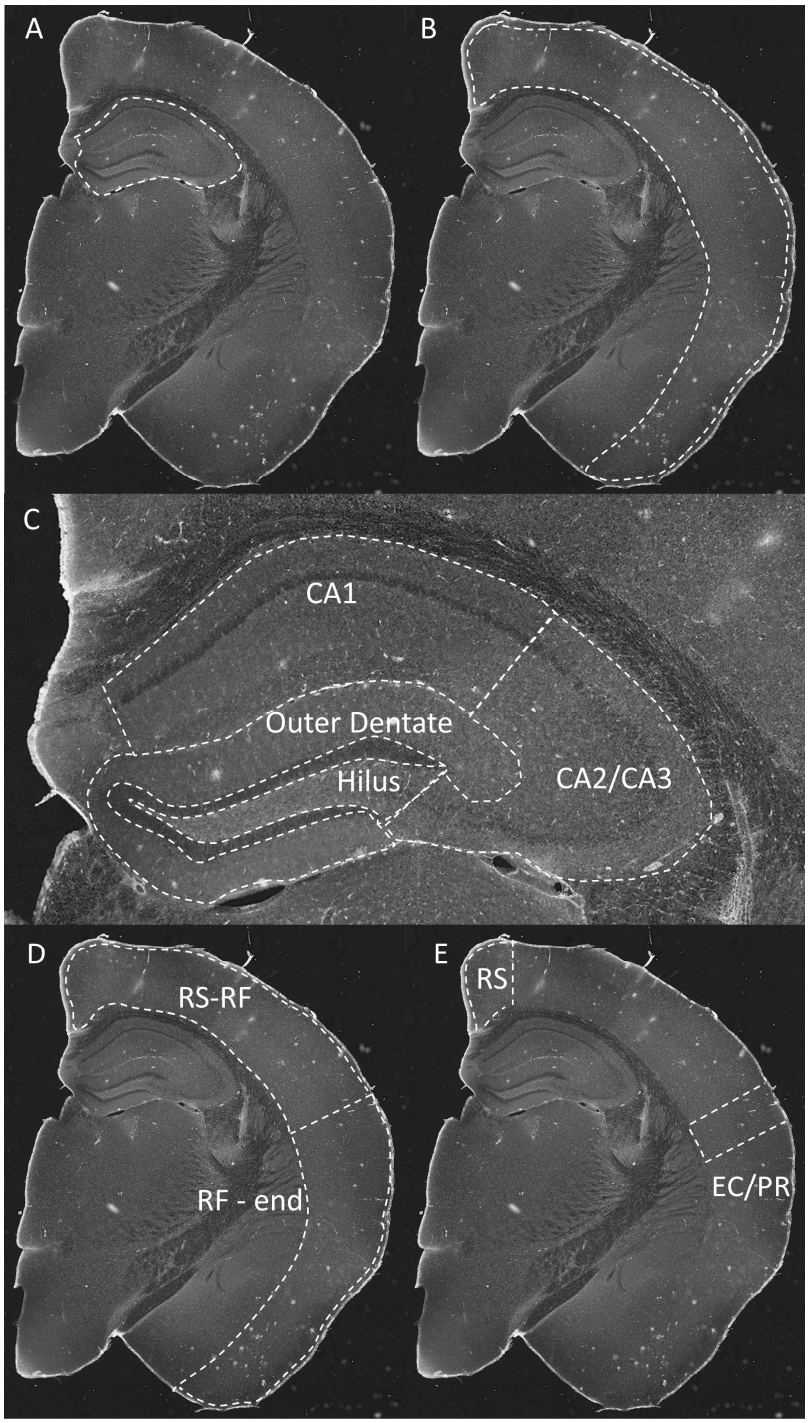


Figure 2. Diagram of regions quantified for Aβ plaques. Regions quantified include (A) Whole Hippocampus, (B) Whole Cortex, (C) CA1 region, CA2 and CA3, Outer Dentate and the Hilus of the hippocampus, (D) Retrosplenial to the Rhinal Fissure of the cortex and from the Rhinal fissure to the end of the cortex, (E) Retrosplenial and the and Ectorhinal/ Perirhinal region.

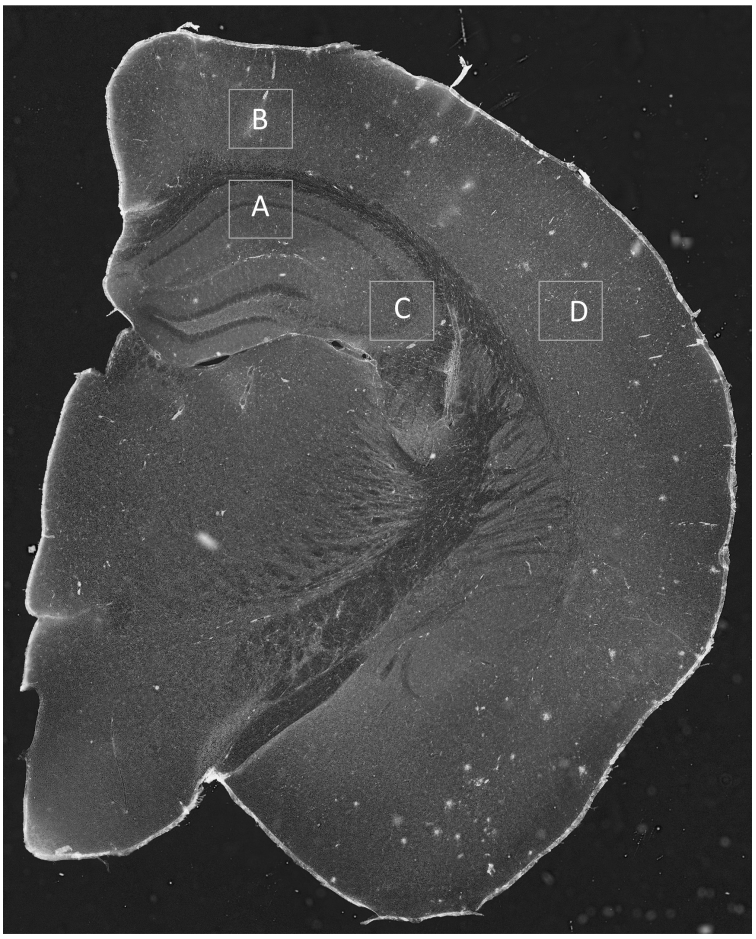


Figure 3. Diagram for regions quantified for Synaptophysin and MAP2. Regions quantified include (A) CA1 pyramidal cells (B) cortex region directly above CA1 (C) CA3 pyramidal cells and (D) cortex region directly above CA3.

