UNIVERSITY OF CALIFORNIA SAN DIEGO

Corticotrophin-Releasing Factor Receptor 1 and chronic stress in Alzheimer's Disease pathogenesis.

A Dissertation submitted in partial satisfaction of the requirements
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in

Biology

by

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The Dissertation of Maya A. Ellisman is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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DEDICATION

In tender memory of Sarah-Rose Bellout and Bee Ellisman. To those who struggle in this culture with neuropsychiatric, neurodevelopmental, and neurodegenerative afflictions. To those working to find better treatments and structures for the distress caused by these variations.

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LIST OF ABBREVIATIONS

PSNS	Parasympathetic nervous system
SNS	sympathetic nervous system
PTSD	post-traumatic stress disorder
AD	Alzheimer's disease
HPA	hypothalamic-pituitary-adrenal
SMA	sympatho-medullo-adrenal
PVN	hypothalamic paraventricular nucleus
CRF	corticotropin-releasing factor
AVP	vasopressin
ACTH	adrenocorticotrophic hormone
MC2R	melanocortin type-2 receptors
CORTs	glucocorticoids
eCB	endocannabinoid
Aβ	amyloid- β
p-tau	hyperphosphorylated tau
fAD	familial AD
APP	amyloid precursor protein
PS1	presenilin 1
PS2	presenilin 2
sAD	sporadic AD
NMDAR	N-methyl-D-aspartate receptor
BACE1	β-secretase

CTF	c-terminal fragment
IDE	insulin-degrading enzyme
NEP	neprilysin
CRFRs	CRF receptors
CRF-BP	CRF binding protein
UCN1-3	urocortins
GRK	GPCR kinase
βarr1 or 2	β-arrestin-1 or β-arrestin-2
AP-2	adaptor protein-2
A	acute restraint stress
R	chronic physical restraint stress
CVS	chronic variable stress
MWM	Morris Water Maze
PCR	polymerase chain reaction
SC	subcutaneous
SRM	Spatial Reference Memory
SWM	Spatial Working Memory
TREM2	triggering receptors expressed on myeloid cells 2
DAMPS	danger associated molecular patterns

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ABSTRACT OF DISSERTATION

Corticotrophin-Releasing Factor Receptor 1 and chronic stress affects Alzheimer's Disease pathogenesis.

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Chronic stress not only decreases the quality of life, but also increases the risk of developing a stress-related neuropsychiatric disorder, cardiovascular diseases, metabolic dysfunction, and/or Alzheimer's disease (AD). Through animal studies, a key stress signaling factor in the hypothalamic-pituitary-adrenal (HPA) axis, corticotropin-releasing factor (CRF), was found to contribute to the development of AD through binding to its cognate type-1 receptor (CRFR1). The two defining cellular pathological phenotypes in AD, amyloid-β (Aβ) plaques and hyperphosphorylated tau (p-tau) tangles are both potentiated by CRFR1 activation. Current treatments for AD offer only a temporary reprieve from symptoms and do not provide ample relief. Unfortunately, the recent experimental therapies targeting amyloidogenic pathways have been unsuccessful in the treatment of human AD. Delineating the therapeutic potential of drugs targeting the CRF system could provide a novel treatment approach to combat AD pathogenesis. To assess how CRFR1-antagonism (vehicle/R121919) and/or stress alters AD pathology, early and mature (6- and 12- month) Wt/PSAPP cohorts were subjected to a series of behavioral assays (measuring learning, memory, and cognitive rigidity), immunohistochemical imaging and

analysis of Aβ plaques in cortical/hippocampal sections, and biochemically probed for soluble/insoluble Aβ species in cortical fractions. Early R121919 treatment reduces AD pathology: improving behavioral task ability, reducing plaques throughout the hippocampus and cortex, and lowering cortical highly toxic soluble Aβ oligomers while increasing the amount of in-soluble monomeric Aβ species. Interestingly, stress improves memory, increases hippocampal plaques, lowers cortical plaques, and increases the amount of cortical insoluble monomeric species of Aβ, in early vehicle groups. While early treatment with R121919 ameliorates AD pathogenesis, mature AD pathology was not affected by R121919 (treatment did lower mature cortical soluble monomeric Aβ species). Parallel to these studies, stress did not alter mature behavioral deficits, but did lower hippocampal and cortical plaques, while increasing levels of mature cortical highly neurotoxic soluble oligomeric Aβ species (vehicle-cohorts) and mature cortical soluble monomeric Aβ (R121919-cohorts). Overall, CRFR1-antagonism may provide novel therapies for AD pathogenesis.

CHAPTER 1: INTRODUCTION

Stress.

Internal responsive signaling of a perceived threat prepares the body to respond to the triggering stressor, sending the body into a *"fight or fight"* response (Cannon, 1929). Stress can be defined as a threat to homeostasis, switching the organism's use of its *parasympathetic nervous system* (PSNS) to its stress-responsive *sympathetic nervous system* (SNS). As the organism adapts to its anticipated environmental demands, a process known as allostasis, the sum of these adaptations to adverse chronic stressors wears on the body, generating an increased allostatic load (Mcewen & Stellar, 1993). Stress can arise from multiple types of adverse stimuli and can result in the development of stress-related neuropsychiatric disorders. For example, exposure to threatening stimuli can leave individuals at risk of developing *posttraumatic stress disorder* (PTSD). Symptoms of PTSD are invasive to a patient's daily life and ability to function, frequently causing disabling distress (American Psychiatric Association, 2013). Adults in the US general population display a 4.7% annual incidence of PTSD, with a lifetime prevalence of 6.1%. The lifetime prevalence of PTSD increases to 13% for combat veterans (Schmeltzer, Herman, & Sah, 2016), yet PTSD treatment is mostly ineffective. The lack of rehabilitation treatment options not only leaves those with PTSD to deal frequently with distressing symptoms, but also leaves them at risk for developing comorbid or associated disorders like anxiety-related disorders, depression, and *Alzheimer's disease* (AD).

Underlying biology of stress responses.

Once stress has been triggered, multiple systems within the body are recruited to assist the organism in responding. The autonomic response to stress switches the body from its resting state, when it is primarily utilizing the PSNS, to the use of the SNS, which releases

catecholamines into the bloodstream (for review (Tsigos, Kyrou, Kassi, & Chrousos, 2000)). Once a threat is processed, the neuroendocrine system responds through activation of the *hypothalamic-pituitary-adrenal* (HPA) axis (reviewed in **Error! Reference source not found.**) and the *sympatho-medullo-adrenal* (SMA) axis. The HPA axis (**Error! Reference source not found.**) integrates stress-processing fibers from the limbic and mid-hindbrain at the hypothalamic *paraventricular nucleus* (PVN) parvocellular neurons. This can trigger the neurons in the hypothalamic median eminence to release *corticotropin-releasing factor* (CRF) and *vasopressin* (AVP) into the pituitary portal vessel's capillaries, resulting in effects at the anterior pituitary. CRF can drive these pituitary neurons to produce and release *adrenocorticotrophic hormone* (ACTH, also known as corticotropin) into the bloodstream. CRF can promote additional alterations to the body once released into the bloodstream. ACTH is shuttled through the blood to the adrenocortical cells of the adrenal gland zona fasciculata, where it can bind to *melanocortin type-2 receptors* (MC2R) generating the production and release of *glucocorticoids* (CORTs) into the bloodstream. CORTs provide feedback inhibition on both the PVN and the anterior pituitary, reducing the production of respective hormones (CRF and ACTH) (Baumeister & Pariante, 2015). Details of this system can be visualized in Figure 1. *In-vivo* stress responses are additionally complicated by longer-acting effects of transcription factors and by the actions of other neurotransmitter systems, such as the *endocannabinoid* (eCB) system. While the short-term activation of stress pathways is necessary for survival, the long-term effects of chronic stress on increased allostatic load are associated with an increased risk for AD pathogenesis (Wilson, et al., 2003).

Alzheimer's Disease.

The most common form of dementia, AD, is predicted to affect 24 million individuals

worldwide and is characterized by two neuropathological hallmarks: amyloid- β (Aβ) plaques and hyperphosphorylated tau (p-tau) tangles. While a heritable genetic form of AD exists, familial AD (fAD) only accounts for <3% of cases. Mutations in three associated genes have been identified in fAD: amyloid precursor protein (*APP)*, *presenilin 1* (PS1), and *presenilin 2* (PS2) (Selkoe, 2001); while the causes of the more common form, *sporadic AD* (sAD), remain elusive, environmental factors (Li, et al., 2015) and susceptibility to stress is linked to sAD and hippocampal degeneration. Current therapies for AD include immunotherapy for Aβ (Aducanamab), cholinesterase inhibition (Rivastigmine, memantine, and Galatamine), and glutamatergic N-methyl-D-aspartate receptor (NMDAR). While these therapies have been approved by the FDA for the treatment of AD, most of them induce adverse side effects (such as edema, convulsions, chest pain, headache, nausea, vomiting, diarrhea, decreased appetite, dizziness, confusion, aggressive behavior, fatigue, hypertension, hallucinations, and pain) which lower patient adherence to treatment, and have limited therapeutic windows (for review (Pardo-Moreno, et al., 2022)). The development of new medication is paramount, considering a large aging population could lead to a predicted threefold rise in US incidence of AD by 2050.

AD Pathology.

The mechanism of stress-induced potentiation of the pathological AD proteins, Aβ and p-tau, is currently unknown. To understand the mediators of this AD-related system, the detailed process that generates these species must be inspected.

Neuronal scaffolding is integral to synaptic stability. Proper neuronal scaffolding relies on networks of microtubules stabilized by various isoforms of a soluble tau protein, encoded from a single gene (Goedert, Spillantini, Jakes, Rutherford, & Crowther, 1989). In AD and other tauopathies, tau becomes hyperphosphorylated and insoluble. The p-tau has a lowered

probability of binding to stabilize microtubules; instead it aggregates into insoluble filaments that further accumulate into tangles (Gustke, Steiner, Biernat, Meyer, & Mandelkow, 1992) (Alonso, Grundke-Iqbal, & Iqbal, 1996) (for further delineation of this process, Querfurth and Laferla detail p-tau aggregation in their review (Querfurth & Laferla, 2010)). Mutations in *tau* are sufficient to generate AD cognitive impairments and neurodegeneration (Hutton, et al., 1998), and tangle generation is highly associated with disease pathogenesis (Arriagada, Growdon, Hedley-Whyte, & Hyman, 1992). While p-tau has these associations, Aβ plaques do not correlate well with disease severity, though there is a correlation between severity and amount of soluble and/or insoluble Aβ (Wang, Dickson, Trojanowski, & Lee, 1999) (McLean, et al., 1999) and immunotherapy targeting Aβ is one of the few approved treatments for AD.

