UNIVERSITY OF CALIFORNIA, SAN DIEGO

Impact of Stress Exposure and Modulation of Stress Pathways on Alzheimer's Neuropathology

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

in

Biology

by

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The thesis of Kevin Nguyen is approved and it is acceptable in quality and form for publication on microfilm and electronically:

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Co-Chair

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DEDICATION

This thesis is dedicate to my family.

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I cannot thank Dr. Robert Rissman enough for allowing me to start on this research project as soon as I joined the lab. As a transfer student from community college, I struggled to obtain lab opportunities when first starting at UCSD due to my lack of experience. Despite that, Dr. Rissman gave me the opportunity to learn what it was like to be a part of a novel research project. Through his sincere guidance and mentorship, I had such a positive educational experience that I will take with me wherever I go in my future academic pursuits. I am also grateful for Kimberley Mullen for her positive attitude and helping me with scheduling meetings and my thesis defense. This whole process would not have been so smooth if it was not for her.

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ABSTRACT OF THE THESIS

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By

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Professor Robert Rissman, Chair

Professor Alissa Huffaker, Co-Chair

Alzheimer's Disease (AD) is the most common form of dementia. Chronic stress has been suggested to influence the progression of AD. Moreover, corticotropin-releasing factor (CRF) signaling in AD has been shown to be upregulated by stress and influence AD pathology such as amyloid-beta (Aβ) accumulation. In rodent models, similar AD-related pathology and

cognitive impairments are produced when exposed to either stress or exogenous CRF. Previous studies have shown that PSAPP (AD) mice treated with CRFR1 antagonist (R121919) resulted in reduced Aβ accumulation and improvements in behavioral tests. However, the influence of stress while underdoing drug treatment is unknown. In this comparison study, we subjected cohorts of AD mice to R121919 in the presence and absence of restraint stress treatment. AD mice were aged to 6 and 12 months to investigate the pre-clinical and mature timepoints of AD pathology. Daily drug/vehicle treatment began at 2 months of age. Stress for the 6 and 12 month groups started at 2 and 6 months, respectively. Overall, R121919 treated AD mice resulted in a significant reduction of Aβ levels. Additionally, it was shown that chronic restraint stress exacerbated Aβ levels. This study suggests that CRFR1 antagonist treatment provides a potential preventative and disease-modifying therapy for populations subjugated to stress and at risk for AD development. Our data provides an important relationship between stress signaling in the CNS and the neuropathology of AD.

INTRODUCTION

Dementia is characterized as a syndrome that causes severe cognitive decline for affected patients; this impediment especially impacts the elderly population (World Health Organization, 2017). Alzheimer's disease (AD), the most common form of dementia, progressively leads to permanent neurodegeneration, which impairs normal aspects of human cognition, memory, and behavior. AD's early signs of pathology presents itself through impairments in working and explicit memory (Jahn, 2013). The later signs which characterize neural atrophy include the diminishing of normal language production and comprehension, executive functions, and visuospatial awareness (Grady et al., 1988). As a result, AD patients slowly and permanently lose their memories while experiencing confusion about their roles and responsibilities in life.

The deleterious effects of AD manifest at the individual level but caregivers, family members, and loved ones may take a toll as well. AD is also increasingly becoming a major concern at the local, state, and national level (CDC, 2011). In fact, its prevalence in the United States is estimated to rise from 5 million adults in 2014 to 13.9 million in 2060 for individuals' ≥ 65-year-olds (Matthews et al., 2019). A smaller amount of AD cases arise from genetic predisposition while approximately 90% occur through sporadic development and affects those within this age group (Bekris et al., 2010). There is an alarming economic burden for caring for individuals with AD in long-term care facilities; the cost of hospice care for AD patients was $236 billion in 2016, an amount that is projected to increase to $1 trillion by 2050 (Alzheimer's Association, 2016).

To date, all clinical efforts toward generating safe and effective disease-modifying treatments that reduce or reverse the trajectory of AD pathology has ultimately been unsuccessful. Between 2002 through 2012, the attrition rate for failing AD treatments was an

alarming 72% in Phase 1, 92% in Phase 2, and 98% in Phase 3 clinical trials (Cummings et al, 2014). Thus, currently approved therapies on the market only treat symptoms of AD. Cholinesterase inhibitors are intended for treating mild-to-moderate AD while N-methyl-D-aspartate (NMDA) inhibitors is a therapeutic option for moderate-to-severe cases (Yiannopoulou and Papageorgiou, 2012). Still, these and other symptomatic treatments only provide mild relief. The main issues with many of the current AD drug candidates toward treating or reversing the deleterious effects of AD is related to failure towards demonstrating translatability in AD populations. The inability to demonstrate the positive effects of tested compounds between animal models and actual human subjects themselves is a major barrier to finding an effective treatment. Still, there is hope, a pharmacological intervention called Aducanumab produced by Biogen and Eisai is currently being considered by the Food and Drug Administration (FDA) for approval to treating AD pathology and cognitive decline.

Even long-term use with currently tested treatments have raised safety concerns of toxicity in patients. Although cholinesterase inhibitors have been approved to treat AD symptoms, long-term use has been shown to produce adverse effects like urinary incontinence and an increased risk of bradycardia that will eventually result in hospitalization (Gill et al., 2005; Hernandez et al., 2009). Semegacestat, a gamma (γ)-secretase inhibitor, was a potential disease modifying treatment that was terminated during its phase 3 clinical trials after long-term use resulted in patients experiencing rapid weight loss, skin cancer, and weakening immune systems (Doody et al., 2013). Non-pharmaceutical treatments for AD mostly rely on theory-based approaches to improve individual's overall cognition. Among the many well-studied interventions, randomized control trials have shown that Cognitive Stimulation Training (CST) achieves the most significant improvements in quality of life and cognition for populations with

AD (Ballard et al, 2011). Furthermore, the proper timing to implement treatment is a major challenge to consider. A majority of tested drug candidates targets pathology that is observed at the later stages of AD when individuals are most severely impacted. This problem makes any chance of treating and reversing AD ineffective. Compounds produced to address earlier pathology in the prodromal stage of AD would perhaps have greater efficacy in slowing neurodegeneration.