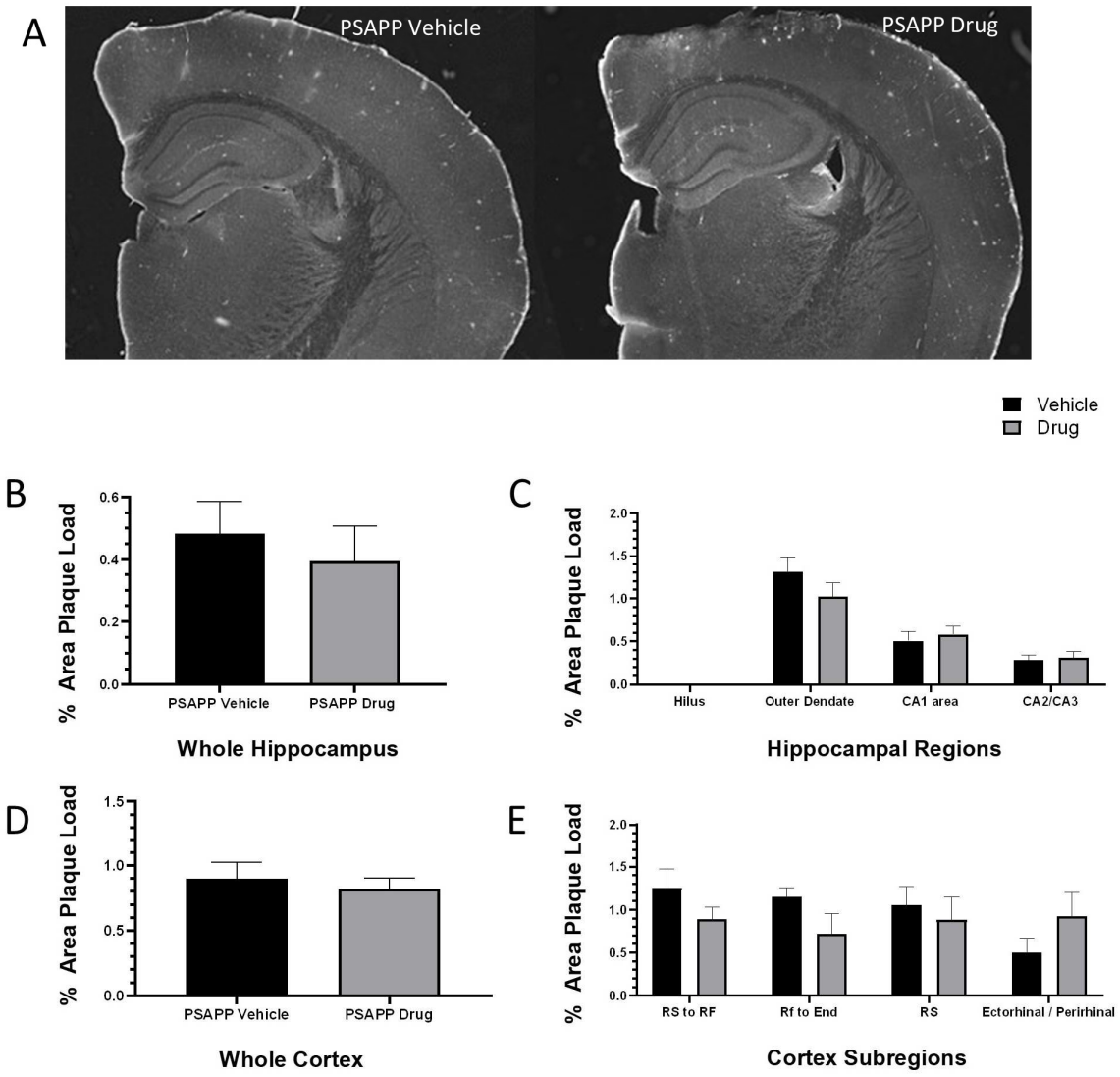


Figure 4. Early age effects of R121919 on Aβ accumulation: 6 Month Non Stressed. Aβ plaques were labeled in vehicle and drug (R121919) treated 6-month-old non-stressed PSAPP mice using 82E1 antibody (greyscale, A). Percent area of plaque load was quantified from coronal plane brain sections in the hippocampus and the cortex (B-E). Between vehicle and drug treatment of R121919, data displays no significance in the hippocampus, cortex or subsequent sub-regions of both areas. All values are expressed as ± SEM, n= 5 for each PSAPP mice group.

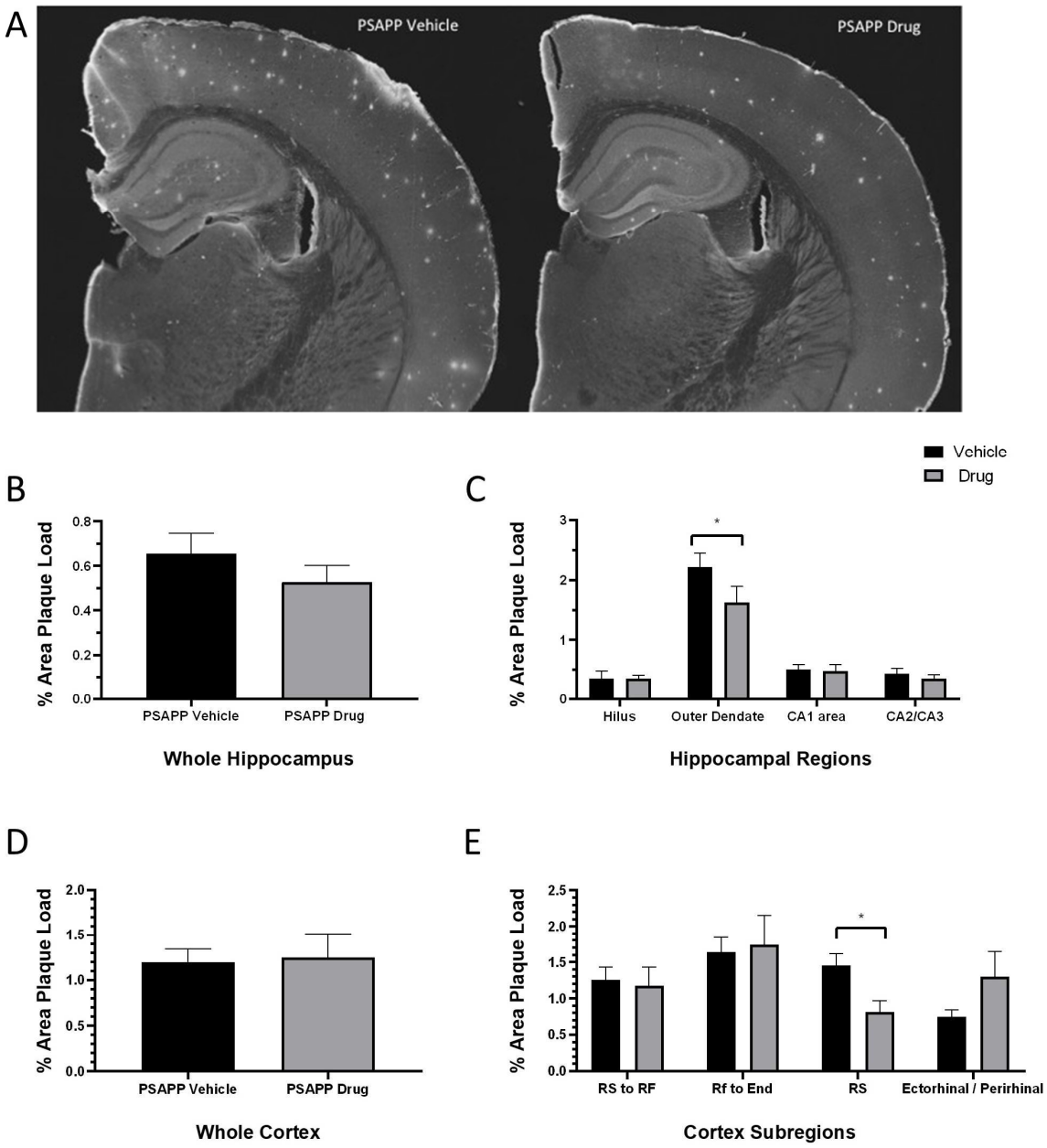


Figure 5. Early age effects of R121919 on Aβ accumulation in the presence of stress: 6 Month Stressed. Aβ plaques were labeled in vehicle and drug (R121919) treated 6-month-old stressed PSAPP mice using 82E1 antibody (greyscale, A). Percent area of plaque load was quantified from coronal plane brain sections using stereology (B-E). In the outer dentate of the hippocampus ($p \leq 0.05$; C) and the retrosplenial (RS) ($p \leq 0.05$; E), a decreased amount of plaque load is observed in drug treated PSAPP mice in comparison to vehicle treated counterparts. All values are expressed as ± SEM, n=6 for vehicle treated and n=7 for drug treated mice.