Aβ is the proteolytic product of a transmembrane protein, *amyloid precursor protein* (APP), that is cleaved by a family of proteases termed secretases (reviewed in (Querfurth & Laferla, 2010)). The initial cleavage of APP by either α-secretase or *β-secretase* (BACE1) directs further processing to either a non-amyloidogenic or an amyloidogenic pathway, respectively (Esch, et al., 1990) (Sinha, et al., 1999). Through the amyloidogenic pathway, BACE1 cleaves APP (Vassar, et al., 1999) while in the lumen of an acidic intracellular compartment, such as within endosomes or other areas of the secretory pathway, releasing sAPPβ and leaving *c-terminal fragment* (CTF) within the membrane (C99) (Das, et al., 2016). The C99 fragment is further processed by γ-secretase, releasing pathogenic Aβ species at various sizes. Aβ is a hydrophobic peptide whose most common species are 38, 40, or 42 amino acids long. Each size has varying degrees of toxicity, with Aβ42 displaying the greatest toxicity and propensity to aggregate, followed by Aβ40 (Yan & Wang, 2006). Soluble Aβ oligomers are highly neurotoxic, while the formation of insoluble plaques into fibrils is neuroprotective (Tolar, Hey, Power, &

Abushakra, 2021). Under non-pathologic states, Aβ is still produced, but is able to be subsequently degraded by several proteases (e.g., *insulin-degrading enzyme* (IDE) (Vekrellis, et al., 2000), *neprilysin* (NEP) (Iwata, et al., 2001), and endothelin-converting enzyme (Eckman, Watson, Marlow, Sambamurti, & Eckman, 2003)).

The production of Aβ is also regulated by the membrane localization of Aβ-generating substrates to lipid rafts domains. Lipid rafts are specialized cellular membrane microdomains where specific cellular processes can occur. These areas are named rafts because, in theory, they have differing membrane fluidities from the surrounding non-raft region, generated by their increased amount of cholesterol and saturated fat. High-fat and high-cholesterol diets are known to increase the risk of developing cardiovascular diseases or obesity, and metabolic disorders, which are all linked to AD. This may be due to the effects of cholesterol on plasma membrane domains: i.e. the presence of high plasma membrane cholesterol alters the translocation of APP to lipid rafts, and subsequently internalized into the early endocytic pathway, thus altering which secretase initially cleaves and fating APP to be amyloidogenic (Lim, 2014).

Several studies have found a link between *G-protein-coupled receptors* (GPCRs) and AD pathogenesis. During the hydrolytic processing of APP, some GPCR signaling pathways (Supplemental Table 2) and GPCRs (Supplemental Table 3) have been shown to change dynamics of β- and/or γ-secretase through various proposed mechanisms (reviewed in (Zhao, Deng, Jiang, & Qing, 2016)). The activation of GPCRs can impact cellular trafficking dynamics by changing the localization of proteins to lipid rafts (Thathiah & De Strooper, 2009) (Teng, Zhao, Wang, Ma, & Pei, 2010) (Park H. J., et al., 2015). CRFR1 is a GPCR known to increase amyloidogenesis when activated by CRF, yet the underlying mechanism of CRFR1-induced AD pathogenesis is unknown.

As stated above, CRF is released upstream of glucocorticoid activation in the HPA-axis, from neurons in the hypothalamic PVN, and is shuttled directly to the anterior pituitary, triggering the release of ACTH into the bloodstream. This area of the HPA-axis was elucidated at the Salk Institute (La Jolla, CA) in 1981, when Vale et al. isolated, purified, and sequenced the ovine hypothalamic secretagogue (CRF), which was named for its ability to regulate pituitary corticotropin (ACTH) and β-endorphin release (Vale, Spiess, Rivier, & Rivier, 1981). Similar molecules in this family have been identified in many other species (Rivier, Spiess, & Vale, Characterization of rat hypothalamic corticotropin-releasing factor., 1983) (Shibahara, et al., 1983) (Ling, Esch, Böhlen, Baird, & Guillemin, 1984) (Esch, et al., 1984) (Patthy, et al., 1985) (Okawara, et al., 1988) (Stenzel-Poore, Heldwein, Stenzel, Lee, & Vale, 1992). Further probing of CRF in rat brains led to the identification of three localized functionally specific systems that contain CRF-stained cells: (1) the parvocellular and magnocellular PVN, which are affected by adrenalectomy, (2) cell groups that regulate autonomic responses, such as the brain stem, hypothalamus, and the basal telencephalon, and (3) cerebral cortical neurons in layers II and III (Swanson, Sawchenko, Rivier, & Vale, 1983).

Within the CRF family, there are two CRF receptors (CRFRs), a CRF binding protein (CRF-BP), and three other related peptides known as urocortins (UCN1-3) (reviewed in Figure 1). These receptors are class-B GPCRS, displaying structural similarity to receptors in the secretin/glucagon family. CRF binds with high affinity to CRFR1. G-protein coupling of CRFR1 is predominantly Gs, activating the adenylyl cyclase cascade upon agonist binding, but this receptor can promiscuously bind to other G-proteins, such as Go, Gq/11, Gi1/2, and Gz.

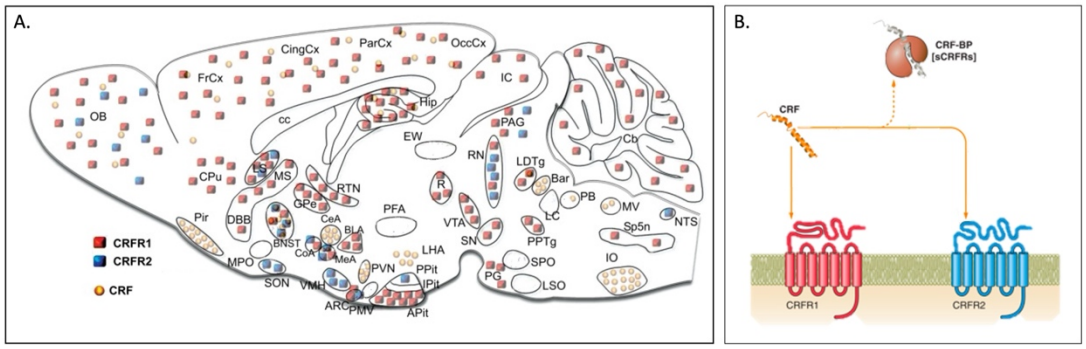

Figure 1. CRF and CRFR1 localization, and binding of ligands and receptors.

Distribution of CRF and its receptors, CRFR1 and CRFR2 in rodent brain (A). Four distinct CRF ligands (only CRF shown here) have different binding profiles to CRFRs and the CRF binding protein (CRF-BP)(B). CRF binds to CRFR1 and CRF-BP; Ucn 2 and 3 (not shown above) are CRFR2-selective ligands. Ucn 1 (not shown above) has an affinity for both CRFRs and CRF-BP. Adapted from *(Dedic, Chen, & Deussing, 2018)*.

The trafficking of CRFR1 regulates the duration and strength of its contribution to stress, and variations that are found in the endocytic desensitization may differentially contribute to CRFR1-mediated potentiation of AD pathology. Receptors, once activated by an agonist, are desensitized through *GPCR kinase* (GRK) mediated phosphorylation of either the C-terminus or the third intracellular loop. This phosphorylation can signal for arrestins to bind to the GPCR, which sterically "arrests" the signaling of the G protein (for review (Walther, Ferguson, & Taylor, 2013) (Smith & Rajagopal, 2016)). β-arrestin-1 or β-arrestin-2 (βarr1 or 2 respectively) can then direct clatherin-coated pit-mediated endocytosis by linking the GPCR to clatherin and *adaptor protein-2* (AP-2) (Laporte, et al., 1999.). Once endocytosed, GPCRs can be recycled or degraded. The rate of recycling resensitization can be regulated by GPCR binding stability with arrestin, which can be either short and transient (class A GPCRs) or can form a more stable complex (class B GPCRs) (Oakley, Laporte, Holt, Caron, & Barak, 2000). Arrestins also can activate G-protein-independent signaling cascades by complexing the signaling proteins with the GPCR (Kohout & Lefkowitz, 2003). CRFR1 binds to both βarr1 and 2, yet it preferentially binds with βarr2. Once agonist activation of CRFR1 occurs, βarr2 is rapidly recruited to bind to

CRFR1. This is dependent on CRFR1 phosphorylation of motifs in the C-terminal and in the IC3. The binding of βarr-2 with CRFR1 is transient, and the arrestin does not traffic with the GPCR into the cell; thus, CRFR1 is categorized as an *"arrestin class A"* GPCR (Oakley, et al., 2007).

Stress and AD pathogenesis continued.

Multiple theories have been proposed linking aspects of the stress system with AD pathogenesis. Further downstream in the HPA-axis, glucocorticoids have been shown to increase with age, conferring an associated enhanced neuronal vulnerability in the hippocampus (Sapolsky, Krey, & McEwen, 1985). In addition to the associations between CRF and AD, evidence also links glucocorticoids to AD pathogenesis. For example, non-AD patients with increased levels of cortisol (a glucocorticoid) display memory impairments and reductions in hippocampal volume (Lupien, et al., 2004), yet this finding has been criticized for not considering the contribution of an upstream regulator, CRF. Genetic studies considering the genetic associations with sAD link a rare haplotype in the 5' regulatory region of the gene HSD11B1, resulting in a reduction in transcription of an enzyme that inactivates cortisol (de Quervain, et al., 2003). Interestingly, the application of glucocorticoids to a mouse model of AD increased transcript and protein levels of APP and BACE1, as well as Aβ accumulation and mislocalized tau, but there were no changes in p-tau levels (Lupien, et al., 2004).

While glucocorticoid-related memory deficits may contribute to AD, it is important to consider that the CRF-system displays AD-associated abnormalities and may play a key role in stress-associated AD pathogenesis. CRF-expressing dystrophic neurites localize with Aβ deposits (Powers, et al., Immunohistochemical study of neurons containing CRF in Alzheimer's disease, 1987) and abnormalities in the CRF system are found early in the brains of postmortem

AD patients. These abnormalities include reductions in CRF immunoreactivity (CRF-IR) in the cortex (Davis, Mohs, Marin, Purohit, & Perl, 1999), caudate (Bissette, Reynolds, Kilts, Widerlöv, & Nemeroff, 1985), and amygdala of AD patients. Increases in AD hypothalamic PVN CRF-immunostaining and mRNA transcripts have also been observed (Powers, et al., 1987) (Raadsheer, et al., 1995). While cortical CRF-IR is reduced in AD, CRF binding increases as AD-related decrements in ChAT activity progress (De Souza, Whitehouse, Kuhar, Price, & Vale, 1986). The increase in bound CRF detection could be due to increased binding of CRF to CRF-BP or to CRFRs. Interrupting the binding of CRF-BP to CRF pharmacologically, thus increasing the bioavailable CRF, has been suggested as a potential therapy for AD (Behan, et al., 1995). Additionally, when CRF is conditionally overexpressed (CRF-OE) in a mutant APP AD-mouse model, there is a significant potentiation of Aβ plaques, memory deficits, and neuronal degeneration (Dong, et al., 2011). Bolstering the link between stress and AD, multiple studies have found that various types of stress induce CRFR1-dependent alterations in AD-associated pathways (Supplemental Tables 4 and 5).