Two hallmarks of familial and sporadic AD pathology are neurofibrillary tangles (NFTs) and senile plaques composed of amyloid-beta (Aβ) peptides. Although familial and inherited forms of AD is relatively less prevalent, clinically-typical cases are linked to several genes. Missense mutations in the Amyloid Precursor Protein (APP) gene as well as overexpression of this gene due to duplication or translocation of chromosome 21, which is often observed with Down Syndrome, may predispose individuals to develop AD at an early age (Selkoe, 2001). Other notable gene modifications include missense mutations in the Presenilin 1 (PSEN1) and 2 (PSEN2) genes on chromosomes 14 and 1, respectively, and polymorphism in the Apolipoprotein E (APOE) gene, which presents a major genetic risk factor for developing sporadic AD (Selkoe, 2001).

APP is an integral membrane protein that is cleaved to form Aβ. While APP processing undergoes two major pathways of cleavage, represented in figure 1 below, only one contributes to Aβ accumulation. APP processing that forms Aβ includes the proteases beta (β)-secretase and γ-secretase, which occurs in the amyloidogenic pathway. The nonamyloidogenic pathway is initiated through alpha (α)-secretase and subsequent γ-secretase cleavage (Moehlmann et al., 2002). Both pathways form APP fragments; however, the amyloidogenic pathway gives rise to various size species of Aβ including the two commonly known toxic forms Aβ40 and Aβ42.

(Tan and Gleeson, 2019). Aβ42 is relatively more toxic and has a greater likelihood of aggregating than its Aβ40 counterpart (Wang et al., 2017).

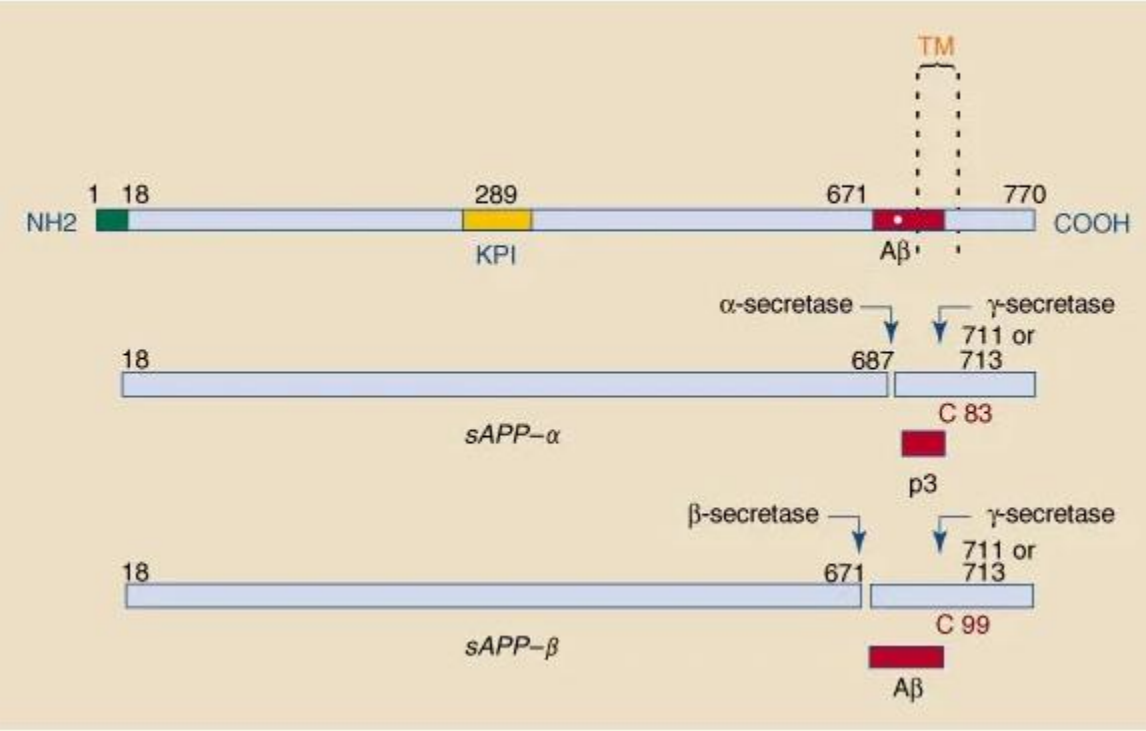

Figure 1. Amyloid Precursor Protein (APP) processing pathways and products of cleavage (Adapted from Selkoe, 1999).

Under normal physiological conditions, Aβ is generated and cleared throughout the brain (Savage, et al., 1998). Aβ is degraded through proteases like neprilysin (NEP), endothelin-converting enzyme, and insulin-degrading enzyme (IDE) without plaque accumulation (B. Funalot, et al., 2004). In pathologically aging brains, issues with Aβ turnover and simultaneous build up results in Aβ accumulation that leads to neurodegeneration and cognitive defects (Wang et al., 1999). NFTs are made of abnormally phosphorylated tau, a microtubule-associated protein (MAP) that stabilize microtubules important in maintaining neuron integrity. (Wang et al., 2017). The mechanism of NFT formation is not fully understood, however, some researchers theorize that under the *Amyloid Cascade Hypothesis*, Aβ accumulation triggers downstream tau

phosphorylation that leads to NFTs (Hardy and Selkoe, 2002). Together, Aβ plaques and NFTs disrupt neural networks and compromise the fragile framework of the human brain.

Research on environmental risk factors to AD also points to psychological stress as a major contributor to the sporadic nature of AD pathogenesis (Zawia and Basha, 2005). There is a strong association between psychological stress and risk of AD development (Wilson et al., 2003). The adverse effects of stress and anxiety on behavior and cognition are linked to the role of the corticotropin-releasing factor (CRF) system and its relationship with hypothalamic-pituitary-adrenal (HPA) signaling. Stress induces HPA signaling through secretion of the CRF peptide; stress-induced CRF activation exacerbating AD pathology is observed widely in animal models (Hostetler and Ryabinin, 2013).