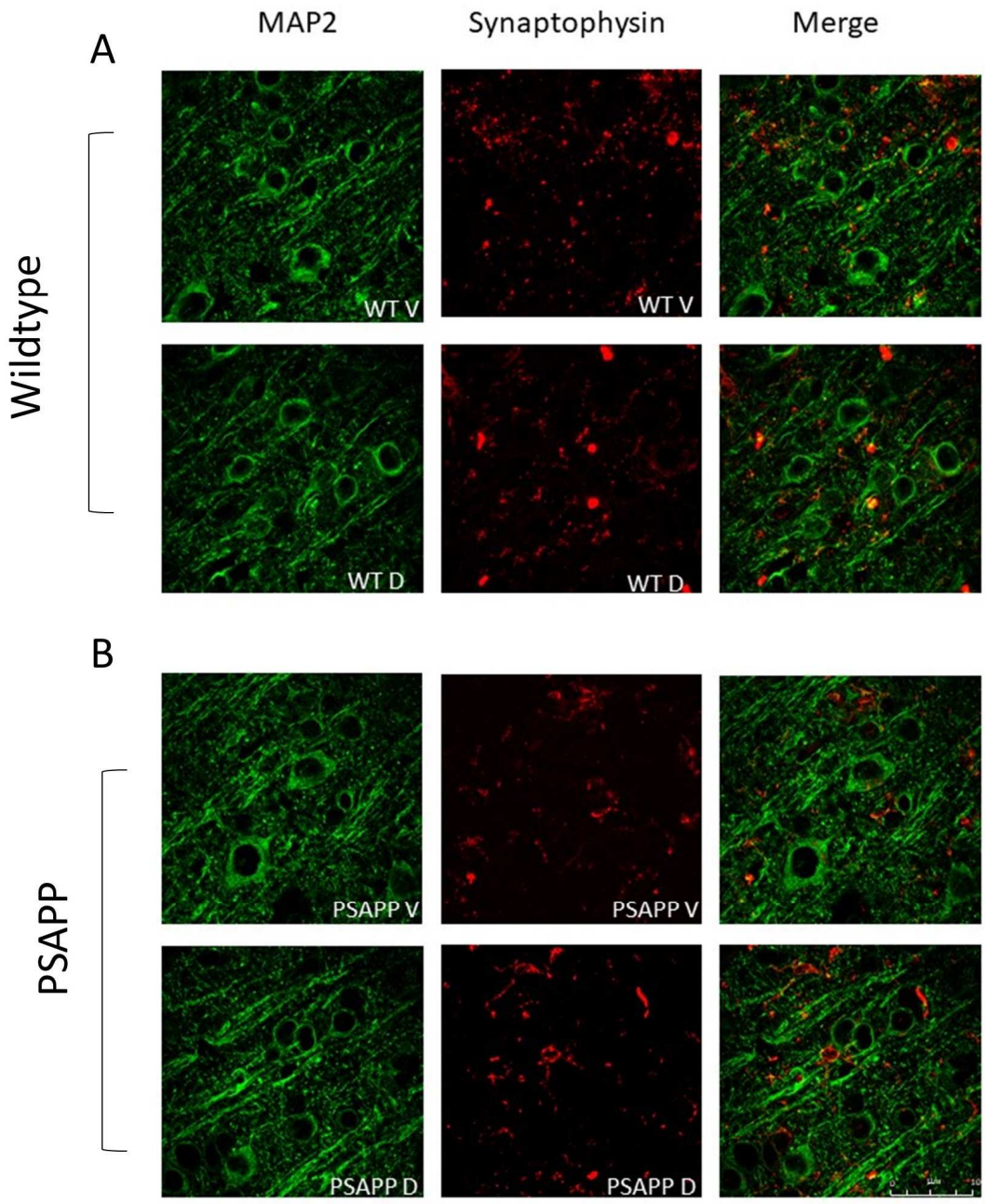
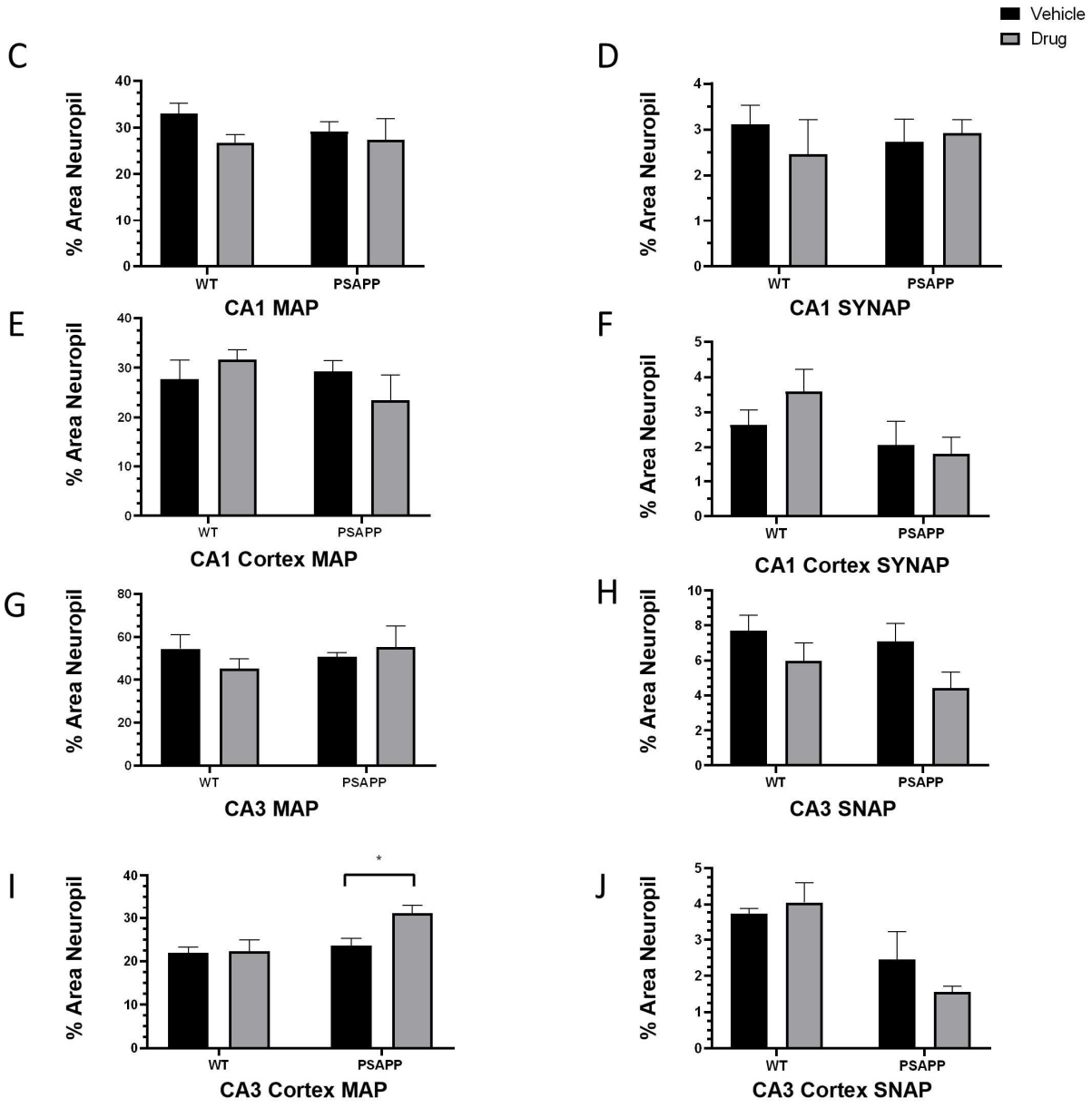


Figure 6. Early age effects of R121919 on Synaptic connections: 6 Month Non-Stressed. Dendrites (MAP2) and axon terminals (Synaptophysin) were double labeled to see changes in dendritic and synaptic connection between neurons in WT and PSAPP mice. Figures presented show images of CA3 cortex region where significance was seen (A-B). Quantifications were done in the CA1 and CA3 region of the hippocampus as well as cortex regions adjacent to these hippocampal regions. Percent area of neuropil containing axon terminals and dendrites were quantified from coronal plane brain sections. Treatment effect showed increased number of dendrites are present in CA3 cortex region ($p \leq 0.05$) in PSAPP mice administered with R121919 (I). All values are expressed as ± SEM; n=6 for vehicle and drug treated PSAPP; n=5 for WT treated mice.

Figure 6 (continued).