All of these aspects make CRFR1 an interesting target for the antagonization of stress-induced activation of ACTH. Prior to the development of true competitive antagonists, CRF-neutralizing antibodies were effective in reducing stress-induced production of ovine ACTH (Rivier, Rivier, & Vale, 1982). In efforts to produce a synthetic competitive antagonist, Rivier et al., (1984) found that CRF residues 4–8 from the N-terminus and the first two amino acids from the C-terminus are highly important for activation of the receptor; thus an antagonist was developed by replacing the first 8 amino acids with α-helical-preferring amino acids, known as α-helical-CRF(9-41) (Rivier, Rivier, & Vale, 1984).

CRFR1 in AD Pathology.

Stress-induced increases in "allostatic load" are associated with multiple disorders and their progression, including AD. While glucocorticoid levels increase with age and prolonged exposure to these stress hormones induces loss of hippocampal neurons (Sapolsky, Krey, & McEwen, 1985), published findings and unpublished preliminary data indicate that the effects of chronic stress on AD pathogenesis are abolished by disruption of CRFR1 signaling and is enhanced in CRFR2-/- mice (Zhang, et al., 2016; Rissman, Lee, Vale, & Sawchenko, 2007; Le, et al., 2016; Bangasser, et al., 2017; Carroll, et al., 2011; Baglietto-vargas, et al., 2015).

Previous studies demonstrate that acute stress, both physiological and emotional, can generate increases in p-tau. While acute stressors induce this change in p-tau transiently (Rissman R. , 2009), chronic physical restraint stress (R) produces enduring toxic p-tau, with a portion accumulating into the insoluble pathogenic tangles (Rissman, Lee, Vale, & Sawchenko, 2007). Additionally, these studies found that CRFR1 and CRFR2 contribute differently to AD pathology. Data suggests that CRFR1 mediates initial stress-induced changes in AD tau pathology prior to downstream glucocorticoid production. Studies published by other groups indicate that CRF-stress signaling, in an AD mouse model, also affects Aβ pathology (Kang, Cirrito, Dong, Csernansky, & Holtzman, 2007). Additionally, CRFR1 and CRFR2 knockout (/KO) mice subjected to acute restraint stress (A) elucidate that CRFR1 is necessary for stress-dependent p-tau generation (Rissman, Lee, Vale, & Sawchenko, 2007).

The potential confounds in these data from the developmental defects in germ-line KO animals can be mitigated by replacing genetic CRFR interference with the direct application of pharmacologic inhibitors. Pretreatment with small molecule antagonist antalarmin blocked A-induced p-tau. Unfortunately, extended antalarmin treatment produced distress phenotypes in mice. Replacing antalarmin treatment with another CRFR1-antagonist, R121919 (20 mg/kg/d),

which has a more favorable partition coefficient (R121919 cLogP = 4.52 v.s. antalarmin cLogP=6.98), reduces stress-induced p-tau 20 min after application of both A and R . Treatment with R121919 significantly reduced the effects of R 24 hrs after stress treatment, yet AT8 and PHF-1 staining was greater than those from non-stressed controls (Rissman, et al., 2012). These data suggest that CRFR1 is a potential therapeutic target for reducing stress-induced impacts on AD pathology.

To view CRFR1-dependent stress-induced changes to Aβ deposition, PSAPP (overexpressing APP-Swe$_{K595N, M596L}$ and PS1$_{dE9}$, this AD mouse model primarily displays Aβ pathology with minor changes in tau), mice were treated with R121919 or vehicle, with or without *chronic variable stress (CVS)*. Groups subjected to CRFR1-antagonism displayed reductions in Aβ plaque deposition. Treatment with stress did not significantly change plaque density, yet CRFR1-antagonism produced significant amelioration of Aβ plaque density (unpublished preliminary data). This indicates that treatment with CRFR1-antagonist, specifically during stress exposure, can mitigate the progression of Aβ pathology in PSAPP AD-model mice. Further studies suggest that PSAPP mice treated during a further progressed disease stage (9-12 months) display a potentially therapeutic effect, though less pronounced than that displayed by groups treated during earlier disease stages.

Effects of CRFR1-/- on AD mouse model pathology.

While these data support that CRFR1 deficiency affects AD pathogenesis in a mouse model, the mechanism is unknown. To further understand this, a PSAPP line was crossed with a CRFR1 null line (CRFR1-/-), generating PSAPP-R1-/- and PSAPP-R1+/- lines, and the development of Aβ pathology was examined at different stages of AD (6- and 12-mo, early and mature, respectively). At early stages, total brain lysates assessed using ELISAs display

significant reductions in Aβ40 and Aβ42; the largest reductions were in Aβ40. Furthermore, IHC viewing Aβ plaque counts in PSAPP mice, with either R1-/- or R1+/-, also show significant CRFR1 genotype effects. PSAPP-R1 groups display a significant reduction in isocortical Aβ deposition. PSAPP-R1 groups at a more mature stage of disease progression continue to display significant reductions, as compared to PSAPP controls, in cortical Aβ plaque load, along with a decrease in the ventricular enlargement, typical for those affected by AD (Campbell, et al., 2015).

Hints at the mechanism of stress-induced CRF-CRFR1 potentiation of Aβ pathology.

While data from these ongoing studies indicate that AD Aβ pathology is potentiated by CRFR1, the mechanism underlying this modulation is currently unknown. In PSAPP, PSAPP-$R1^{-/-}$ and $–R1^{+/-}$ mice, APP levels were not altered by genotype, yet CRFR1 expression lowered CTFs. Interestingly, IDE protein levels decreased within both R1-altered PSAPP groups. Overall, these data indicate that CRFR1 expression does not change APP protein levels, but might alter downstream enzymes that produce amyloidogenic CTFs (BACE1) (Campbell, et al., 2015).

CRFR1 Antagonism Effects on PSAPP Memory Deficits.

Preliminary data from experiments designed to assess the potential of these studies show that 6-month-old PSAPP mice who began treatment at 30 days of age with continuous IP injections of R121919 (20mg/kg) display increased reference memory, as measured by Morris Water Maze (MWM) (Zhang, et al., 2016). *Latency* to, as well as *distance* traveled to, find the platform were both reduced with R121919 treatment. Additionally, working memory deficits were rescued with drug treatment, as measured by SWM tests (protocol detailed in MWM Methods Section). Trials extending these data to mature 12-mo female PSAPP

pathological stages (Zhang, et al., 2016), display similar SWM trends; yet these data need to be reassessed for task competency by viewing recorded locomotor patterns. Mature PSAPP females may show a reduced treatment effect due to the efficacy of the PSAPP transgenes.

Taken together, these findings provide a foundation for the question: *How does stress, via CRF-CRFR1 signaling, mechanistically contribute to the pathological development of p-tau and Aβ in AD? Moreover, will specific pharmacological intervention of CRFR signaling confer a therapeutic benefit for AD?*

To assess how CRFR1-antagonism (vehicle/R121919) and/or stress alters AD pathology, we subjected early and mature (6- and 12- month) Wt/PSAPP cohorts to a series of behavioral assays (measuring learning, memory, and cognitive rigidity), immunohistochemical imaging of Aβ plaques in cortical/hippocampal sections, and biochemically probed soluble/insoluble Aβ species in cortical fractions. We found that early R121919 ameliorates AD pathogenesis, while stress induced better memory toxic species of Aβ increased. While R121919 treatment improved early, AD pathology was not altered by R121919 in mature AD, yet stress increased mature toxic soluble oligomeric Aβ species. Overall, these data suggest the potential for novel AD treatments through CRFR1-antagonism.

Chapter 1,in part, is currently being prepared for submission. Maya Anne Ellisman is the primary researcher.

CHAPTER 2: MATERIALS AND METHODS

Animals.

For the following experiments, a colony of double transgenic PSAPP mouse line 85 (*APP-Swe*$_{K595N, M596L}$ and *PS1*$_{dE9}$; Jackson Labs, Bar Harbor, ME) was the experimental animal model of AD. This line, generated by Dr. D. Borschelt's group (Jankowsky, et al., 2001), expresses two plasmids (Mo/Hu APP-Swe and PS1dE9) controlled by individual elements within the mouse prion protein promoter- which localize expression predominately to neurons in the central nervous system. Transgenes expressed in this line are both linked to fAD. The resulting line 85 PSAPP colony is maintained within our vivarium as hemizygotes, by crossing the transgenic line with B6C3F1/J mice, under standard conditions. Genotype is determined by polymerase chain reaction (PCR). Control cohorts were generated using non-transgenic littermates. Mice were kept in mixed cages (PSAPP and WT) with 2-5 mice per cage. The vivarium was temperature controlled and on a 12:12 hour light/dark cycle, with ad libitum food and water. Cohorts were aged to either 6- (early) or 12- (mature) months before euthanasia.

In-Vivo Pharmacology.

The CRFR1-antagonist used in this study, R121919, primarily acts in the CNS. Each cohort will be treated with subcutaneous (SC) injections five times per week with either vehicle or R121919 (10 mg/kg), from two months-of-age until sacrifice (Figure 3 A and B). The R121919 used in this study was donated by collaborator Dr. K Rice (NIH). A solution of 0.01 M tartaric acid and 5% v/v polyethoxylated castor oil was used to dissolve R121919 and as vehicle, at pH 3. Vehicle/R121919 treatments were assigned to matched littermates at random.

Chronic Restraint Stress.

Both 6- and 12-month cohorts were subjected to chronic restraint stress (CRS or stress), beginning at 2- and 6- months, respectively (outlined in Figure 3). Stress began one-hour post

injections of vehicle or R121919, for 30 minutes consecutively for 5 days every other week after initiation of CRS protocol. Subjects were restrained in a 50 ml conical tube (adapted with multiple drilled holes to provide air and reduce thermal stress) and placed alongside cage mates in a brightly lit room for the full duration of stress treatment.