CRF signaling plays a significant role in the physiological response to stress adaptation (Rissman et al., 2007). The CRF system includes the CRF ligand and G-Protein Coupled Receptors (GPCR) CRF Receptor 1 (CRFR1) and CRF Receptor 2 (CRFR2), however, experiments on repeated psychological stress point to the dependence of CRFR1 on AD pathology (Rissman et al., 2012). CRFR1 is expressed widely in AD-relevant areas like the hippocampus and cerebral cortex. Prolonged CRF signaling seen in chronically stressed animal models show increases in AD development. Likewise, CRF overexpression in AD mice models accelerated Aβ deposition and subsequent behavioral defects (Dong et al., 2012). Since early AD pathologic signs of Aβ is observed within the hippocampus and cerebral cortex, the CRF system is a plausible target for pharmacological intervention.

In rodent models, similar AD-related pathology and cognitive impairments are produced when exposed to either stress or exogenous CRF. Previous studies in our lab have shown that AD mice treated with CRFR1 antagonist (R121919) resulted in a significant reduction of Aβ

accumulation and improvements in behavioral tests (Zhang et al, 2016). Indeed, R121919 may prove to have high efficacy in decreasing Aβ, however, the influence of stress while undergoing this drug treatment is unknown. Building off of previous studies, we hypothesize that AD models undergoing R121919 treatment while exposed to chronic stress will yield significantly less AD pathology than stress-induced mice given placebo. This study postulates that CRFR1 antagonism may serve as a potential disease-modifying treatment for chronically stressed and aging populations susceptible to developing AD.

MATERIALS AND METHODS

Mice Models

Double transgenic AD mice (PSAPP): (B6C3-Tg [APPswe, PSEN1dE9] 85Dbo/Mmjax, (stock no: 34829-JAX). PSAPP mice was maintained as a hemizygote by crossing transgenic mice to a C57BL/6J mouse. Non-transgenic littermates were used as controls. Mice were aged to 6 or 12 months of age before euthanasian. The mice vivarium was maintained in constant temperature-controlled conditions. Each cage contained either 2-5 PSAPP or wild-type (mice rotating in a 12:12 hour light/dark cycles with water and food provided.

In-Vivo Pharmacology Administration and Treatment

CRFR1 antagonist, R121919 was dissolved in 0.01 M tartaric acid and 5% v/v polyethoxylated castor oil. The vehicle solution, 0.01 M tartaric acid with 5% castor oil, served as a control. The pH of the vehicle or R12191 was at pH 3. Drug/Vehicle were randomly assigned to each cohort of mice.

PSAPP and WT mice received subcutaneous 10 mg/kg/d injections with either R121919 or vehicle starting at 2 months of age until euthanized.

Stress

Stress was initiated at least one hour post injection of R121919 or vehicle. PSAPP and WT mice, 6 and 12 month groups, were subjected to restraint stress for 30 minutes on a biweekly schedule beginning at 2 and 6 months, respectively.

Sample Collection

Anesthesia with isoflurane was administered prior to sacrificing mice via decapitation. Trunk blood was collected and centrifuged at 6000 rpm for 6 min to isolate plasma and pellet, which was stored in -80°C freezer. Brains were removed and the right hemisphere cortex and hippocampus were dissected and flash frozen for biochemical assays. The left hemisphere was drop fixed into 4% paraformaldehyde for 48 hours then transferred to 30% sucrose for 24 hours and was used for immunohistochemistry.

Protein Extraction

Cortex brain samples were homogenized and sonicated in PBS buffer with protease inhibitors and centrifuged at 40,000G. The PBS soluble fraction was collected. The pellet was sonicated with RIPA buffer and centrifuged at 40,000G, and the RIPA soluble fraction was collected. The pellet was frozen and saved for future use. Analysis of protein concentration was done using a BCA assay.

Western Blot

82E1 primary antibody (IBL, Cat# 10323) was used to probe for N-terminal human Aβ. β-actin (Sigma-Aldrich, MDL# MFCD00145889) served as a loading control. Biorad 10% Criterion gels were used to separate the proteins via electrophoresis. Gels ran for 3 hours at 80 volts. The gels were then transferred into a PVDF membrane at 200mA for 1 hour. The blots were then blocked with 5% non-fat milk in TBS-0.1% Tween. Secondary antibody for 82E1 and β-actin were mouse and rabbit HRP, respectively.

Aβ Peptide Quantification and Analysis

The western blot bands were measured and quantified using ImageJ software. PRISM software was used to produce graphs while an ANOVA test was utilized to identify significant differences between control and experimental groups.

RESULTS

Analysis and Quantification of Aβ Levels

To determine the efficacy of R121919 on 6-month and 12-month AD mice groups, biochemical assays were used to measure levels of Aβ monomer and oligomer species by probing with a N-terminal-specific anti-human Aβ monoclonal antibody (82E1). The impact of restraint stress on the formation of Aβ was also assessed. Aβ levels were analyzed via Western blot analysis. Aβ monomer and oligomer species were quantified and normalized to β-actin, a loading control.

Probing with 82E1 revealed two prominent bands at ~20 kDa (oligomer Aβ) and ~8kDa (monomeric Aβ). Figure 2 below shows that soluble (PBS) protein samples of the cerebral cortex in 6-month AD mice showed significantly ($p \leq 0.001$) higher levels of oligomeric Aβ in the vehicle-treated group compared to those undergoing R121919 treatment. Furthermore, there was observed to be a trend towards reduction of monomeric Aβ in the non-stressed, drug group. The effect of stress and R121919 treatment was negligible between stressed and non-stressed cohorts. Still, there was trending towards an increase observed in the stressed, monomeric Aβ groups.

Figure 3 represents 12-month soluble samples from the cerebral cortex which demonstrated significantly ($p \leq 0.05$) greater Aβ oligomers in the vehicle-treated, stressed cohort than the non-stressed, vehicle-treated group. The stressed, R121919 group, however, was not significantly different from both non-stressed groups. Furthermore, Aβ monomer production was significantly greater in the R121919-treated, stressed group than any other cohort.

Detergent-soluble (RIPA) protein samples of 6-month cortex, shown in figure 4 revealed no significant differences in oligomeric Aβ. However, stressed AD mice resulted in significantly

($p \leq 0.01$) higher levels of monomeric Aβ than non-stressed AD mice that were also undergoing the vehicle treatment. R121919 treatment seemed to significantly ($p \leq 0.01$) reduce monomeric Aβ in the stressed group.

Lastly, in the 12-month cortex detergent-soluble fractions shown in figure 5, the stressed, vehicle-treated group had significantly ($p \leq 0.001$) greater Aβ oligomer levels than the non-stressed, vehicle group. The stressed, R121919-treated group did not show major differences compared to either of the non-stressed groups. Also, no significant differences were observed for monomeric Aβ in either group.