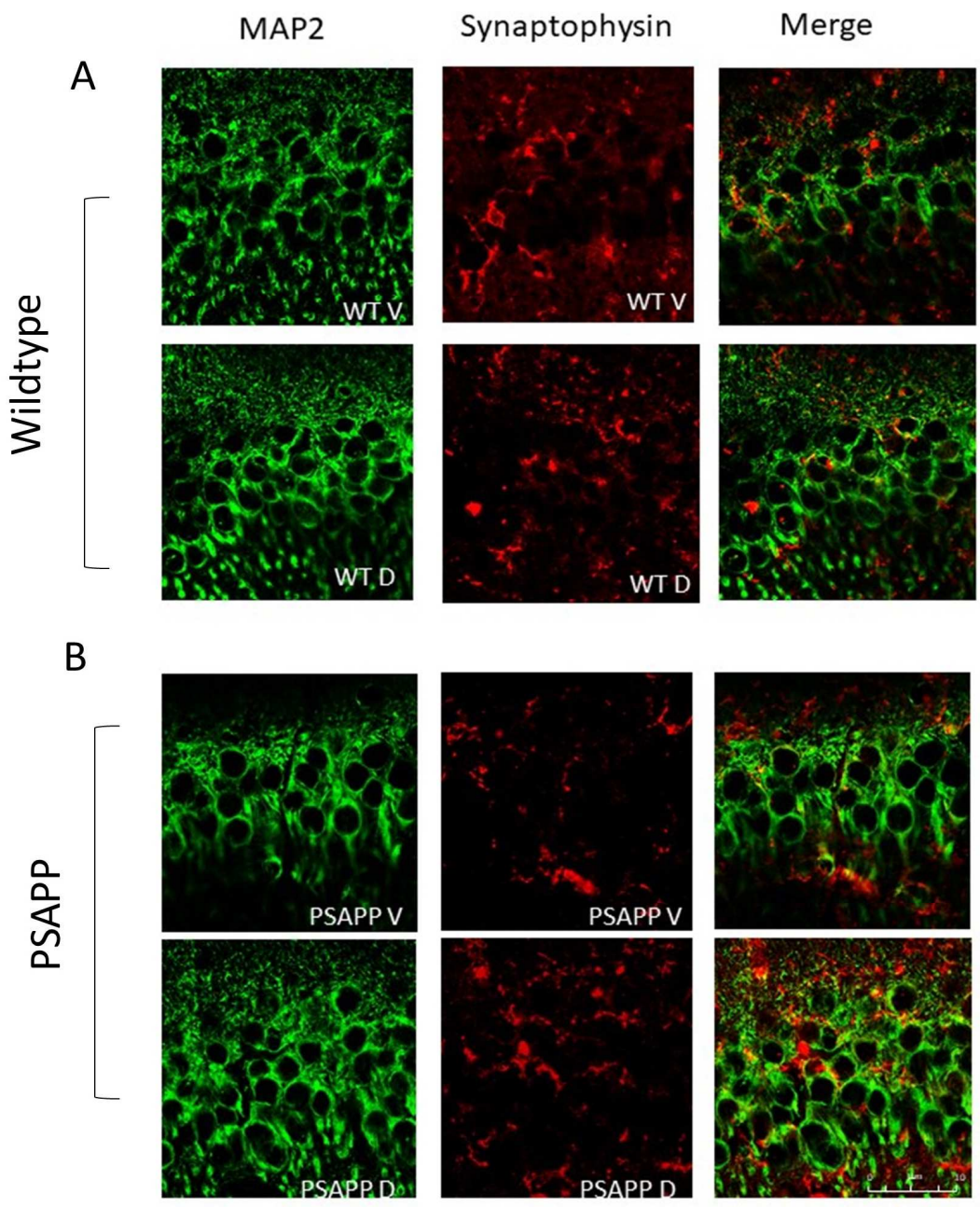
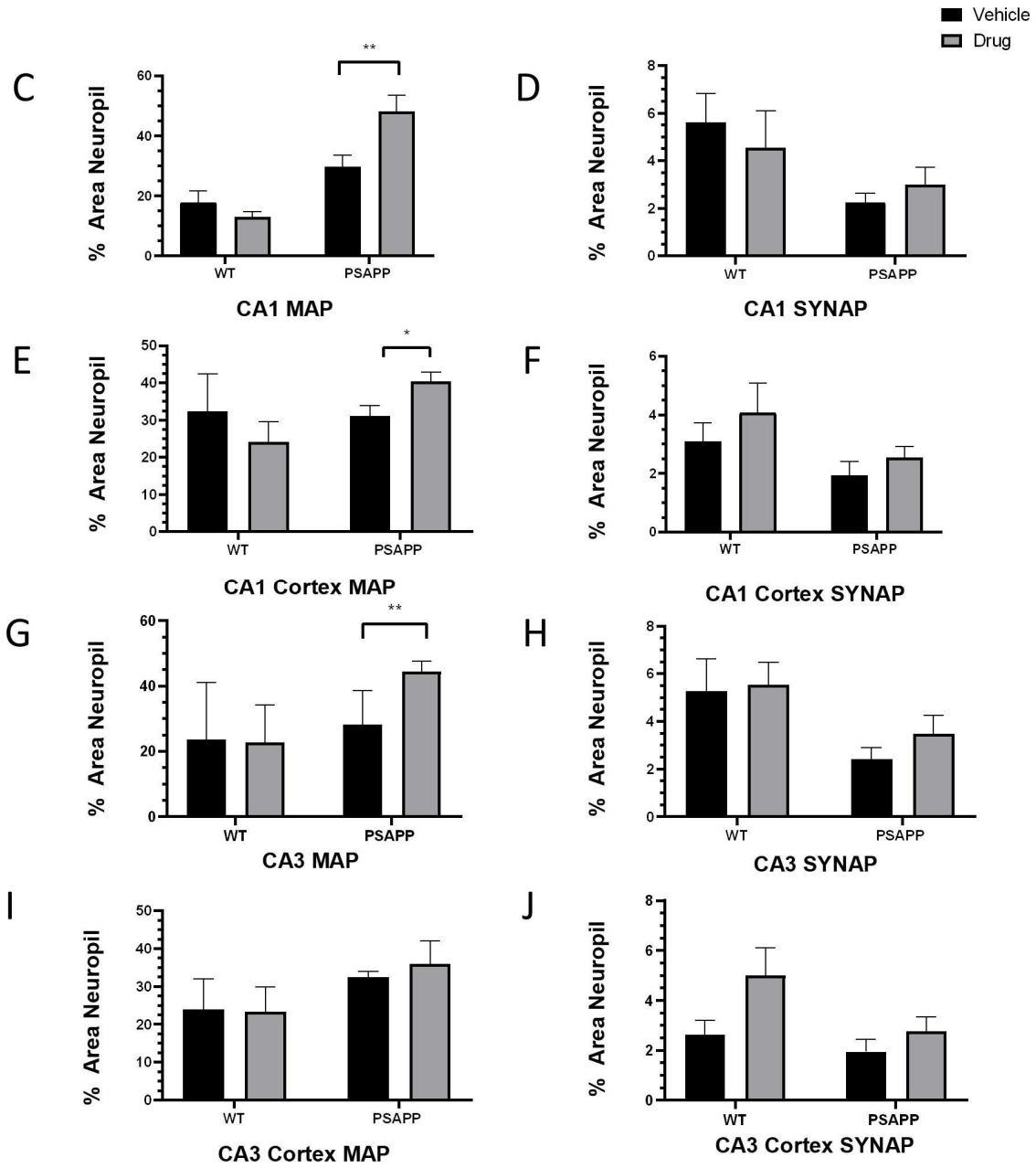


Figure 7. Early age effects of R121919 on Synaptic connections in the presence of stress: 6 Month Stressed. Dendrites (MAP2) and axon terminals (Synaptophysin) were double labeled to see changes in dendritic and synaptic connection between neurons in WT and PSAPP mice. Figures presented show images of CA1 region (A-B). Quantifications were done in the CA1 and CA3 region of the hippocampus as well as cortex regions adjacent to these hippocampal regions. Percent area of neuropil containing axon terminals and dendrites were quantified from coronal plane brain sections. Treatment affects showed increased dendritic density in CA1 ($p \leq 0.01$; C), CA1 cortex ($p \leq 0.05$; E), and CA3 ($p \leq 0.01$;G) in treated PSAPP mice. All values are expressed as ± SEM; n=9 for vehicle treated and n=10 for drug treated PSAPP mice; n= 6 for vehicle and drug treated WT mice.

Figure 7 (continued).