MWM SRM & SWM.

To view changes in various types of memory, PSAPP cohorts were subjected to: **1) the non-spatial acquisition test** – a series of training trials, also used to assess the ability to learn this task *(days 1-3, visible flagged platform)*; **2) a Spatial Reference Memory (SRM) test** – to test their spatial memory of location symbols to find the platform *(days 4-5 & 8-9, hidden platform)*; **3) a probe test** – to assess vision and memory *(day 10, no platform and visible platform)*; **4) a Spatial Working Memory (SWM) test** – to view if task-trained animals abilities to learn new platform locations *(day 10-12, visible flagged platform)*.

The Morris Water Maze (MWM) pool was filled the week prior to the assessment, & daily temperature of pool water was 21°C ± 1°C. While running the trials, animals were acclimated to being handled and distress-associated signals were limited by reducing associated signals (such as smell, noise, and CRS reminders) by conducting behavior and CRS induction in a different room with distinct research technicians per task. Mice were taken from the vivarium to the MWM room 1 hour prior to the behavioral assessment to allow the animals to acclimate to the testing environment. Mice have been familiarized with experimenter handling. At the beginning of a trial, mice were carried in a terry cloth towel from the home cage and placed facing the pool center into the water, thus initiating AnyMaze software tracking of testing trials. The data from recorded trials are analyzed using PRISM. The protocol for behavior was performed as described in Figure 3.

Sample collection.

Mice were anesthetized in isoflurane chambers prior to decapitation. Subsequently, terminal blood was collected, and brains were removed. Brains were halved: (1) with one hemi-brain drop-fixed in 4% paraformaldehyde for 48 hours, then transferred to a cryoprotect (30% sucrose in PBS) for 24 hours, before being sectioned by a freezing-sliding microtome at 30 μm, and subsequently kept in a cryoprotectant (20% glycerol and 30% ethylene glycol in 0.1 M phosphate buffer) in a 4°C fridge; (2) while the other hemi-brain was flash frozen using dry-ice for biochemical assays. Terminal blood was immediately centrifuged (6000 rpm for 6 minutes) to extract the plasma from the pellet (both stored at -80°C).

Immunohistochemical Assays.

Fixed hemi-brains cryoprotected in 30% sucrose for 24 hours, then coronally cut using a Leica sliding freezing microtome into 30 μm sections. Sections were stored in a 30% glycerol/ 30% ethylene glycol in PBS cryoprotectant at -20° C. Slices that contain hippocampus were stained using an N-terminal anti-human Aβ monoclonal antibody (82E1) (IBL #10323) to assess localization, percent area, size, and number of hippocampal and cortical Aβ plaques. To stain Aβ, slices were placed in 50% formic acid for 3 minutes for antigen retrieval. The tissue was then permeabilized in 0.1% TritonX/TBS, followed blocking in a 3% BSA in 0.1% Triton#/TBS solution. To stain the plaques with 82E1, the tissue was transferred to a 1:500 82E1 (0.1 ug/ul) dilution and incubated at room temperature overnight. The following day, the tissue was biotinylated with a mouse secondary antibody (Vector labs #BA-2001), transferred into an ABC solution (Vector Labs #PK6100) prior to DAB reaction (Vector Labs # SK4100).

Aβ Quantification.

To analyze the deposition of Aβ, mosaic- images of 82E1 stained sections were taken using a Leica TCS SPE confocal microscope and LasX software. Quantification of the percent area of plaque-load, the plaque size, and the number of plaques, was done using FIJI sectional analysis of thresholded whole and subdivisions of the hippocampi and cortexes.

Homogenous Lysate for Co-protein and -RNA Extraction.

To extract both the total RNA and protein from correlated areas in each treatment subject, a homogenous lysate from the frozen hippocampus and was generated using cold RNase-free 1xPBS (with protease inhibitors), quickly in an RNase-free environment. A portion of the lysate was transferred to a separate tube for protein processing, while RNA is isolated from the rest of the lysate and centrifuged at 2,000 RPM for 3 min at 4 ºC. The supernatant is removed from the RNA-tube, without disruption of the pellet, and 600 ul of QIAzol was quickly used to re-suspend the pellet. RNA was then isolated using the RNeasy PLUS Universal Mini Kit and protocol from QIAgen. The protein processing tube is then processed using the following RAB/RIPA extraction protocol.

RAB/RIPA Extraction.

To process the protein in the lysate for biochemical analysis, the 1xPBS homogenate (with protease inhibitors) is centrifuged and the supernatant is saved (1xPBS fraction). The resulting pellet is resuspended in a high-salt RAB solution (0.1 M MES, 0.75 NaCl, 1 mM EGTA, and 0.5 mM $MgSO_4$) and then is centrifuged for 40 min at 40,000 G. The RAB-supernatant is collected (RAB-soluble fraction) and the resulting pellet is resuspended in a RIPA buffer (50 Mm Tris-HCl pH 7.4, 0.1% SDS, 1% NP40, 0.25% sodium deoxycholate, 150 nM NaCl, 1mM EDTA, 1mM EGTA, 1 mM Na_3VO_4, & 1 uM okadaic acid) with protease inhibitors (PMSF, NAF 1 mM, aprotinin, leupeptin, & 1ug/mL pepstatin). The solution is then centrifuged

twice at 40,000 G for 20 min, and the resulting supernatant is collected (detergent-soluble fractions). The protein concentration is then determined using a BCA Protein Assay Kit (Pierce Biotechnology).

Biochemical Western Blot analysis of plaques.

To probe plaque species in the cortex, proteins were separated via electrophoresis in 10% Criterion gels, and stained for Aβ with 82E1 (IBL, Cat#10323), and β-actin (Sigma-Aldrich, MDL #MFCD00145889) was quantified as a loading control. Protein electrophoresis gels were run at 80 V for 3 hours. Gels were then transferred to a PVDF membrane at 200 mA for 1 hour. Blots were subsequently blocked in 5% non-fat milk in 0.1% TBS/Tween, and stained with secondary mouse and rabbit HRP antibodies (for 82E1 and β-actin, respectively). FIJI (ImageJ) software was used to measure and quantify the Western Blot bands.

Statistical Analyses.

Statistical analyses and graphs were made using GraphPad Prism 9 software (La Jolla, CA) between cohorts with either t-test, one-way or two-way ANOVA with Tukey's Multiple Comparison with a 95% confidence interval and a $P < 0.05$ value accepted for significance. All values have been displayed as Mean ± SEM.

Chapter 2,in part, is currently being prepared for submission. Maya Anne Ellisman is the primary researcher.

CHAPTER 3: RESULTS

Behavior: Morris Water Maze in early and mature AD.

To assess the efficacy of R121919 in treating AD behavioral pathology, the Morris Water Maze was used to evaluate learning, memory, and cognitive rigidity in both early (6 m) and mature (12 m) AD (Figure 3), during the last month before sacrifice.

Learning and cognitive rigidity were significantly improved with R121919 treatment in early AD ($P<0.01$ and $P<0.05$, respectively) (Figure 4 C and I, respectively). R121919-treatment significantly reduced the latency to escape on day 4-5 and 6-7 in the SRM task ($P<0.01$) (Figure 4 C), and on day 8 in the SWM task ($P<0.05$) (Figure 4 I) in early AD. Stress significantly enhances memory in early AD ($P<0.01$) (Figure 4 F). Early vehicle-treated cohorts who underwent stress spent more time in the platform zone during probe trail 1, when the platform was removed, on day 8 ($P<0.01$) (Figure 4 F). The pathological behavioral deficits in mature AD were not affected by R121919 and stress; no significant change was observed in learning, memory, and cognitive rigidity (Figure 4 K-N, O-P, and Q-T).

Immunohistochemical Analysis 82E1 Staining.

To view the localization and alterations of pathological plaque development, immunohistochemical analysis of 82E1 (an N-terminal-specific anti-human Aβ monoclonal antibody) stained Aβ in the cortex and hippocampus were analyzed and compared.

Early R121919 significantly reduced the percent area and size of plaques in the whole hippocampus ($P<0.05$) (Figure 5 B and C). Additionally, early R121919 altered hippocampal subregions to various extents.

The number of plaques was significantly increased in CA2/3 and the inner dentate ($P<0.05$ and $P<0.01$, respectively) (Figure 5 I and K); drug-affects also significantly increased the size of inner dentate plaques ($P<0.01$) (Figure 5 S).

Stress in early cohorts significantly increased the number of plaques in vehicle-treated early cohorts ($P<0.05$) (Figure 5 D). Plaques in hippocampal subregions also showed various alterations with stress and vehicle treatment. The percent area of plaques was significantly increased by stress in CA2/3, CA3 inner, and the outer dentate hippocampal subregions ($P<0.01$, $P<0.01$, and $P<0.05$, respectively). The number of plaques in CA2/3, CA3, and inner dentate significantly increases with stress in vehicle-treated groups ($P<0.01$, $P<0.001$, and $P<0.05$, respectively) (Figure 5 K, N, and T). The size of inner dentate plaques significantly increase with stress in vehicle-treated early cohorts ($P<0.05$) (Figure 5 S). Stress in R121919-treated groups significantly decrease the number of plaques in CA1 and the inner dentate ($P<0.05$ and $P<0.01$, respectively) (Figure 5 H and T, respectively).

Treatment with R121919, in early stress cohorts, significantly reduces hippocampal CA3 the percent area of plaques, as compared to vehicle-treated stress controls ($P<0.01$) (Figure 5 I), while no other area in the hippocampus was altered by these treatments.

Early R121919 significantly reduced the percent area and size of early cortical plaques ($P<0.05$) (Figure 6 B and C). Stress reduces the percent area and size of cortical plaques in vehicle early cohorts ($P<0.01$ for both) (Figure 6 B and C); while R121919 applied to stress cohorts significantly decreases the number of cortical plaques, as compared to stress vehicle and non-stress R121919 treated cohorts ($P<0.05$) (Figure 6 D).

Plaque size in cortical subregions RS, RS-RF, and RF-End were significantly reduced with R121919-treatment ($P<0.05$, $P<0.05$, and $P<0.01$, respectively) (Figure 6 G, J, and M). Stress also significantly reduced plaque percent area in RF-End and Entorhinal cortical subregions ($P<0.05$ and $P<0.01$, respectively) (Figure 6 L and O, respectively); size in RS, RS-RF, RF-End, and Entorhinal cortical subregions ($P<0.01$, $P<0.01$, $P<0.05$, and $P<0.05$,

respectively) (Figure 6 G, J, M, and P, respectively); and plaque number in RF-End cortical subregion ($P<0.05$) (Figure 6 N). Stress in R121919 cohorts significantly reduces the number of plaques in cortical RS, RS-RF, and Entorhinal subregions ($P<0.01$ in all) (Figure 6 H, K, and Q). R121919 in stress cohorts increases the size of plaques in cortical RS subregion ($P<0.05$) (Figure 6 G), and decreases the number of plaques in the RS and RS-RF cortical subregions ($P<0.01$ and $P<0.05$, respectively) (Figure 6 H and K, respectively).