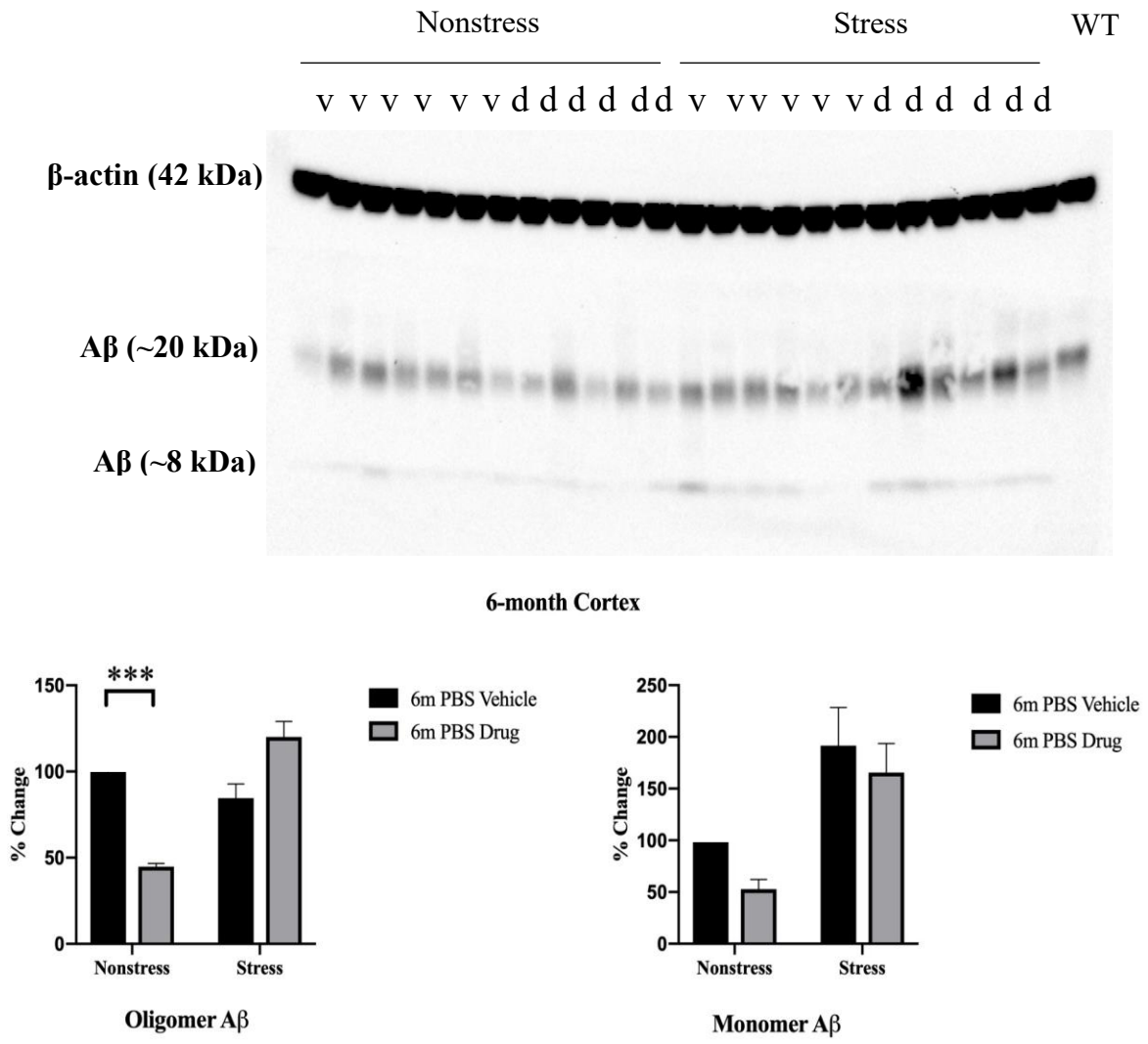

Figure 2. Effects of drug treatment and stress on soluble fractions of 6-month cortex. The cerebral cortex from 6-month AD mice (drug vs vehicle) was homogenized and sonicated in PBS buffer and separated using a 10% Criterion gel. The gel was then transferred into a membrane. Aβ was detected with primary antibody 82E1. β-actin served as a loading control. *p<0.05, **p<0.01, ***p<0.001. All values are expressed as mean ± SEM, n = 6 AD mice/group. v = vehicle; d = drug.