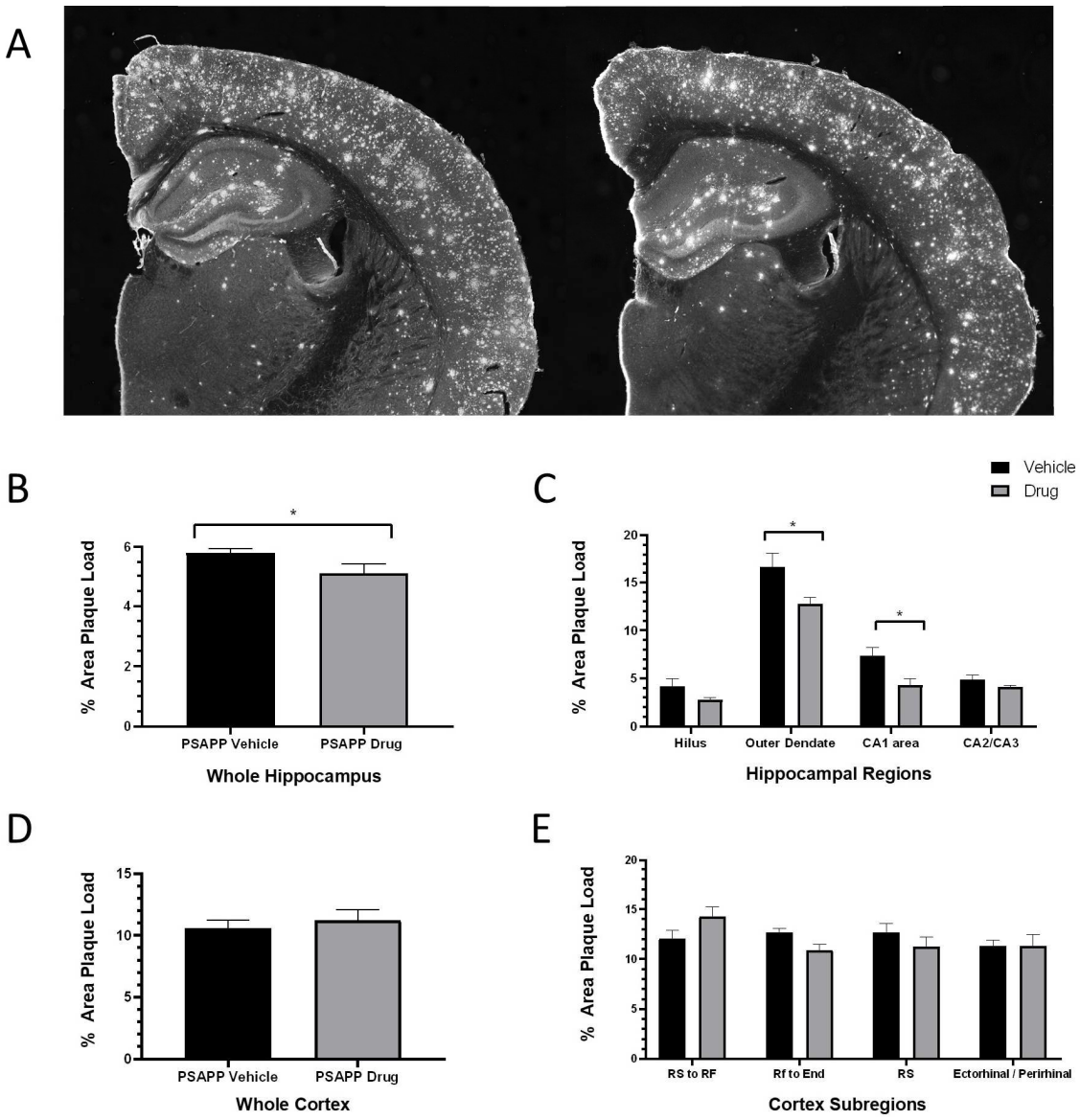


Figure 8. Late age effects of R121919 on Aβ accumulation: 12 Month Non Stress. Aβ plaques were labeled in vehicle and drug (R121919) treated 12-month-old stressed PSAPP mice using 82E1 antibody (greyscale; A). Percent area of plaque load was quantified from coronal plane brain sections using stereology (B-E). Aβ plaque load in drug treated mice exhibited a decrease in the whole hippocampus ($p \leq 0.05$; B), outer dentate ($p \leq 0.05$; C), and CA1 ($p \leq 0.05$; C). All values are expressed as ± SEM; n=9 for vehicle treated and n=7 for drug treated PSAPP mice.

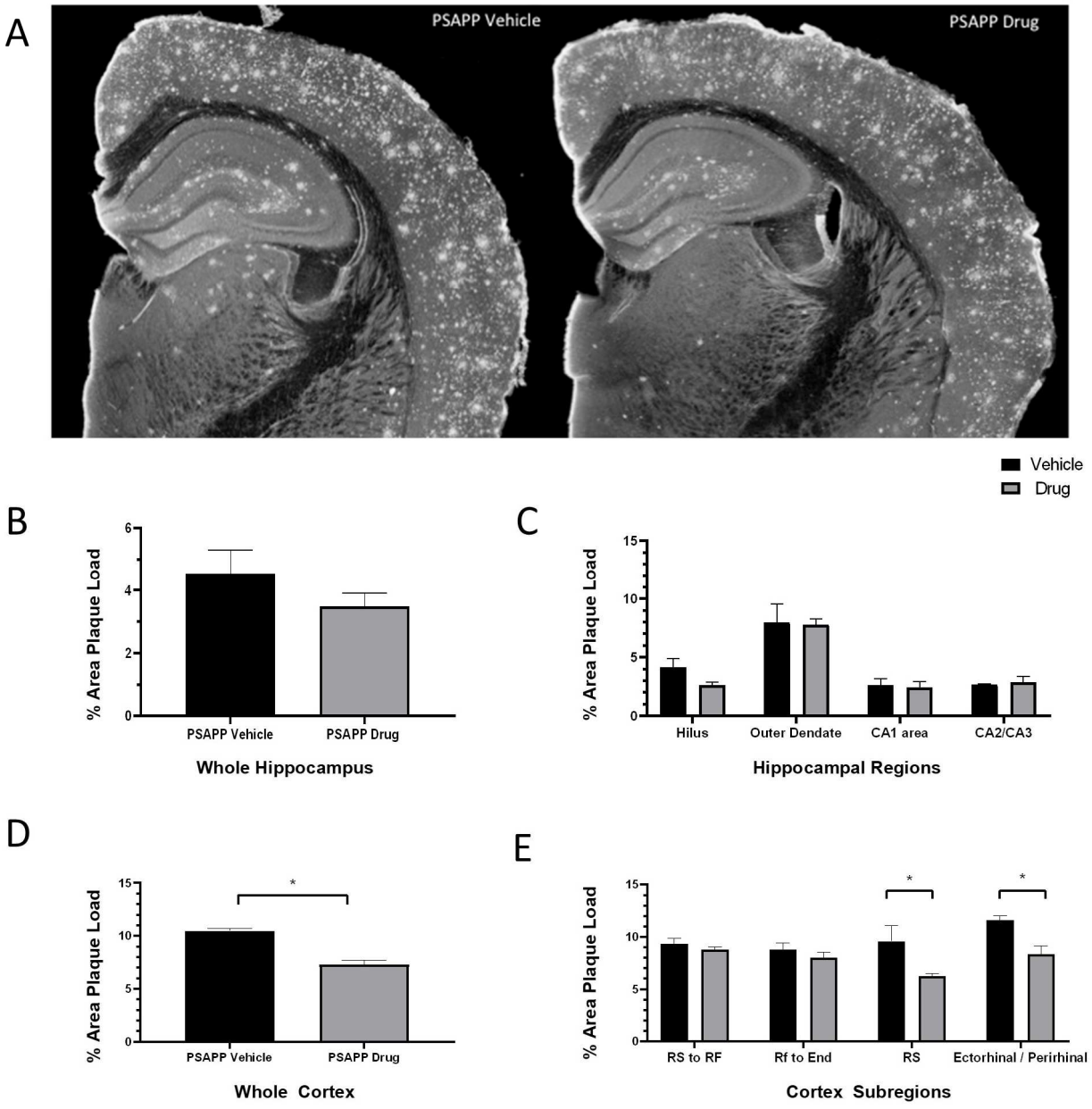


Figure 9. Late age effects of R121919 on Aβ accumulation in the presence of stress: 12 Month Stressed. Aβ plaques were labeled in vehicle and drug (R121919) treated 12-month-old stressed PSAPP mice using 82E1 antibody (greyscale, A). Percent area of plaque load was quantified from coronal plane brain sections using stereology (B-E). Aβ plaque load displayed a decreased amount in the cortex ($p \leq 0.05$; D), retrosplenial (RS) ($p \leq 0.05$: E), and ectorhinal/ perirhinal regions ($p \leq 0.05$; E). All values are expressed as ± SEM, n= 4 for both vehicle and drug PSAPP mice groups.