In mature cohorts, R121919 did not significantly alter plaques within the whole hippocampus (Figure 7 B, C, and D); yet stress significantly decreases the number of plaques in both vehicle and R121919 mature cohorts ($P<0.001$ and $P<0.05$, respectively) (Figure 7 D), and reduces the percent area of plaques in R121919-treated groups ($P<0.01$) (Figure 7 B). These changes occur within specific hippocampal subregions. Stress reduces the percent area of CA2/3 hippocampal plaques ($P<0.05$) (Figure 7 I) in R121919-treated cohorts. Stress also significantly decreases the number of plaques in CA1, CA2/3, CA3 inner, and outer dentate hippocampal subregions ($P<0.001$, $P<0.05$, $P<0.01$, and $P<0.05$, respectively) in vehicle groups (Figure 7 H, K, N, and Q) and CA2/3, CA3 inner, and outer dentate hippocampal subregions ($P<0.05$ in all) (Figure 7 K, N, and Q, respectively). The plaque size was significantly reduced with stress in the outer dentate ($P<0.05$) (Figure 7 P). R121919 treatment significantly reduced the size of mature plaques in CA1 and the outer dentate hippocampal subregions ($P<0.05$ and $P<0.01$, respectively) (Figure 7 G and P, respectively).

Stress significantly reduces mature cortical plaque percent area and number, in R121919 and vehicle cohorts ($P<0.05$ in both) (Figure 8 B and D, respectively). Specifically, stress alters the percent area of plaques in RF-End ($P<0.05$) (Figure 8 L) and the number of plaques in RS-

RF, RF-End, and Entorhinal cortical subregions ($P<0.01$, $P<0.05$, and $P<0.01$, respectively) (Figure 8 K, N, and Q).

Biochemical analysis: Western Blot Analysis and Quantification of Cortical Aβ.

To further investigate the efficacy of R121919 and CRS, the size and solubility of cortical Aβ were determined using 82E1 antibody probe in Western Blot biochemical assays. The quantification of monomeric (~8 kDa) and oligomeric (~20 kDa) Aβ species are normalized to β-actin loading control.

Early soluble Aβ oligomers were significantly lowered in the cortex of cohorts with R121919 treatment ($P<0.001$) (Figure 9 A and C), while in-soluble monomeric Aβ was increased in the same group ($P<0.05$) (Figure 9 D and E), as compared to vehicle-treated cohorts.

Stress significantly increased early cortical in-soluble monomeric Aβ ($p<0.01$), while these species are significantly reduced with R121919 ($p<0.01$) to levels similar to non-stressed cohorts (Figure 9 D and E). Additionally, R121919 treatment in stressed groups significantly increased soluble oligomeric Aβ ($P<0.05$) (Figure 9 A and C).

Mature cohorts treated with R121919 displayed a significant reduction in soluble monomeric Aβ species ($P<0.05$) (Figure 9 G and H), yet no other species showed significant changes. Stress in mature cohorts significantly increased soluble oligomeric Aβ ($P<0.05$) (Figure 9 G and I). Treatment with R121919 in stress-exposed mature cohorts significantly increased soluble monomeric Aβ ($P<0.001$) (Figure 9 G and H).

Chapter 3,in part, is currently being prepared for submission. Maya Anne Ellisman is the primary researcher.

CHAPTER 4: DISCUSSION

AD cases are predicted to rise at an alarming rate, yet current interventions are insufficient in preventing progressive pathological neurodegeneration and the corresponding symptoms. Without sufficient therapeutic interventions, patients experience progressive irreversible memory impairments that isolate them from their community. The distress caused by AD bolsters stress-dependent pathogenesis and illustrates the necessity to identify effective palliative therapies. Chronic stress is strongly associated with an increase in susceptibility to AD, which hints at a potential avenue for preventative treatment. Stress-dependent AD pathogenesis is dependent on CRF-CRFR1 signaling, thus we assess in the current study the efficacy of a CRFR1-antagonist (R121919) as a potential therapy for populations susceptible to chronic stress-induced allostatic overload at both early and mature disease stages.

To evaluate how CRFR1-antagonism (vehicle/R121919) and/or stress alters AD pathology, we subjected early and mature (6- and 12- month) Tw/PSAPP cohorts to a series of behavioral assays (measuring learning, memory, and cognitive rigidity), immunohistochemical imaging of Aβ plaques in cortical/hippocampal sections, and biochemically probed soluble/insoluble Aβ species in cortical fractions.

We found that early R121919 ameliorates AD pathology, specifically improving learning while decreasing total cortical and hippocampal plaque burden, lowering toxic soluble Aβ oligomers, and increasing in-soluble monomeric Aβ species in the cortex, as compared to vehicle-treated controls. Stress-induced improvements in memory, while also increasing the number of hippocampal plaques and lowering the percent area and size of cortical plaques in the early vehicle group. Stress also increases in-soluble monomeric species of Aβ, and R121919

abolishes this stress-induced increase. Alongside to these findings, early stress under R121919 treatment lowers the number of cortical plaques and increases the levels of highly toxic soluble oligomeric Aβ species in the cortex.

While marked improvements are shown in early AD with R121919 treatment, the pathological behavioral deficits, as well as cortical and hippocampal plaque deposition, was not altered by R121919 in mature AD; while R121919 did reduce mature cortical soluble monomeric Aβ species. Lack of efficacy of R121919 at mature AD stages was expected due to the pathological development of plaques in this transgenic mouse model.

Stress also did not alter the behavioral deficits in mature AD, but does reduce the number of hippocampal plaques and both the percent area and the number of cortical plaques in both vehicle/R121919 treated mature cohorts. Stress is found to increase toxic soluble oligomeric Aβ and insoluble oligomeric Aβ species in the cortex of mature vehicle groups, (while mature cortical soluble monomeric Aβ increases with stress exposure in R121919-cohorts).

These changes in Aβ could result from alterations in APP processing or Aβ metabolism. As discussed above, CRFR1 may alter the localization of APP to lipid rafts; increasing the probability of internalization of APP into the early endocytic pathway and thus increasing the likelihood of amyloidogenic BACE1 cleavage. Stress is also linked to alterations in immunological responses through multiple avenues (Schramm & Waisman, 2022) (Kempuraj, et al., 2020) (Bisht, Sharma, & Tremblay, 2018) (Sathyanesan, Haiar, Watt, & Newton, 2017) (Fleshner, 2013). Additionally increases in soluble Aβ may lead to more efficient plaque clearance. To parse what aspect of plaque metabolism is affected by R121919, a future direction for this study may be to quantify AD-associated triggering receptors expressed on myeloid cells

2 (TREM2), danger-associated molecular patterns (DAMPS) alongside microglia, astrocytes, and neurons (Venegas & Heneka, 2016) (Li, et al., 2022).

It is important to note that the chronic stress protocol may not induce stress but instead may serve as enrichment. To understand the significance of this study, analysis of stress hormones in the plasma collected at intervals throughout the experiment would serve to identify how our chronic stress paradigm affects allostatic load. Furthermore, analysis and quantification Aβ species in the hippocampus, alongside cortical protein analysis, could help us determine how R121919 and/or stress affects AD-associated hippocampal atrophy. To delineate the mechanism that CRFR1 alters pathology, future studies could utilize the isolated RNA for transcriptomic changes.

Our findings support the therapeutic potential of CRFR1-antagonism, yet the antagonist used has already been rejected in clinical phase 2a studies for human depression treatment due to hepatic toxicity (Chen & Grigoriadis, 2005). New CRFR1-antagonists are currently being developed and will be analyzed for their therapeutic potential in AD. This exciting drug development research could lead to an effective treatment for AD pathogenesis.

Chapter 4,in part, is currently being prepared for submission. Maya Anne Ellisman is the primary researcher.

References

Aguilar, B. J. (2017). Rho GTPases as therapeutic targets in Alzheimer's disease. *Alzheimer's Research & Therapy, 9*(97).

Aisen, P. S., Davis, K. L., Berg, J. D., Schafer, K., Campbell, K., Thomas, R. G., & … Thal, L. J. (2000). A randomized controlled trial of prednisone in Alzheimer's disease. Alzheimer's Disease Cooperative Study. *Neurology, 54*(3), 588–93.

Aisen, P. S., Davis, K. L., Berg, J. D., Schafer, K., Campbell, K., Thomas, R. G., & … Thal, L. J. (2000). A randomized controlled trial of prednisone in Alzheimer's disease. Alzheimer's Disease Cooperative Study. *Neurology, 54*(3), 588–93.

Akhtar, M. W., Sanz-Blasco, S., Dolatabadi, N., Parker, J., Chon, K., Lee, M. S., . . . Lipton, S. A. (2016). Elevated glucose and oligomeric β-amyloid disrupt synapses via a common pathway of aberrant protein S-nitrosylation. *Nature Communications, 7*, 10242.

Alonso, A., Grundke-Iqbal, I., & Iqbal, K. (1996). Alzheimer's disease hyperphosphorylated tau sequesters normal tau into tangles of filaments and disassembles microtubules. *Nat Med*(2), 783-787.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (Vol. 5th ed.). Washington DC.

Andres, A. L., Regev, L., Phi, L., Seese, R. R., Chen, Y., Gall, C. M., & Baram, T. Z. (2013). NMDA Receptor Activation and Calpain Contribute to Disruption of Dendritic Spines by the Stress Neuropeptide CRH. *The Journal of Neuroscience, 33*(43), 16945–16960.

Arendash, G. W., Mori, T., Cao, C., Mamcarz, M., Runfeldt, M., Dickson, A., & al., e. (2009). Caffeine reverses cognitive impairment and decreases brain amyloid-β levels in aged Alzheimer's disease mice. *J. Alzheimers Dis. , 1*, 661-680.

Ariga, T., McDonald, M., & Yu, R. (2008). Role of ganglioside metabolism in the pathogenesis of Alzheimer's disease--a review. *J Lipid Res. , 49*(6), 1157-75.

Arriagada, P., Growdon, J., Hedley-Whyte, E., & Hyman, B. (1992). Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. . *Neurology*(42), 631-639.