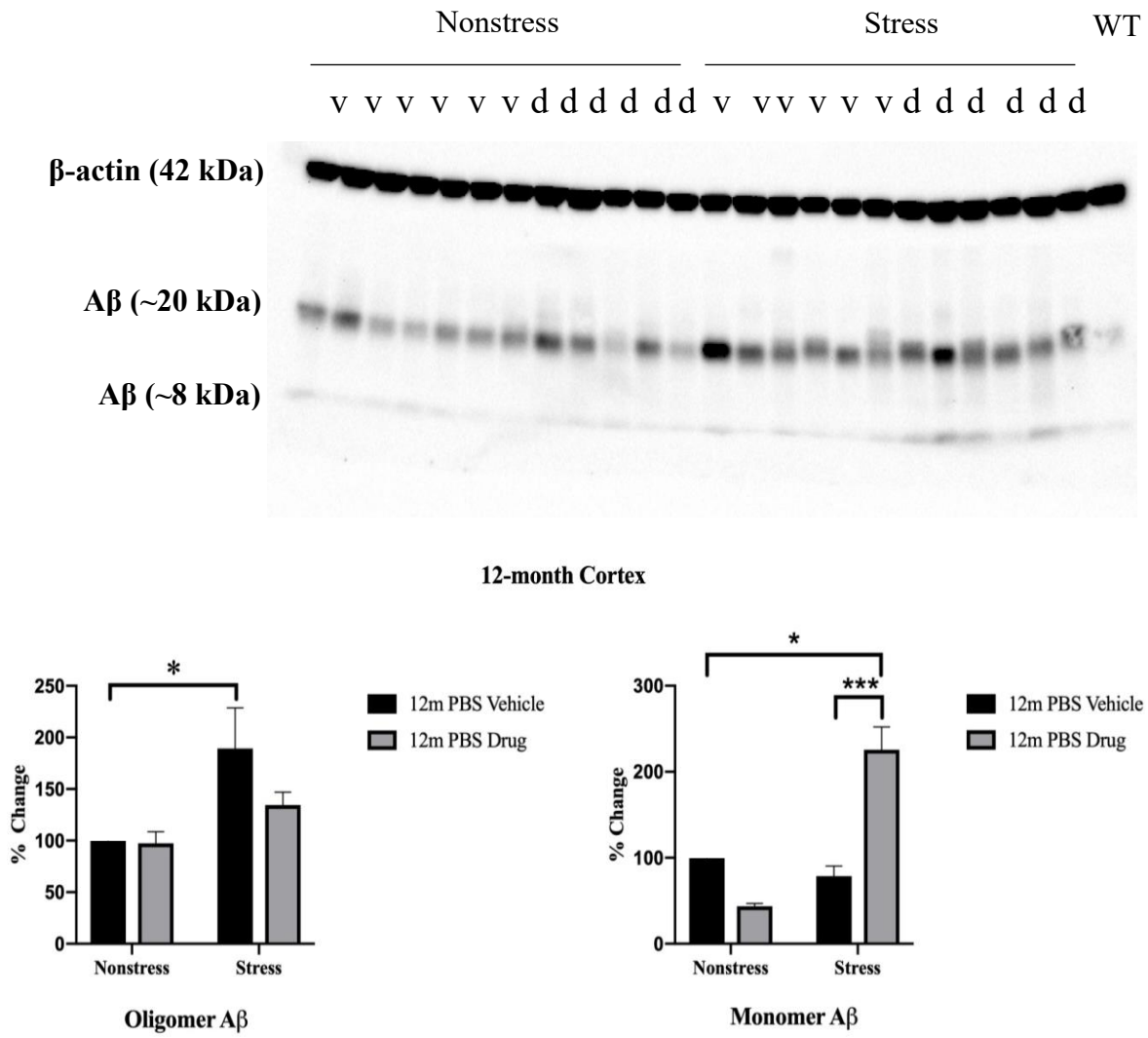

Figure 3. Effects of drug treatment and stress on soluble fractions of 12-month cortex. The cerebral cortex from 12-month AD mice (drug vs vehicle) was homogenized and sonicated in PBS buffer and separated using a 10% Criterion gel. The gel was then transferred into a membrane. Aβ was detected with primary antibody 82E1. β-actin served as a loading control. *p<0.05, **p<0.01, ***p<0.001. All values are expressed as mean ± SEM, n = 6 AD mice/group. v = vehicle; d = drug.

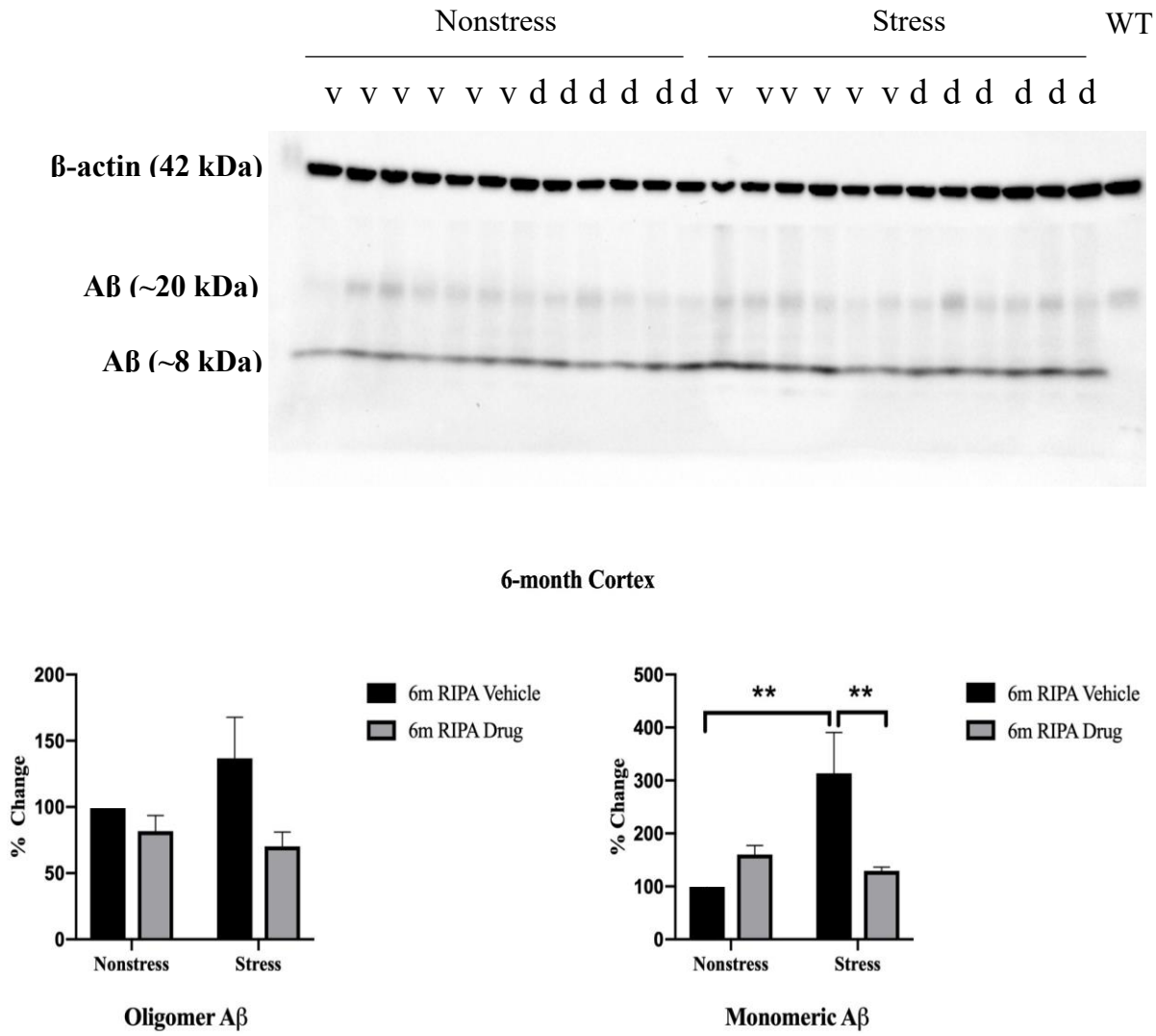

Figure 4. Effects of drug treatment and stress on detergent-soluble fractions of 6-month cortex. The cerebral cortex from 6-month AD mice (drug vs vehicle) was homogenized and sonicated in RIPA buffer and separated using a 10% Criterion gel. The gel was then transferred into a membrane. Aβ was detected with primary antibody 82E1. β-actin served as a loading control. *p<0.05, **p<0.01, ***p<0.001. All values are expressed as mean ± SEM, n = 6 AD mice/group. v = vehicle; d = drug.