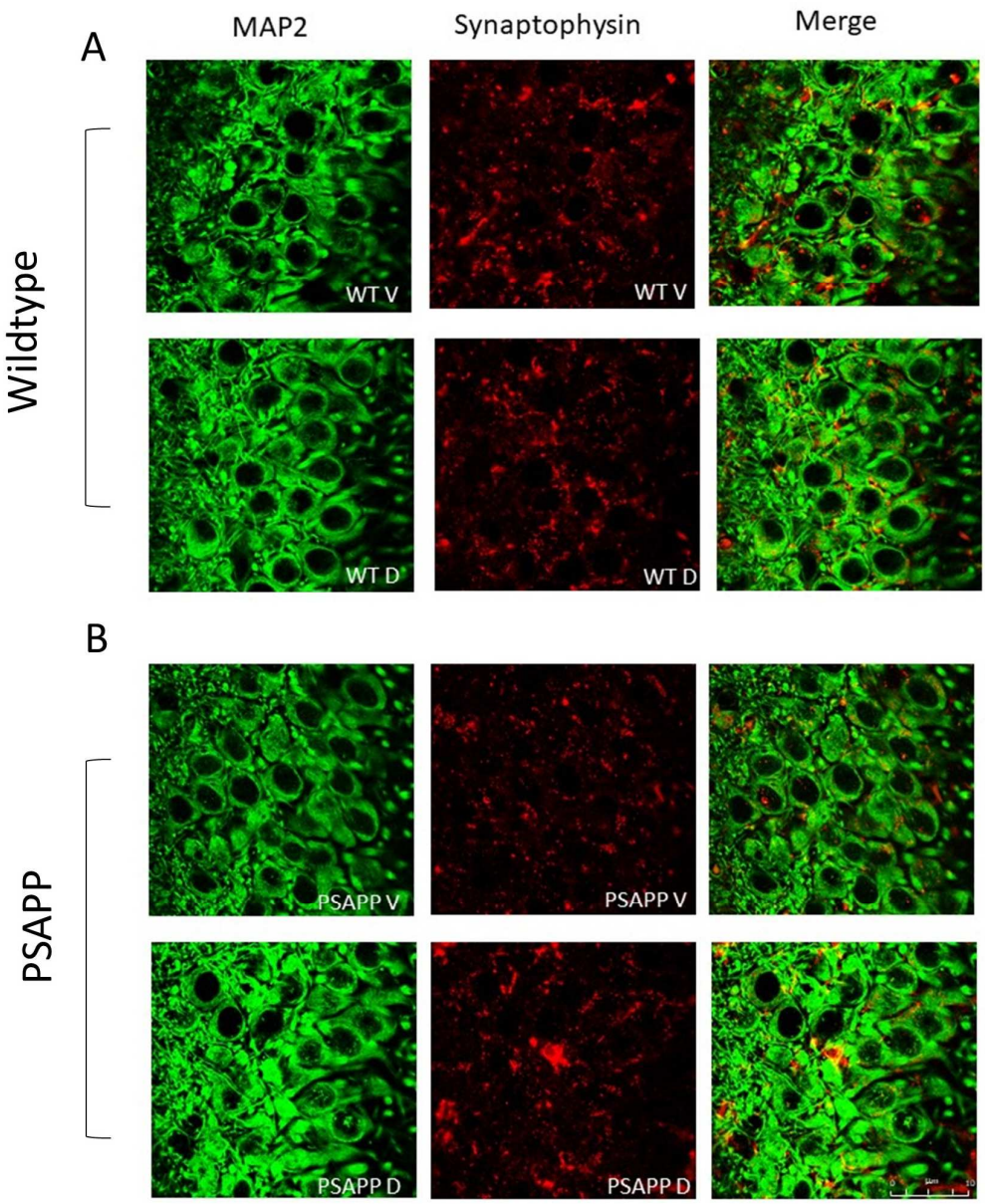
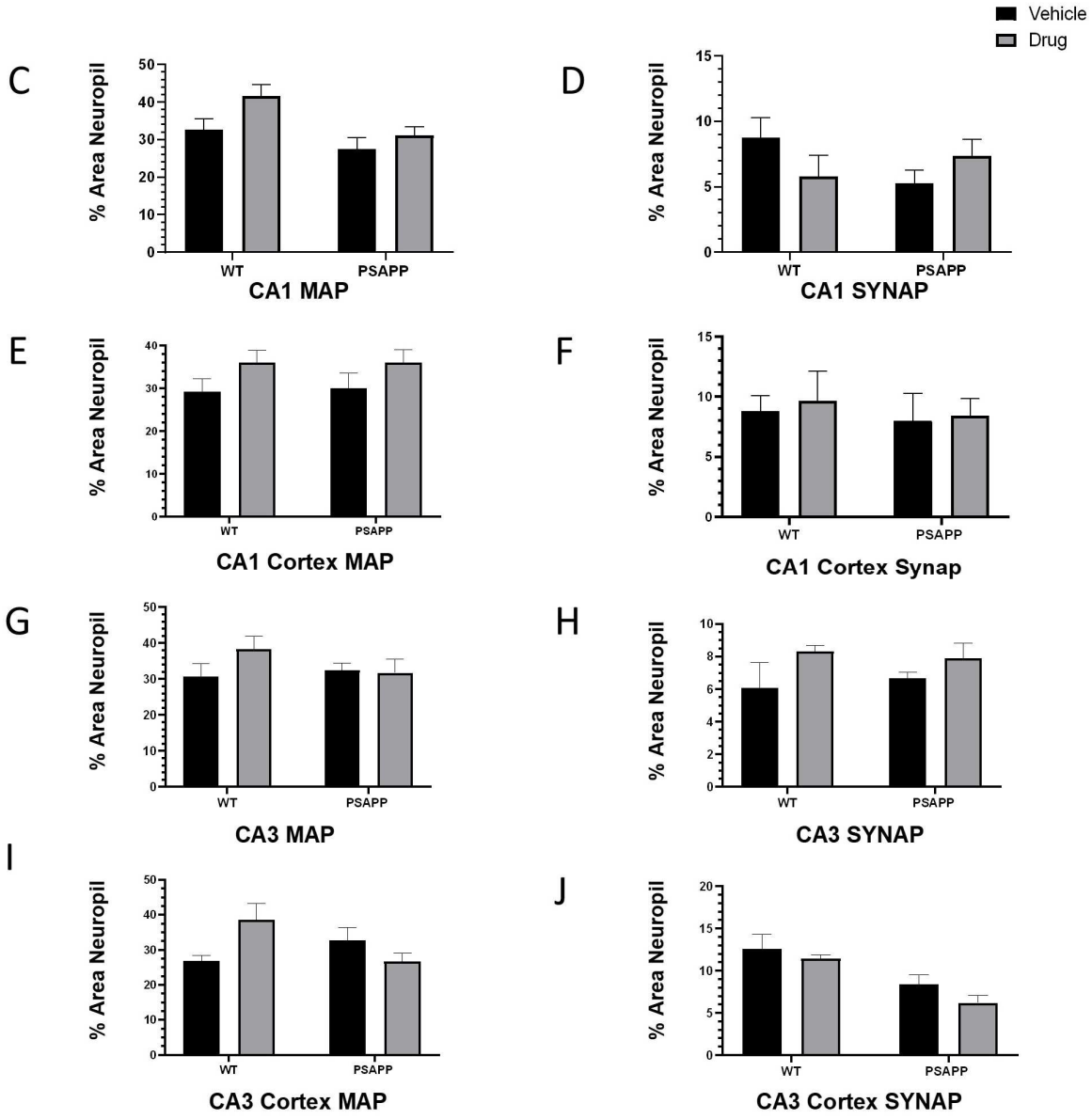


Figure 10. Late age effects of R121919 on Synaptic connections: 12 Month Non-Stress. Dendrites (MAP2) and axon terminals (Synaptophysin) were double labeled to see changes in dendritic and synaptic connection between neurons in WT and PSAPP mice. Pictures presented are images of CA3 region (A-B). Quantifications were done in the CA1 and CA3 region of the hippocampus as well as cortex regions adjacent to these hippocampal regions. Percent area of neuropil containing axon terminals and dendrites were quantified from coronal plane brain sections. Between vehicle and drug treatment of R121919, data displays no significance in the hippocampus or subsequent cortex regions (C-J). All values are expressed as ± SEM; n= 10 for vehicle and drug treated PSAPP mice group; n=7 for vehicle and n=6 for drug treated WT mice group.

Figure 10 (continued).