Baglietto-vargas, D., Chen, Y., Suh, D., Ager, R. R., Carlos, J., Mederios, R., . . . LaFerla, F. (2015). Short-term modern life-like stress exacerbates Aβ-pathology and synapse loss in 3xTg-AD mice. *J Neurochem, 134*(5), 915–926.

Bagot, R. C., Labonté, B., Peña, C. J., & Nestler, E. J. (2014). Epigenetic signaling in psychiatric disorders: stress and depression. *Dialogues in Clinical Neuroscience, 16*(3), 281–295.

Bangasser, D. A., Dong, H., Carroll, J., Plona, Z., Ding, H., Rodriguez, L., . . . Valentino, R. J. (2017). Corticotropin-releasing factor overexpression gives rise to sex differences in Alzheimer ' s disease-related signaling. *Molecular Psychiatry, 22*(8), 1126–1133.

Bangasser, D., Curtis, A., Reyes, B., Bethea, T., Parastatidis, I., Ischiropoulos, H., & … Valentino, R. (2010). Sex differences in corticotropin-releasing factor receptor signaling and trafficking: potential role in female vulnerability to stress-related psychopathology. *Molecular Psychiatry, 1566*, 896–904.

Baumeister, D., & Pariante, C. M. (2015). The Interface of Stress and the HPA Axis in Behavioural Phenotypes Of Mental Illness. (S. L. Lightman, Ed.) *Behavioral Neurobiology of Stress-related Disorders, 18*, 13-24.

Behan, D., Heinrichs, S., Troncoso, J., Liu, X., Kawas, C., Ling, N., & De Souza, E. (1995). Displacement of CRF from its binding protein as a possible treatment for Alzheimer's disease. *Nature, 378*(6554), 284-287.

Benhar, M., Forrester, M., & Stamler, J. (2009). Protein denitrosylation: enzymatic mechanisms and cellular functions. *Nat Rev Mol Cell Biol. , 10*(10), 721-32.

Bisht, K., Sharma, K., & Tremblay, M.-È. (2018). Chronic stress as a risk factor for Alzheimer's disease: Roles of microglia-mediatedsynaptic remodeling, inflammation, and oxidative stress. *Neurobiology of Stress*.

Bissette, G., Reynolds, G. P., Kilts, C. D., Widerlöv, E., & Nemeroff, C. B. (1985). Corticotropin-Releasing Factor-like Immunoreactivity in Senile Dementia of the Alzheimer Type. *JAMA, 254*(21), 3067.

Bodovitz, S., & Klein, W. (1996). Cholesterol modulates a-secretase cleavage of amyloid precursor protein. *J. Biol. Chem*(271), 4436-4440.

Buggia-Prévot, V., Fernandez, C. G., Riordan, S., Vetrivel, K. S., Roseman, J., Waters, J., & al., e. (2014). Axonal BACE1 dynamics and targeting in hippocampal neurons: a role for Rab11 GTPase. *Mol. Neurodegener, 9*(1).

Campbell, S. N., Zhang, C., Monte, L., Roe, A. D., Rice, K. C., Taché, Y., . . . Rissman, R. A. (2015). Increased tau phosphorylation and aggregation in the hippocampus of mice overexpressing corticotropin-releasing factor. , 43(3), 967–976. . *Journal of Alzheimer's Disease, 43*(3), 967–976.

Campbell, S. N., Zhang, C., Roe, A. D., Lee, N., Lao, K. U., Monte, L., & Donohue, M. R. (2015). Impact of CRFR1 ablation on amyloid-β production and accumulation in a mouse model of Alzheimer's disease. . *Journal of Alzheimer's Disease, 45*(4), 1175–1184.

Cannon, W. (1929). *Bodily Changes in Pain, Hunger, Fear and Rage.* Appleton, New York.

Carroll, J., Iba, M., Bangasser, D., Valentino, R., James, M., Brunden, K., . . . Trojanowski, J. (2011). Chronic stress exacerbates tau pathology, neurodegeneration, and cognitive performance through a CRF receptor-dependent mechanism in a transgenic mouse model of tauopathy. *J. Neurosci, 31*, 14436-14449.

Chacon, P. J., Garcia-Mejias, R., & Rodriguez-Tebar, A. (2011). Inhibition of RhoA GTPase and the subsequent activation of PTP1B protects cultured hippocampal neurons against amyloid β toxicity. *Molecular neurodegeneration, 6*(1), 14.

Chakravarty, S., Reddy, B. R., Sudhakar, S. R., Saxena, S., Das, T., Meghah, V., & … Idris, M. M. (2013). Chronic Unpredictable Stress (CUS)-Induced Anxiety and Related Mood Disorders in a Zebrafish Model: Altered Brain Proteome Profile Implicates Mitochondrial Dysfunction. *PLoS ONE, 8*(5), e63302.

Chen, C., & Grigoriadis, D. E. (2005). NBI 30775 (R121919), an orally active antagonist of the corticotropin-releasing factor (CRF) type-1 receptor for the treatment of anxiety and depression. *Drug Development Research, 65*, 216-226.

Chen, C., Wilcoxen, K. M., Huang, C. Q., Xie, Y. F., McCarthy, J. R., Webb, T. R., & … 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.

Chen, R., Lewis, K. A., Perrin, M. H., & Vale, W. W. (1993). Expression cloning of a human corticotropin-releasing-factor receptor. *Proceedings of the National Academy of Sciences of the United States of America, 90*(19), 8967–8971.

Chen, Y., Andres, A. L., Frotscher, M., & Baram, T. Z. (2012). Tuning synaptic transmission in the hippocampus by stress: the CRH system. *Frontiers in Cellular Neuroscience, 6*(13).

Chen, Y., Molet, J., Lauterborn, J. C., Trieu, B. H., Bolton, J. L., Patterson, K. P., . . . Baram, T. Z. (2016). Converging, Synergistic Actions of Multiple Stress Hormones Mediate Enduring Memory Impairments after Acute Simultaneous Stresses. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 36*(44), 11295–11307.

Cheng, A., Wan, R., Yang, J.-L., Kamimura, N., Son, T. G., Ouyang, X., & … Mattson, M. P. (2012). Involvement of PGC-1α in the Formation and Maintenance of Neuronal Dendritic Spines. . *Nature Communications, 3*(1250).

Cheng, H., Wang, M., Li, J.-L., Cairns, N. J., & Han, X. (2013). Specific changes of sulfatide levels in individuals with preclinical Alzheimer's disease: an early event in disease pathogenesis. *Journal of Neurochemistry, 127*(6).

Das, U., Wang, L., Ganguly, A., Saikia, J. M., Wagner, S. L., Koo, E. H., & Roy, S. (2016). Visualizing APP and BACE-1 approximation in neurons yields insight into the amyloidogenic pathway. *Nature Neuroscience*(19), 55-64.

Davis, E. G., Keller, J., Hallmayer, J., Pankow, H. R., Murphy, G. M., Gotlib, I. H., & Schatzberg, A. F. (2018). Corticotropin-releasing factor 1 receptor haplotype and cognitive features of major depression. *Translational Psychiatry, 8*(5).

Davis, K., Mohs, R., Marin, D., Purohit, D., & Perl, D. (1999). Neuropeptide abnormalities in patients with early Alzheimer disease. *Arch Gen Psychiatry , 56*, 981-987.

de Quervain, D. J.-F., Poirier, R., Wollmer, M. A., Grimaldi, L. M., Tsolaki, M., Streffer, J. R., & Papassotiropoulos, A. (2003). Glucocorticoid-related genetic susceptibility for Alzheimer's disease. . *Human Molecular Genetics, 13*(1), 47–52.

De Souza, E., Whitehouse, P., Kuhar, M., Price, D., & Vale, W. (1986). Reciprocal changes in CRF-like immunoreactivity and CRF receptors in cerebral cortex of AD. *Nature, 319*, 593-595.

Dedic, N., Chen, A., & Deussing, J. M. (2017). The CRF Family of Neuropeptides and their Receptors - Mediators of the Central Stress Response. *Current Molecular Pharmacology, 10*.

Deussing, J. M., & Chen, A. A. (2018). The Corticotropin-Releasing Factor Family: Physiology of the Stress Response. . *Physiol Rev, 98*, 2225–2286.

Dong, H., Murphy, K., Meng, L., Montalvo-Ortiz, J., Zeng, Z., & Csernansky, J. (2011). Corticotrophin releasing factor accelerates neuropathology and cognitive decline in a mouse model of Alzheimer's. . *J Alz Dis, 28*, 579-92.

Du, H., Guo, L., Fang, F., Chen, D., Sosunov, A. A., Mckhann, G. M., . . . Yan, D. (2008). Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease. *Nature Medicine, 14*(10), 1097-1105.

Eckman, E., Watson, M., Marlow, L., Sambamurti, K., & Eckman, C. (2003). Alzheimer's disease beta-amyloid peptide is increased in mice deficient in endothelin-converting enzyme. *J Biol Chem, 278*(4), 2081-4.

Esch, F., Keim, P., Beattie, E., Blacher, R., Culwell, A., Oltersdorf, T., . . . Ward, P. (1990). Cleavage of amyloid beta peptide during constitutive processing of its precursor. *Science*(248), 1122-1124.

Esch, F., Ling, N., Bohlen, P., Baird, A., Benoit, R., & Guillemin, R. (1984). Isolation and characterization of the bovine hypothalamic corticotropin-releasing factor. *Biochemical and Biophysical Research Communications, 122*(3), 899-905.

Fleshner, M. (2013). Stress-evoked sterile inflammation, danger associated molecular patterns (DAMPs),microbial associated molecular patterns (MAMPs) and the inflammasome. *Brain, Behavior, and Immunity, 27*, 1-7.

Gamba, P., Testa, G., Sottero, B., Gargiulo, S., Poli, G., & Leonarduzzi, G. (n.d.). 2012. The link between altered cholesterol metabolism and Alzheimer's disease. *Ann. N. Y. Acad. Sci.* (1259), 54-64.

Goedert, M., Spillantini, M., Jakes, R., Rutherford, D., & Crowther, R. (1989). Multiple isoforms of human microtubule-associated protein tau: sequences, localization in neurofibrillary tangles of AD. . *Neuron*(3), 519-526.

Goodenowe, D., Cook, L., Liu, J., Lu, Y., Jayasinghe, D., Ahiahonu, P., . . . Wood, P. (2007). Peripheral ethanolamine plasmalogen deficiency: a logical causative factor in Alzheimer's disease and dementia. *J Lipid Res, 48*(11), 2485-98.

Gougoura, S., Liakos, P., & Koukoulis, G. (2010). Effect of CRH on NO bioavailability, ROS production and antioxidant defense systems in endothelial EAhy926 cells. *Free Radical Research, 44*(5), 803-812.