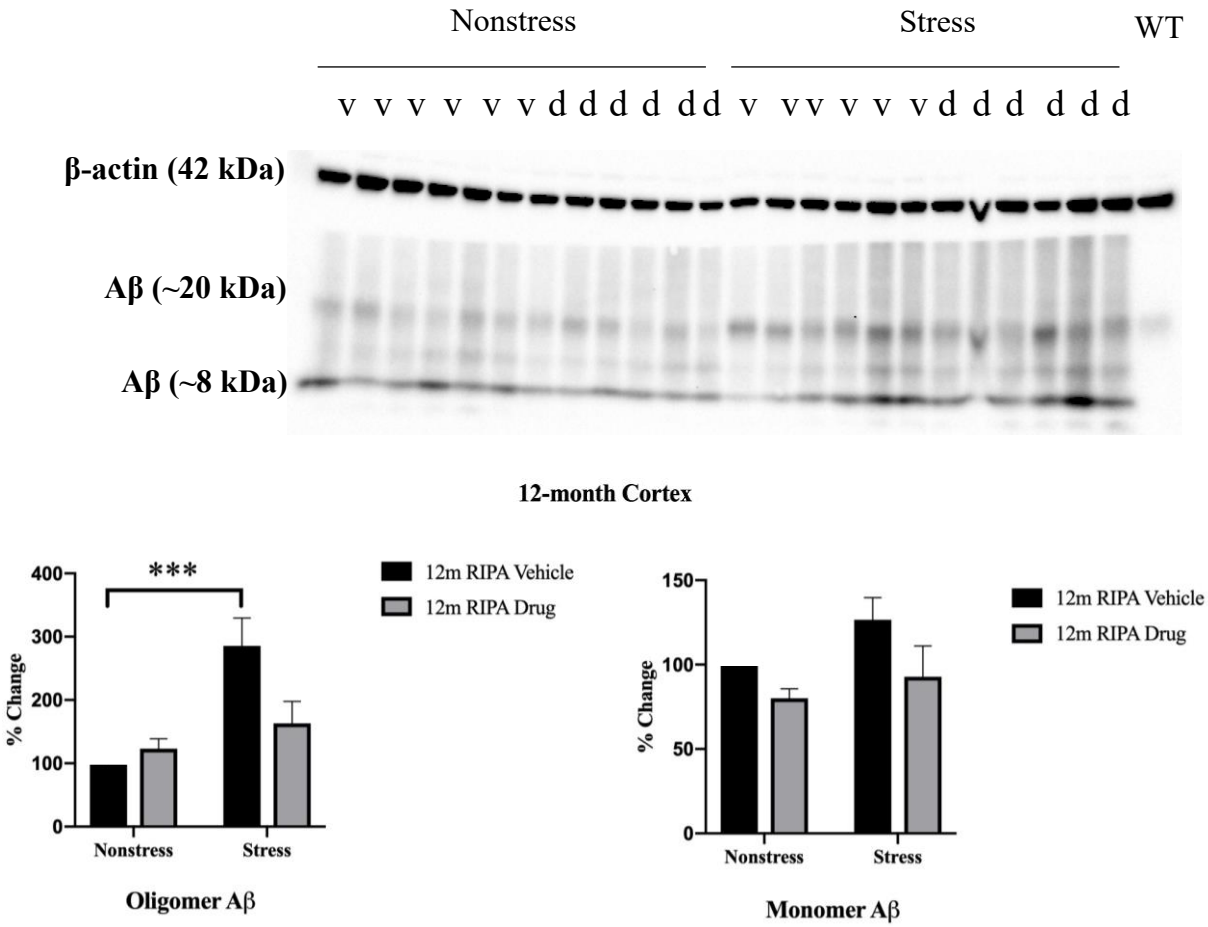

Figure 5. Effects of drug treatment and stress on detergent-soluble fractions of 12-month cortex. The cerebral cortex from 12-month AD mice (drug vs vehicle) was homogenized and sonicated in RIPA buffer and separated using a 10% Criterion gel. The gel was then transferred into a membrane. Aβ was detected with primary antibody 82E1. β-actin served as a loading control. *p<0.05, **p<0.01, ***p<0.001. All values are expressed as mean ± SEM, n = 6 AD mice/group. v = vehicle; d = drug.

DISCUSSION

The alarming projected rise in cases of AD demonstrates the need for safe and effective disease-modifying interventions. While the impact of chronic stress is associated with an increased susceptibility to developing AD, some demographics especially aging veterans who suffer from post-traumatic stress disorder (PTSD) are certainly more at risk than others.

Within the field of AD research, this study was the first to assess the efficacy of CRFR1 antagonist, R121919, as a potential AD therapy for populations susceptible to chronic stress. Our biochemical assays revealed two notable Aβ species with molecular weights at about 20 kDa and 8 kDa. Aβ at around 20 kDa may represent oligomer species while monomeric Aβ would perhaps be represented by the lower band at around 8 kDa in figures 2-5. Soluble protein fractions may comprise of free-floating Aβ whereas the detergent-soluble fractions may contain more commonly aggregated forms.

To examine two time points in the development of AD, there was breeding of 6-month (pre-clinical AD stage) and 12-month (mature AD stage) cohorts. Compared to the 6-month groups, oligomeric Aβ production was greater in the soluble and detergent-soluble fractions of 12-month groups. However, monomeric Aβ was greatest in the detergent-soluble fractions. These findings were reasonable as AD mice in the mature stages of neurodegeneration are expected to accumulate higher levels of Aβ due to the later progression of the disease. In addition, the detergent-soluble fractions may be able to dissolve greater levels of monomeric Aβ that have been aggregated due to plaque build-up.