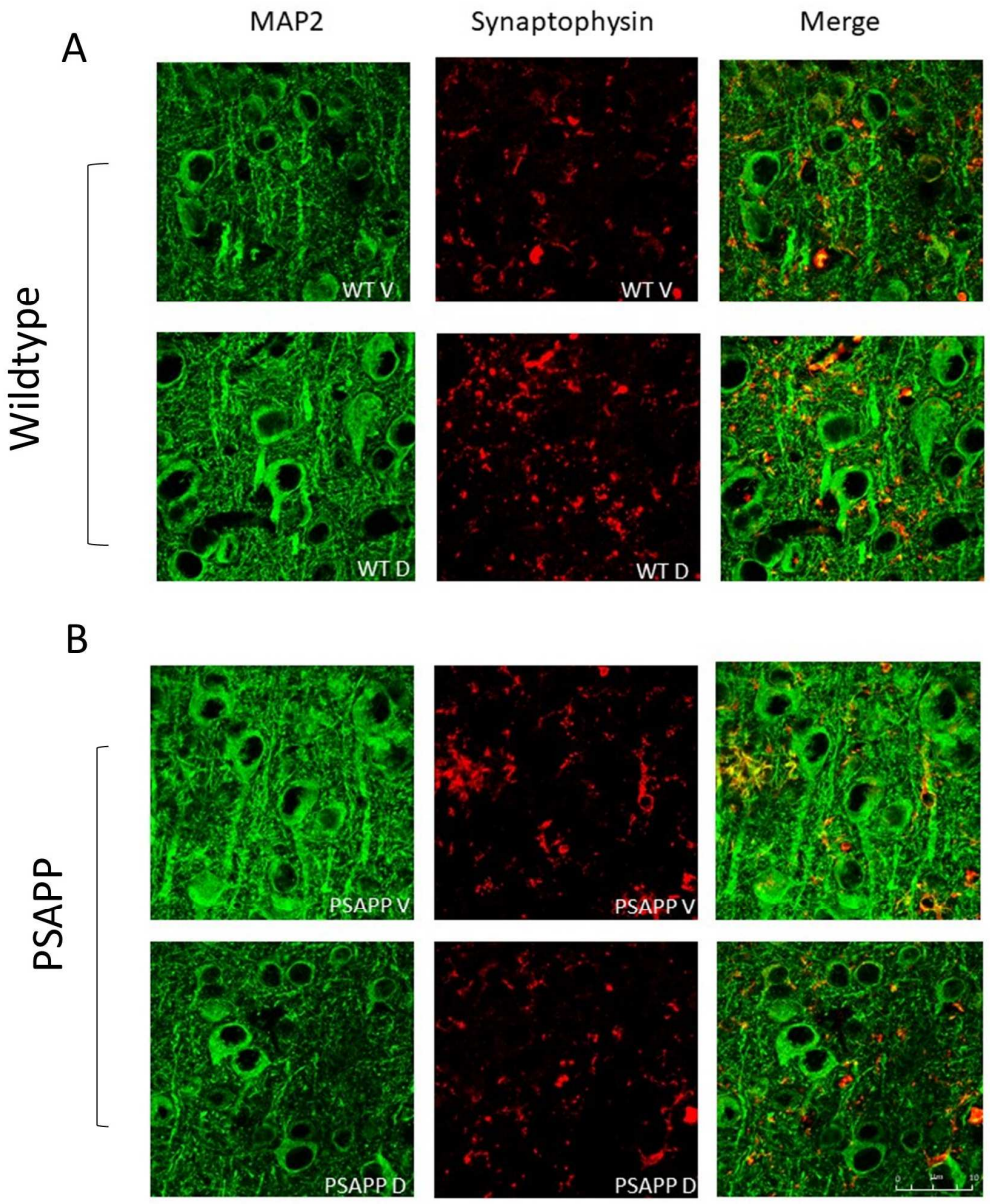
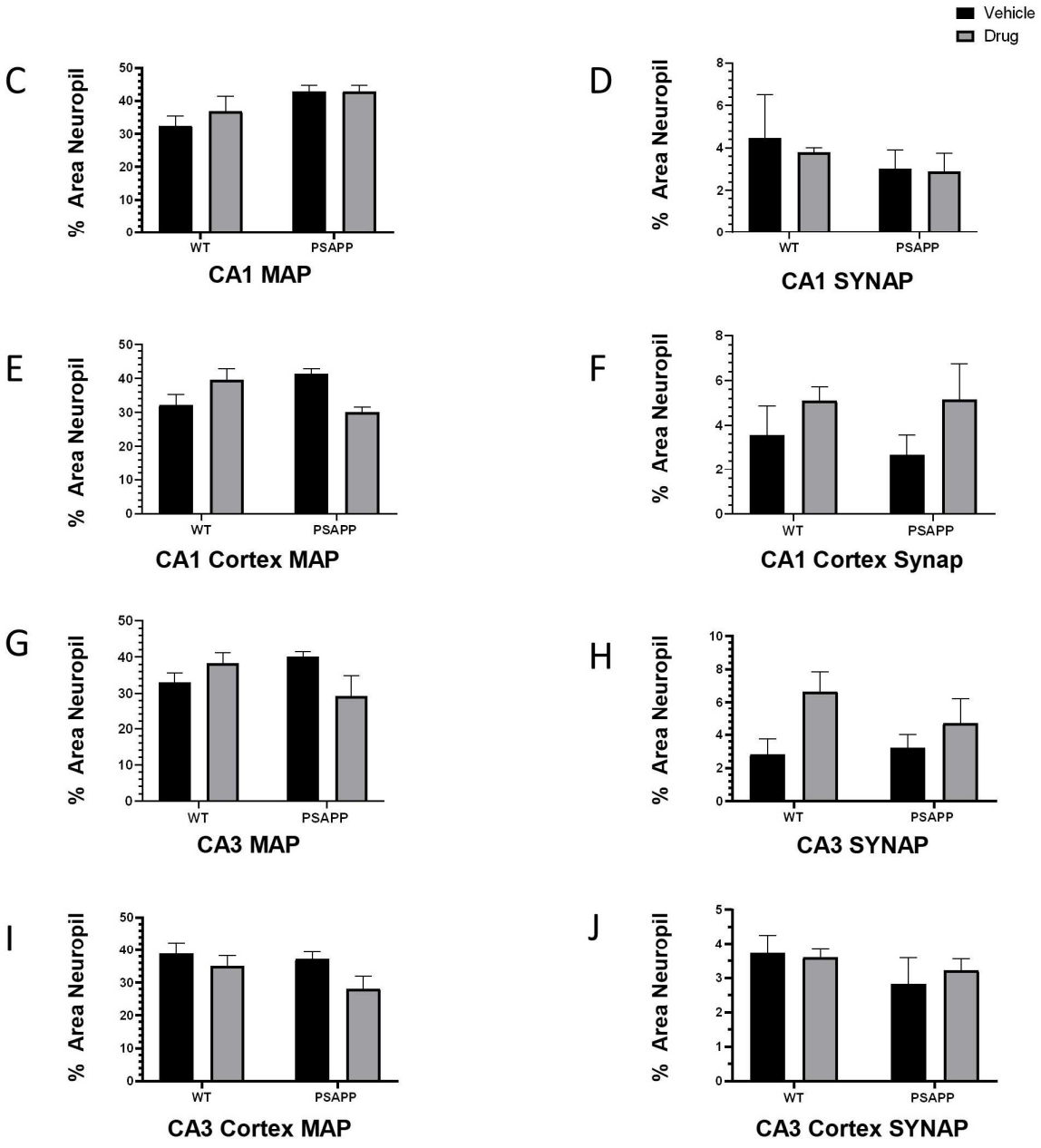


Figure 11. Late age effects of R121919 on Synaptic connections in the presence of stress: 12 Stressed. Dendrites (MAP2) and axon terminals (Synaptophysin) were double labeled to see changes in dendritic and synaptic connection between neurons in WT and PSAPP mice. Pictures shown present images from CA1 cortex region (A-B). Quantifications were done in the CA1 and CA3 region of the hippocampus as well as cortex regions adjacent to these hippocampal regions (C-J). Percent area of neuropil containing axon terminals and dendrites were quantified from coronal plane brain sections. All values are expressed as ± SEM; n=6 for vehicle and n= 5 for drug treated PSAPP mice group; n=5 for each WT mice group.

Figure 11 (continued).



References

Adamec, R., Fougere, D., & Risbrough, V. (2010). CRF receptor blockade prevents initiation and consolidation of stress effects on affect in the predator stress model of PTSD. *Int J Neuropsychopharmacol*, *13*(6), 747–757. https://doi.org/10.1017/S1461145709990496.CRF

Alzheimer's Association(2009). 10 Early Signs and Symptoms of Alzheimer's. <https://www.alz.org/alzheimers-dementia/10_signs>. [accessed 2019 Jan 27]

Baglietto-Vargas, D., Chen, Y., Suh, D., Ager, R. R., Rodriguez-Ortiz, C. J., Medeiros, R., Myczek, K., Green, K. N., Baram, T. Z., LaFerla, F. M. (2015). Short-term modern life-like stress exacerbates Aβ-pathology and synapse loss in 3xTg-AD mice. Journal of Neurochemistry, 134(5), 915–926. https://doi.org/10.1111/jnc.13195

Bejanin, A., Schonhaut, D. R., La Joie, R., Kramer, J. H., Baker, S. L., Sosa, N., Ayakta, N., Cantwell, A., Janabi, M., Lauriola, M., O'Neil, J. P., Gorno-Tempini, M. L., Miller, Z. A., Rosen, H. J., Miller, B. L., Jagust, W. J., Rabinovici, G. D. (2017). Tau pathology and neurodegeneration contribute to cognitive impairment in Alzheimer's disease. Brain, 140(12), 3286–3300. https://doi.org/10.1093/brain/awx243

Bisht, K., Sharma, K., & Tremblay, M. È. (2018). Chronic stress as a risk factor for Alzheimer's disease: Roles of microglia-mediated synaptic remodeling, inflammation, and oxidative stress. Neurobiology of Stress, 9(May), 9–21. https://doi.org/10.1016/j.ynstr.2018.05.003

Calcia, M. A., Bonsall, D. R., Bloomfield, P. S., Selvaraj, S., Barichello, T., & Howes, O. D. (2016). Stress and neuroinflammation: A systematic review of the effects of stress on microglia and the implications for mental illness. *Psychopharmacology*, *233*(9), 1637–1650. https://doi.org/10.1007/s00213-016-4218-9

Campbell, S. N., Zhang, C., Roe, A. D., Lee, N., Lao, K. U., Monte, L., Donohuea, M.C., & Rissman, R.A. Jolla, L. (2015). Impact of CRFR1 Ablation on Amyloid-β Production and Accumulation in a Mouse Model of Alzheimer's Disease Shannon. *J Alzheimers Dis*, *45*(4), 1175–1184. https://doi.org/10.3233/JAD-142844.Impact

Chen, C., Wilcoxen, K. M., Huang, C. Q., Xie, Y. F., McCarthy, J. R., Webb, T. R., Zhu, Y.F., Saunders, J., Liu, X.J., Chen, T.K, Bozigian, H., Grigoriadis, D. E. (2004). Design of 2,5-dimethyl-3-(6-dimethyl-4-methylpyridin-3-yl)-7-dipropylamino- pyrazolo[1,5-a]pyrimidine (NBI 30775/R121919) and structure-activity relationships of a series of potent and orally active corticotropin-releasing factor receptor antagonists. Journal of Medicinal Chemistry, 47(19), 4787–4798. https://doi.org/10.1021/jm040058e