Gray, J. M., Vecchiarelli, H. A., Morena, M., Lee, T. T., Hermanson, D. J., Kim, A. B., . . . Hill, M. N. (2015). Corticotropin-Releasing Hormone Drives Anandamide Hydrolysis in the Amygdala to Promote Anxiety. *The Journal of Neuroscience, 35*(9), 3879–3892.

Grimm, M., Mett, J., Stahlmann, C., Haupenthal, V., Zimmer, V., & Hartmann, T. (2013). Neprilysin and Aβ Clearance: Impact of the APP Intracellular Domain in NEP Regulation and Implications in Alzheimer's Disease. *Frontiers in aging neuroscience, 5*(98).

Guo, Q., Zheng, H., & Justice, N. J. (2012). Central CRF system perturbation in an Alzheimer's disease knockin mouse model. *Neurobiology of Aging, 33*(11), 2678–2691.

Gustke, N., Steiner, B., Biernat, J., Meyer, H., & Mandelkow, E. (1992). The Alzheimer-like phosphorylation of tau protein reduces microtubule binding and involves Ser-Pro and Thr-Pro motifs. *FEBS Lett*(307), 199-205.

Hauger, R. L., Olivares-Reyes, J. A., Dautzenberg, F. M., Lohr, J. B., Braun, S., & Oakley, R. H. (2012). Molecular and cell signaling targets for PTSD pathophysiology and pharmacotherapy. *Neuropharmacology, 62*(2), 705–714.

Hemley, C. F., McCluskey, A., & Keller, P. A. (2007). Corticotropin releasing hormone - a GPCR drug target. *Current Drug Targets, 8*(1), 105–115.

Holcomb, L. G. (1998). Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nature Medicine, 4*(1), 97-100.

Holcomb, L., Gordon, M., Jantzen, P. H., Duff, K., & Morgan, D. (1999). Behavioral changes in transgenic mice expressing both amyloid precursor protein and presenilin-1 mutations: lack of association with amyloid deposits. *Behav Genet.*(3), 177-85.

Hopperton, K. E., Mohammad, D., Trépanier, M. O., Giuliano, V., & Bazinet, R. P. (2018). Markers of microglia in post-mortem brain samples from patients with Alzheimer's disease: a systematic review. *Molecular Psychiatry, 23*(2), 177–198.

Hroudová, J., Singh, N., & Fišar, Z. (2014). Mitochondrial Dysfunctions in Neurodegenerative Diseases: Relevance to Alzheimer's Disease. *BioMed Research International*(175056), 9.

Huesa, G., Baltrons, M. A., Gómez-Ramos, P., Morán, A., García, A., Hidalgo, J., . . . Galea, E. (2010). Altered Distribution of RhoA in Alzheimer's Disease and AβPP Overexpressing Mice. *Journal of Alzheimer's Disease, 19*(1), 37-56.

Hutton, M., Lendon, C., Rizzu, P., Baker, M., Froelich, S., Houlden, H., . . . Heutink, P. (1998). Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature*(393), 702-705.

Igarashi, M., Ma, K., Gao, F., Kim, H., Rapoport, S., & Rao, J. (2011). Disturbed choline plasmalogen and phospholipid fatty acid concentrations in Alzheimer's disease prefrontal cortex. *J Alzheimers Dis. , 24*(3), 507-17.

Iwata, N., Tsubuki, S., Takaki, Y., Shirotani, K., Lu, B., Gerard, N., . . . Saido, T. (2001). Metabolic regulation of brain Abeta by neprilysin. *Science, 292*(5521), 1550-2.

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.

Jiang, S., Wang, Y., Ma, Q., Zhou, A., Zhang, X., & Y.W., Z. (2012). M1 muscarinic acetylcholine receptor interacts with BACE1 and regulates its proteosomal degradation. *Neurosci Lett. , 515*, 125-130.

JS, B. (2004). The GGA proteins: Adaptors on the move. *Nat Rev Mol Cell Biol, 5*, 23-32.

Justice, N. J., Huang, L., Tian, J., Cole, A., Pruski, M., Hunt, A., . . . Zheng, H. (2015). Posttraumatic Stress Disorder-Like Induction Elevates Beta-Amyloid Levels, Which Directly Activates Corticotropin-Releasing Factor Neurons to Exacerbate Stress Responses. *Neurobiology of Disease, 35*(6), 2612–2623.

Kakio, A., Nishimoto, S., Yanagisawa, K., Kozutsumi, Y., & Matsuzaki, K. (2002). Interactions of Amyloid β-Protein with Various Gangliosides in Raft-Like Membranes: Importance of GM1 Ganglioside-Bound Form as an Endogenous Seed for Alzheimer Amyloid. *Biochemistry, 41*(23), 7385-90.

Kanekiyo, T., & Bu, G. (2014). The low-density lipoprotein receptor-related protein 1 and amyloid-beta clearance in Alzheimer's disease. *Frontiers in Aging Neuroscience, 6*(93).

Kang, J., Cirrito, J. R., Dong, H., Csernansky, J. G., & Holtzman, D. M. (2007). Acute stress increases interstitial fluid amyloid- ␤ via corticotropin-releasing factor and neuronal activity. *Proc Natl Acad Sci U S A, 104*(25), 10673–10678.

Kelmendi, B., Adams, T. G., Yarnell, S., Southwick, S., Abdallah, C. G., & Krystal, J. H. (2016). PTSD: from neurobiology to pharmacological treatments. (1), 1-11.

Kempuraj, D., Ahmed, M., Selvakumar, G., Thangavel, R., Raikwar, S., Zaheer, S., . . . Zaheer, A. (2020). Psychological StresseInduced Immune Response andRisk of Alzheimer's Disease in Veterans fromOperation Enduring Freedom and OperationIraqi Freedom. *Clinical Therapeutics, 42*(6), 974-982.

Kempuraj, D., Selvakumar, G. P., Thangavel, R., Ahmed, M. E., Zaheer, S., Raikwar, S. P., & … Zaheer, A. (2017). Mast Cell Activation in Brain Injury, Stress, and Post-traumatic Stress Disorder and Alzheimer's Disease Pathogenesis. . *Frontiers in Neuroscience, 11*, 703.

Kohout, T., & Lefkowitz, R. (2003). Regulation of G protein-coupled receptor kinases and arrestins during receptor desensitization. *Mol Pharmacol, 63*, 9-18.

Kojro, E., Gimpl, G., Lammich, S., Marz, W., & Fahrenholz, F. (2001). Low cholesterol stimulates the nonamyloidogenic pathway by its effect on alpha-secretase ADAM 10. *Proc. Natl. Acad. Sci.* (98), 5815-5820.

Kwak, Y.-D., Wang, R., Li, J., Zhang, Y.-W., Xu, H., & Liao, F.-F. (2011). Differential regulation of BACE1 expression by oxidative and nitrosative signals. *Molecular Neurodegeneration, 6*(1), 17.

Laporte, S., Oakley, R., Zhang, J., Holt, J., Ferguson, S., Caron, M., & Barak, L. (1999.). The beta2-adrenergic receptor/beta-arrestin complex recruits the clathrin adaptor AP-2 during endocytosis. *Proc Natl Acad Sci USA , 96*, 3712–3717.

Le, M. H., Weissmiller, A. M., Monte, L., Lin, P. H., Hexom, T. C., Natera, O., . . . Rissman, R. A. (2016). Functional Impact of Corticotropin-Releasing Factor Exposure on Tau Phosphorylation and Axon Transport. *PLoS ONE, 11*(1), 1–15.

Li, G., Kim, C., Kim, J., Yoon, H., Zhou, H., & Kim, J. (2015). Common Pesticide, Dichlorodiphenyltrichloroethane (DDT), Increases Amyloid-β Levels by Impairing the Function of ABCA1 and IDE: Implication for Alzheimer's Disease. *Journal of Alzheimer's Disease*(46), 109-122.

Li, R.-Y., Qin, Q., Yang, H.-C., Wang, Y.-Y., Mi, Y.-X., Yin, Y.-S., . . . Tang, Y. (2022). TREM2 in the pathogenesis of AD: a lipid metabolism regulator and potential metabolic therapeutic target. *Mol Neurodegeneration, 17*(40).

Lim, W. L. (2014). The involvement of lipids in alzheimer's disease. *Journal of Genetics and Genomics. Institute of Genetics and Developmental Biology.*

Ling, N., Esch, F., Böhlen, P., Baird, A., & Guillemin, R. (1984). Isolation and characterization of caprine corticotropin-releasing factor. *Biochemical and Biophysical Research Communications, 122*(3), 1218-1224.

Lupien, S. J., Fiocco, A., Wan, N., Maheu, F., Lord, C., Schramek, T., & Tu, M. T. (2004). Stress hormones and human memory function across the lifespan. *Psychoneuroendocrinology, 30*, 225–242.

Lupien, S. J., Fiocco, A., Wan, N., Maheu, F., Lord, C., Schramek, T., & Tu, M. T. (2004). Stress hormones and human memory function across the lifespan. . *Psychoneuroendocrinology, 30*, 225–242.

Magalhaes, A., Holmes, K., Dale, L., Comps-Agrar, L., Lee, D., Yadav, P., . . . al., e. (2010). CRF receptor 1 regulates anxiety behavior via sensitization of 5-HT2 receptor signaling. *Nat Neurosci, 12*, 622-629.

Marquer, C., Devauges, V., Cossec, J., Liot, G., Le´cart, S., Saudou, F., ... (2011). Local cholesterol increase triggers amyloid precursor protein-BACE 1 clustering in lipid rafts and rapid endocytosis. *FASEB J.* (25), 1295-1305.

Mcewen, B., & Stellar, E. (1993). Stress and the individual. Mechanisms leading to disease. *E Arch Intern Med*(153), 2093–2101.

McLean, C., Cherny, R., Fraser, F., Fuller, S., Smith, M., Beyreuther, K., . . . Master, C. (1999). Soluble pool of amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann Neurol*(46), 860-866.

Mishra, M., & Heese, K. (2011). P60TRP interferes with the GPCR/secretase pathway to mediate neuronal survival and synaptogenesis. *J. Cell. Mol. Med. , 15*, 2462-2477.

Murat, B., Devost, D., Andrés, M., Mion, J., Boulay, V., Corbani, M., . . . Guillon, G. (2012). V1b and CRHR1 receptor heterodimerization mediates synergistic bi-ological actions of vasopressin and CRH. *Mol Endocrinol, 26*, 502-520.

Nakamura, T., Cieplak, P., Cho, D.-H., Godzik, A., & Lipton, S. A. (2010). S-Nitrosylation of Drp1 links excessive mitochondrial fission to neuronal injury in neurodegeneration. *Mitochondrion, 10*(5), 573-578.