R121919 was effective at reducing Aβ in the pre-clinical AD mice, which is consistent with previous findings (Zhang et al, 2016). Still, R121919-treated 12-month groups were trending toward a reduction of Aβ levels in oligomeric and monomeric species of mature AD

models, thus, efficacy of R121919 in mature AD mice remains uncertain. Moreover, monomeric Aβ levels of mature AD mice in the detergent-soluble fractions were not substantially different between the groups that received vehicle or drug. Perhaps, R121919 is most effective at reducing larger Aβ species in the prodromal stage of AD. Aggregated forms of Aβ oligomers has been shown to exert neurotoxicity in AD (Nag et al, 2011). Aβ monomers, on the other hand, serves a neuroprotective role in pathogenesis (Zou et al, 2002). It is not clear how R121919 specifically impacts both the production of oligomeric and monomeric Aβ within this mice model.

The effects of stress was most prominent on the 12-month soluble and detergent-soluble fractions. Stress seemed to increase levels of oligomeric Aβ the greatest between these groups. In addition, it was promising to observe that the stressed-induced 6-month groups were mostly trending toward an increase in Aβ. Detergent-soluble, 6-month AD mice treated with vehicle achieved a significant increase in monomeric Aβ with stress. These findings suggests that chronic stress is associated with greater accumulation of aggregated and oligomeric Aβ in mature AD. This was expected as the oligomeric forms are more toxic.

The effectiveness of R121919 under chronic stress was most prominent in the 6-month detergent-soluble fractions. This suggests that CRFR1 antagonism through R121919 is most effective with reducing the more likely to aggregate forms of Aβ when AD is in its pre-clinical stage. Furthermore, an unexpected finding observed in the 12-month soluble fractions was that the stress-induced group also undergoing R121919 treatment achieved the greatest levels of monomeric Aβ compared to others. One explanation may be that R121919 helps to increase the production of soluble Aβ, leading to more efficient clearance. Still, this data remains inconclusive till further experimentation is done while also testing a larger sample size of AD mice. Overall, the effect of R121919 was distinct in different Aβ fractions.

It is important to note that implementing chronic restraint stress alone as the single contributor of stress to these AD mice throughout this study was quite difficult. These mice are subjected to stress during the handling process of vehicle or drug administration as well. Furthermore, since mice are nocturnal beings, experimentation that disrupts normal sleep-wake cycles may be another factor of added stress to consider. Certainly, limitations of this procedure is that stress comes in many forms, thus, testing restraint stress alone may not give sufficient insight to the efficacy of R121919 while undergoing stress. Alternatively, other forms of stress that may be tested on AD mice with R121919 include being in isolation, administering painful shocks, and chronic noise. Changing the diet of our rodent models may also give insight on how to synergize the positive effects of routine R121919 treatment.

Moving forward, the results obtained from measuring Aβ levels in the cortex will be compared with the histology and behavior data from the same mice to further confirm whether levels of oligomeric and/or monomeric Aβ are associated with plaque load in brain tissue, cognitive defects, and impairments in learning and working memory. Furthermore, our main reason for analyzing samples of cortex is that Aβ initially accumulates in this region first within these mice models before developing in other AD-relevant regions like the hippocampus. Atrophy of the hippocampus is associated with AD pathology (Woodard et al, 2010). Thus, we will perform Aβ protein analysis and quantification on our extracted hippocampus samples as well.

Though CRFR1 antagonism seems to possess therapeutic potential for treating AD, it will take time for these types of pharmaceutical interventions to be approved as a treatment. Unfortunately, many tested forms like R121919, Antalarmin, Pexacerfont, Verucerfont and others have shown clinical failure due to many reasons that include toxicity, lack of efficacy, and

the inability to show positive effects beyond AD animal models (Spierling and Zorrilla, 2017). Still, we hope to learn from the failures of the past to develop new CRFR1 antagonist drugs that show human compatibility. Moreover, we hope to advocate researchers to continue to explore this pathway as a means for one day having an effective treatment to halt the development of or reverse the deleterious effects of AD for our aging populations.

REFERENCES

1. World Health Organization. (2017). Global action plan on the public health response to dementia 2017-2025. *World Health Organization*, License: CC BY-NY-SA 3.0 IGO, 1-52.

2. Jahn, H. (2013). Memory loss in Alzheimer's disease. *Dialogues Clinical Neurosciences, 15*(4), 445-454.

3. Grady, C. L., Haxby, J. V., Horwitz, B., Sundaram, M., Berg, G., Schapiro, M., Rapoport, S. I. (1988). Longitudinal study of the early neuropsychological and cerebral metabolic changes in dementia of the Alzheimer type. *Journal of Clinical and Experimental Neuropsychology, 10*(5), 576-596.

4. National Center for Disease Control and Prevention. (2011). The CDC Healthy Brain Initiative: Progress 2006–2011. *National Center for Disease Control and Prevention,* 1-11.

5. Bekris, L. M., Yu, C. E., Bird, T. D., Tsuang, D. W. (2010). Genetics of Alzheimer Disease. *Journal of Geriatric Psychiatry and Neurology, 23*(4), 213–227.

6. Alzheimer's Association. (2016). Alzheimer's Association Report: 2016 Alzheimer's disease facts and figure. *Alzheimer's and Dementia,* 12, 459-509.

7. Matthews, K. A., Wei, X., Gagliotic, A. H., Holta, J. B., Crofta, Janet B., Mack, D., McGuirea, L. C. (2019). Racial and ethnic estimates of Alzheimer's disease and related dementias in the United States (2015–2060) in adults aged ≥ 65 years. *Alzheimer's and Dementia*, 15(1), 17-24.

8. 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.

9. Savage, M.J., Trusko, S. P., Howland, D. S., Pinsker, L. R., Mistretta, S., Reaume, A.G., Greenberg, B. D., Siman, R., Scott, R. W. (1998). Turnover of a-beta in mouse brain & acute reduction by phorbol ester. *Journal of Neuroscience,* 18(5), 1743-1752.

10. Wang, J., Dickson, D. W., Trojanowski, J.Q., Lee, V. M. (1999). The levels of soluble versus insoluble brain Abeta distinguish Alzheimer's disease from normal and pathologic aging. *Experimental Neurology*, 158(2), 328-337.