Carmen, S. (2013). Stress and cognition. WIREs Cognitive Science, 4(June), 245–261. https://doi.org/10.1002/wcs.1222

Dong, H., & Csernansky, G. J. (2010). Effects of Stress and Stress Hormones on Amyloid-β Protein and Plaque Deposition Hongxin. Psychiatry: Interpersonal and Biological Processes, 18(2), 459–469. https://doi.org/10.3233/JAD-2009-1152.Effects

Dong, H., Keegan, J. M., Hong, E., Gllardo, C., Montalvo-Ortiz, J., Wang, B., Rice, K., Csernansky, J. (2018). Corticotrophin releasing factor receptor 1antagonists prevent chronic stress-induced behavioral changes and synapse loss in aged rats. Psychoneuroendocrinology, 90, 92–101. https://doi.org/doi:10.1016/j.psyneuen.2018.02.013

Dautzenberg, F. M., & Hauger, R. L. (2002). The CRF peptide family and their receptors: Yet more partners discovered. *Trends in Pharmacological Sciences*, *23*(2), 71–77. https://doi.org/10.1016/S0165-6147(02)01946-6

Golde, T. E., Eckman, C. B., & Younkin, S. G. (2000). Biochemical detection of Aβ isoforms: implications for pathogenesis, diagnosis, and treatment of Alzheimer's disease. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1502*(1), 172–187. https://doi.org/10.1016/S0925-4439(00)00043-0

Mendiola-Precoma, J., Berumen, L. C., Padilla, K., & Garcia-Alcocer, G. (2016). Therapies for Prevention and Treatment of Alzheimer's Disease. *BioMed Research International*, *2016*(2), 1–17. https://doi.org/10.1155/2016/2589276

Mortimer, J. A., Van Duijn, C. M., Chandra, V., Fratiglioni, L., Graves, A. B., Heyman, A., Jorm, A. F., Kokmen, E., Kondo, K., Rocca, W. A. (1991). Head trauma as a risk factor for Alzheimer's disease: a collaborative re-analysis of case-control studies. EURODEM Risk Factors Research Group. *International Journal of Epidemiology*, *20 Suppl 2*(2), S28--35. Retrieved from http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=med3&NEWS=N&AN=1833351

Hauger, R. L., Risbrough, V., Oakley, R. H., Olivares-Reyes, J. A., & Dautzenberg, F. M. (2009). Role of CRF Receptor Signaling in Stress Vulnerability, Anxiety, and Depression. *Ann N Y Acad Sci*, *1179*(1), 120–143. https://doi.org/doi:10.1111/j.1749-6632.2009.05011.x. Role

Hardy, J., & Selkoe, D. J. (2002). the Amyloid Hypothesis of Alzheimer ' S Disease : Progress and Problems on the. *Science*, *297*(July), 353–356.

Hollensteina, K., de Graafb, C., Bortolato, A., Wang, M.-W., Marshall, F. H., & Stevens, R. C. (2014). Insights into the structure of class B GPCRs. Trends in Pharmacological Sciences, 35(1), 12–22. https://doi.org/10.1016/j.tips.2013.11.001

Jankowsky, J. L., Slunt, H. H., Ratovitski, T., Jenkins, N. A., Copeland, N. G., & Borchelt, D. R. (2001). Co-expression of multiple transgenes in mouse CNS: A comparison of strategies. Biomolecular Engineering, 17(6), 157–165. https://doi.org/10.1016/S1389-0344(01)00067-3

Justice, N. J., Huang, L., Tian, J.-B., Cole, A., Pruski, M., Hunt, A. J., Flores, R., Zhu, M.X., Arenkiel, B.R, & Zheng, H. (2015). Posttraumatic Stress Disorder-Like Induction Elevates -Amyloid Levels, Which Directly Activates Corticotropin-Releasing Factor Neurons to Exacerbate Stress Responses. *Journal of Neuroscience*, 35(6), 2612–2623. https://doi.org/10.1523/JNEUROSCI.3333-14.2015

Justice, N. J. (2018). The relationship between stress and Alzheimer's disease. Neurobiology of Stress, 8(January), 127–133. https://doi.org/10.1016/j.ynstr.2018.04.002

Jutkiewicz, E. M., Wood, S. K., Houshyar, H., Hsin, L. W., Rice, K. C., & Woods, J. H. (2005). The effects of CRF antagonists, antalarmin, CP154,526, LWH234, and R121919, in the forced swim test and on swim-induced increases in adrenocorticotropin in rats. Psychopharmacology, 180(2), 215–223. https://doi.org/10.1007/s00213-005-2164-z

Kametani, F., & Hasegawa, M. (2018). Reconsideration of amyloid hypothesis and tau hypothesis in Alzheimer's disease. *Frontiers in Neuroscience*, *12*(JAN). https://doi.org/10.3389/fnins.2018.00025

Kim, H. K., Nunes, P. V., Oliveira, K. C., Young, L. T., & Lafer, B. (2016). Neuropathological relationship between major depression and dementia: A hypothetical model and review. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *67*, 51–57. https://doi.org/10.1016/j.pnpbp.2016.01.008

Kirkwood, C. M., Ciuchta, J., Ikonomovic, M. D., Fish, K. N., Abrahamson, E. E., Murray, P. S., Klunk, W.E., Sweet, R. A. (2013). Dendritic spine density, morphology, and fibrillar actin content surrounding amyloid-β plaques in a mouse model of amyloid-β deposition. *Journal of Neuropathology and Experimental Neurology*, *72*(8), 791–800. https://doi.org/10.1097/NEN.0b013e31829ecc89

Klevanski, M., Herrmann, U., Weyer, S. W., Fol, R., Cartier, N., Wolfer, X. P., Caldwell, J. H., Korte, M., Müller, U. C. (2015). The APP intracellular domain is required for normalsynaptic morphology, synaptic plasticity, and hippocampus-dependent behavior. *Journal of Neuroscience*, *35*(49), 16018–16033. https://doi.org/10.1523/JNEUROSCI.2009-15.2015

Masters, C. L., Bateman, R., Blennow, K., Rowe, C. C., Sperling, R. A., & Cummings, J. L. (2004). Alzheimer's Disease. *Nature Reviews Disease Primers*, *54*(13), 1461–1462. https://doi.org/10.1038/nrdp.2015.56

Okamoto, K., Nagai T, Miyawaki, A., Hayashi, Y. (2004). Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity. *Nature Neuroscience*, 7(10), 1104-1112. https://doi.org/10.1038/nn1311

Qureshi, S. U., Kimbrell, T., Pyne, J. M., Magruder, K. M., Hudson, T. J., Petersen, N. J., Yu, H., Schulz, P. E., Kunik, M. E. (2010). Greater Prevalence and Incidence of Dementia in

Older Veterans with Posttraumatic Stress Disorder. *Journal of the American Geriatrics Society*, *58*(9), 1627–1633. https://doi.org/10.1111/j.1532-5415.2010.02977.x

Rissman, R. A., Lee, K.-F., Vale, W., & Sawchenko, P. E. (2007). Corticotropin-Releasing Factor Receptors Differentially Regulate Stress-Induced Tau Phosphorylation. *Journal of Neuroscience*, *27*(24), 6552–6562. https://doi.org/10.1523/JNEUROSCI.5173-06.2007

Selkoe, D. J. (2001). Alzheimer ' s Disease : Genes, Proteins, and Therapy. *Physiological Reviews*, *81*(2), 741–767. https://doi.org/10.1186/s13195-018-0373-z

Tan, J., & Gleeson, P. (2019). The role of membrane trafficking in the processing of amyloid precursor protein and production of amyloid peptides in Alzheimer's disease. *Alzheimer's Disease*. doi:https://doi.org/10.1016/j.bbamem.2018.11.013

Takahashi, M., Miyata, H., Kametani, F., Nonaka, T., Akiyama, H., Hisanaga, S. ichi, & Hasegawa, M. (2015). Extracellular association of APP and tau fibrils induces intracellular aggregate formation of tau. Acta Neuropathologica, 129(6), 895–907. https://doi.org/10.1007/s00401-015-1415-2

Wang, J., Gu, B. J., Masters, C. L., & Wang, Y.-J. (2017). A systemic view of Alzheimer disease insights from amyloid-β metabolism beyond the brain. *Nature Reviews Neurology*, 13(10), 612–623. https://doi.org/10.1038/nrneurol.2017.111

Zhang, C., Kuo, C. C., Moghadam, S. H., Monte, L., Campbell, S. N., Rice, K. C., Sawchenko, P.E., Masliah, E., & Rissman, R. A. (2016). Corticotropin-releasing factor receptor-1 antagonism mitigates beta amyloid pathology and cognitive and synaptic deficits in a mouse model of Alzheimer's disease. *Alzheimer's and Dementia*. https://doi.org/10.1016/j.jalz.2015.09.007