11. Hardy, J., Selkoe, D. J. (2002). The Amyloid Hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science*, 297 (5580), 353–356.

12. Moehlmann, T., Winkler, E., Xia, X., Edbauer, D., Murrell, J., Capell, A., Steiner, H., Kaether, C., Zheng, H., Ghetti, B., Hasss, C. (2002). Presenilin-1 mutations of leucine 166 equally affect the generation of the Notch and APP intracellular domains independent of

their effect on Aβ42 production. *Proceedings of the National Academy of Sciences of the United States*, 99(12), 8025-8030.

13. Tan, J. A., Gleeson, P. A. (2019) The Role of Membrane Trafficking in the Processing of Amyloid Precursor Protein and Production of Amyloid Peptides in Alzheimer's Disease. *Biochimica et Biophysica Acta (BBA) – Biomembranes*, 1861(4), 697-712.

14. 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.

15. Zawia, N. H., Basha, M. R. (2005). Environmental risk factors and the developmental basis for Alzheimer's disease. *Reviews in the Neurosciences*, 16(4), 325-337.

16. Wilson, R. S., Evans, D. A., Bienias, J. L., Mendes de Leon, C. F., Schneider, J. A., Bennett, D.A. (2003). Proneness to psychological distress is associated with risk of Alzheimer's disease. *Neurology*, 61(11), 1479-1485.

17. Hostetler, C. M., Ryabinin, A. E. (2013). The CRF system and social behavior: a review. *Frontiers of Neuroscience*.

18. Rissman, R. A., Lee, K. F., Vale, W., Sawchenko, P. E. (2007). Corticotropin-Releasing Factor Receptors Differentially Regulate Stress-Induced Tau Phosphorylation. *The Journal of Neuroscience*, 27(24), 6552-6562.

19. Rissman, R. A., Staupa, M. A., Lee, A. R., Justice, N. J., Rice, K. C., Vale, W., and Sawchenko, P. E. (2012). Corticotropin-releasing factor receptor-dependent effects of repeated stress on tau phosphorylation, solubility, and aggregation. *Proceedings of the National Academy of Sciences of the United States of America*, 109(16), 6277-6288.

20. Dong, H., Murphya, K. M., Menga, L., Montalvo-Ortiza, J., Zengb, Z., Kolber, B. J., Zhang, S., Mugliad, L. J., Csernanskya, J. G. (2012). Corticotrophin Releasing Factor Accelerates Neuropathology and Cognitive Decline in a Mouse Model of Alzheimer's Disease. *Journal of Alzheimer's Disease*, 28(3), 579-592.

21. 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). CRF receptor-1 antagonism mitigates Aβ pathology and cognitive and synaptic deficits in a mouse model of Alzheimer's disease. *Alzheimer's Dementia*, 12(5), 527-537.

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

23. Cummings, J. L., Morstorf, T., Zhong, K. (2014) Alzheimer's disease drug development pipeline: few candidates, frequent failures. *Alzheimer's Research and Therapy*, 6(37), 1-7.

24. Gill, S. S., Mamdani, M., Naglie, G., Streiner, D.L., Bronskill, S. E., Kopp, A., Shulman, K. I., Lee, P. E., Rochon, P. A. (2005). A prescribing cascade involving cholinesterase inhibitors and anticholinergic drugs. *Archives of internal medicine,* 165(7), 808-813.

25. Hernandez, R. K., Farwell, W., Cantor, M. D., Lawler, E. V. (2009) Cholinesterase inhibitors and incidence of bradycardia in patients with dementia in the veterans affairs new England healthcare system. *Journal of the American Geriatrics Society*, 57(11), 1997-2003.

26. Doody, R. S., Raman, R., Farlow, M., Iwatsubo, T., Vellas, B., Joffe, S., Kieburtz, K., He, F., Sun, X., Thomas, R. G., Aisen, P. S., Alzheimer's Disease Cooperative Study Steering Committee, Siemers, E., Sethuraman G., Mohs, R., Semagacestat Study Group. (2013). A phase 3 trial of semagacestat for treatment of Alzheimer's disease. *The New England journal of medicine,* 369(4), 341-350.

27. Ballard, C., Khan, Z., Clack, H., Corbett, A. (2011). Nonpharmacological treatment of Alzheimer disease. *Canadian journal of psychiatry*, 56(10), 589-595.

28. Selkoe, D. J. (2001) Alzheimer's Disease: Genes, Proteins, and Therapy. *Physiologic reviews, 81(2), 741-766.*

29. Nag, S., Sarkar, B., Bandyopadhyay, A., Sahoo, B., Screenivasan, V. K., Kombrabail, M., Muralidharan, C., Maiti, S. (2011). Nature of the amyloid-beta monomer and the monomer-oligomer equilibrium. *The Journal of biological chemistry,* 286(16), 13827-13833.

30. Zou, K., Gong, J. S., Yanagisawa, K., Michikawa, M. (2002). A novel function of monomeric amyloid beta-protein serving as an antioxidant molecule against metal-induced oxidative damage. *The Journal of neuroscience: the official journal of the Society of Neuroscience*, 22(12), 4833-4841.

31. Woodard, J. L., Seidenberg, M., Nielson, K. A., Smith, J. C., Antuono, P., Durgerian, S., Guidotto, L., Zhang, Q., Butts, A., Hantke, N., Lancaster, M., Rao, S. M. (2010). Prediction of cognitive decline in healthy adults using fMRI. *Journal of Alzheimer's disease*, 21(3), 871-885.

32. Spierling, S. R., Zorrilla, E. P. (2017). Don't stress about CRF: Assessing the translational failures of CRF1 antagonists. *Psychopharmacology (Berl)*, 234(9-10), 1467-1481.

33. Selkoe, D. J. (1999). Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature*, 399(6738 Suppl), A23-A31.

34. Funalot, B., Ouimet, T., Claperon, A., Fallet, C., Delacourte, A., Epelbaum, J., Subkowski, T., Leonard, N., Codron, V., David, J. P. Amouyel, P., Schwartz, J. C., Helbecque, N. (2004). Endothelin-converting enzyme-1 is expressed in human cerebral cortex and protects against Alzheimer's disease. *Molecular psychiatry*, 9(12), 1122-1128.