Nakamura, T., Tu, S., Akhtar, M. W., Sunico, C. R., Okamoto, S.-I., & Lipton, S. A. (2013). Aberrant Protein S-Nitrosylation in Neurodegenerative Diseases. *Nature, 78*.

Navarro, G., Quiroz, C., Moreno-Delgado, D., Sierakowiak, A., McDowell, K., Moreno, E., . . . al., e. (2015). Orexin-corticotropin-releasing factor receptor heteromers in the ventral tegmental area as targets for cocaine. *J Neurosci, 35*, 6639-6653.

Oakley, R. H., Laporte, S. A., Holt, J. A., Caron, M. G., & Barak, L. S. (2000). Differential affinities of visual arrestin, beta arrestin1, and beta arrestin2 for G protein-coupled receptors delineate two major classes of receptors. *The Journal of Biological Chemistry, 275*(22), 17201–109.

Oakley, R. H., Olivares-Reyes, J. A., Hudson, C. C., Flores-Vega, F., Dautzenberg, F. M., & Hauger, R. L. (2007). Carboxyl-terminal and intracellular loop sites for CRF1 receptor phosphorylation and β-arrestin-2 recruitment: a mechanism regulating stress and anxiety responses. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology, 293*(1), R209-R222.

O'Connor, T., Doherty-Sadleir, K. R., Maus, E., Velliquette, R. A., Zhao, J., Cole, S. L., . . . Vassar, R. (2008). Phosphorylation of the Translation Initiation Factor eIF2α Increases BACE1 Levels and Promotes Amyloidogenesis. *Neuron, 60* (6), 988–1009.

Okawara, Y., Morley, S. D., Burzio, L. O., Zwiers, H., Lederis, K., & Richter, D. (1988). Cloning and sequence analysis of cDNA for corticotropin-releasing factor precursor from the teleost fish Catostomus commersoni. *Proceedings of the National Academy of Sciences of the United States of America, 85*(22), 8439-8443.

Panneerselvam, M., Ali, S. S., Finley, J. C., Kellerhals, S. E., Migita, M. Y., Head, B. P., & … Patel, H. H. (2013). Epicatechin regulation of mitochondrial structure and function is opioid receptor dependent. *Mol Nutr Food Res, 57*(6).

Pardo-Moreno, T., González-Acedo, A., Rivas-Domínguez, A., García-Morales, V., García-Cozar, F., Ramos-Rodríguez, J., & Melguizo-Rodríguez, L. (2022). Therapeutic Approach to Alzheimer's Disease: Current Treatments and New Perspectives. *Pharmaceutics, 14*(1117).

Parikh, P. K., Troyer, A. K., Maione, A. M., & Murphy, K. J. (2016). The Impact of Memory Change on Daily Life in Normal Aging and Mild Cognitive Impairment. . *The Gerontologist, 56*(5), 877–885.

Park, H. J., Ran, Y., Jung, J. I., Holmes, O., Price, A. R., Smithson, L., . . . Felsenstein, K. (2015). The stress response neuropeptide CRF increases amyloid-β production by regulating γ -secretase activity. *EMBO J., 34*, 1674-1686.

Park, H., Ran, Y., Jung, J. I., Holmes, O., Price, A. R., Smithson, L., . . . Felsenstein, K. M. (2015). The stress response neuropeptide CRF increases amyloid- b production by regulating c -secretase activity, . *The EMBO Journal, 34*(12), 1674-1686.

Patthy, M., Horvath, J., Mason-Garciat, M., Szoke, B., Schlesingert, D. H., & Schally, A. V. (1985). Isolation and amino acid sequence of corticotropin-releasing factor from pig hypothalami. . *Medical Sciences, 82*, 8762–8766.

Perrin, M., Donaldson, C., Chen, R., Blount, A., Berggren, T., Bilezikjian, L., & … Vale, W. (1995). Identification of a second corticotropin-releasing factor receptor gene and characterization of a cDNA expressed in heart. *Biochemistry, 92*, 2969–2973.

Podcasy, J. L., & Epperson, C. N. (2016). Considering sex and gender in Alzheimer disease and other dementias. *Dialogues in Clinical Neuroscience, 18*(4), 437–446.

Powers, R., Walker, L., De Souza, E., Vale, W., Struble, R., Whitehouse, P., & Price, D. (1987). Immunohistochemical study of neurons containing CRF in Alzheimer's disease. *Synapse, 1*, 405-410.

Powers, R., Walker, L., De Souza, E., Vale, W., Struble, R., Whitehouse, P., & Price, D. (1987). Immunohistochemical study of neurons containing CRF in Alzheimer's disease. *Synapse, 1*, 405-410.

Querfurth, H. W., & Laferla, F. M. (2010). Mechanism of Disease: Alzheimer's Disease. *The New England Journal of Medicine, 362*(4), 329-344.

Raadsheer, F., van Heerikhuize, J., Lucassen, P., Hoogendijk, W., Tilders, F., & Swaab, D. (1995). CRH mRNA in the paraventricular nucleus of patients with Alzheimer's disease and depression. *Am J Psych , 152*(9), 1372-76.

Reddy, P. H. (2011). Abnormal Tau, Mitochondrial Dysfunction, Impaired Axonal Transport of Mitochondria, and Synaptic Deprivation in Alzheimer's Disease. *Brain Res.* (1415), 136–148.

Refolo, L., Pappolla, M., Malester, B., LaFrancois, J., Bryant-Thomas, T., Wang, R., . . . Duff, K. (2000). Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. *Neurobiol. Dis.* (7), 321-331.

Reul, J. M., & Holzboer, F. (2002). Corticotropin-releasing factor receptors 1 and 2 in anxiety and depression. *Current Opinion in Pharmacology, 2*, 23-33.

Rissman, R. (2009). Stress induced tau-P: functional neuroplasticity,neuronal vulnerability. *J Alz Dis* , 453-57.

Rissman, R., Lee, K., Vale, W., & Sawchenko, P. (2007). Corticotropin-releasing factor receptors differentially regulate stress-induced tau phosphorylation. . *J Neurosci , 27*, 6552-6562.

Rissman, R., Staup, M., Lee, A., Justice, N., Rice, K., Vale, W., & Sawchenko, P. (2012). CRF receptor-dependent effects of repeated stress on tau phosphorylation, solubility, aggregation. *PNAS, 109*, 6277-82.

Rivier, C., Rivier, J., & Vale, W. (1982). Inhibition of adrenocorticotropic hormone secretion in the rat by immunoneutralization of corticotropin-releasing factor. . *Science, 218*(4570), 377-9.

Rivier, J., Rivier, C., & Vale, W. (1984). Synthetic competitive antagonists of corticotropin-releasing factor: effect on ACTH secretion in the rat. . *Science , 224*(4651), 889–91.

Rivier, J., Spiess, J., & Vale, W. (1983). Characterization of rat hypothalamic corticotropin-releasing factor. *Proceedings of the National Academy of Sciences of the United States of America, 80*(15), 4851-4855.

Salemi, J., Obregon, D. F., Cobb, A., Reed, S., Sadic, E., Jin, J., & … Giunta, B. (2011). Flipping the switches: CD40 and CD45 modulation of microglial activation states in HIV associated dementia (HAD). *Molecular Neurodegeneration, 6*(3).

Sannerud, R., Declerck, I., Peric, A., Raemaekers, T., Menendez, G., Zhou, L., & al., e. (2011). ADP ribosylation factor 6 (ARF6) controls amyloid precursor protein (APP) processing by mediating the endosomal sorting of BACE1. *Proc. Natl. Acad. Sci. , 108*, E559-E568.

Sapolsky, R., Krey, L., & McEwen, B. (1985). Prolonged glucocorticoid exposure reduces hippocampal neuron number: implications for aging. *J Neurosci , 5*, 1222-1227.

Sathyanesan, M., Haiar, J., Watt, M., & Newton, S. (2017). Restraint stress differentially regulates inflammation and glutamate receptor gene expression in the hippocampus of C57BL/6 and BALB/c mice. *Stress, 20*(2), 197–204.

Schmeltzer, S. N., Herman, J. P., & Sah, R. (2016). Neuropeptide Y (NPY) and posttraumatic stress disorder (PTSD): A translational update. *Experimental Neurology*(284), 196–210.

Schramm, E., & Waisman, A. (2022). Microglia as Central Protagonists in the Chronic Stress Response. *Neurology Neuroimmunology Neuroinflammation, 9*(e200023).

Scullion, G. A., Hewitt, K. N., & Pardon, M.-C. (203). Corticotropin-Releasing Factor Receptor 1 Activation During Exposure to Novelty Stress Protects Against Alzheimer's Disease-Like Cognitive Decline in APP/PS1 Mice. *Journal of Alzheimer's Disease, 34*, 781–793.

Scullion, G., Hewitt, K., & Pardon, M. (2013). Corticotropin-releasing factor receptor 1 activation during exposure to novelty stress protects against Alzheimer's disease-like cognitive decline in AβPP/PS1 mice. *J Alzheimers Dis. , 34*(3), 781-93.

Selkoe, D. (2001). Alzheimer's disease: genes, proteins, and therapy. *Physiol. Rev.*(81), 741-766.

Shahani, N., Pryor, W., Swarnkar, S., Kholodilov, N., Thinakaran, G., Burke, R. E., & al., e. (2014). Rheb GTPase regulates β-secretase levels and amyloid β generation. *J. Bio. Chem. , 289*, 5799-5808.

Shibahara, S., Morimoto, Y., Furutani, Y., Notake, M., Takahashi, H., Shimizu, S., & … Numa, S. (1983). Isolation and sequence analysis of the human corticotropin-releasing factor precursor gene. *The EMBO Journal, 2*(5), 775-779.

Shie, F.-S., Jin, L.-W., Cook, D., Leverenz, J., & LeBoeuf, R. (2002). Dietinduced hypercholesterolemia enhances brain Ab accumulation in transgenic mice. (13), 455-459.

Simons, M., Keller, P., Strooper, B., Beyreuther, K., Dotti, C., & Simons, K. (1998). Cholesterol depletion inhibits the generation of b-amyloid in hippocampal neurons. *Proc. Natl. Acad. Sci.*(95), 6460-6464.

Sinha, S., Anderson, J. P., Barbour, R., Basi, G. S., Caccavello, R., & Davis, D. e. (1999). Purification and cloning of amyloid precursor protein β-secretase from human brain. *Nature, 402*, 537–540.

Smith, G., Aubry, J., Dellu, F., Contarino, A., Bilezikjian, L., & al., e. (1998). CRF-1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. *Neuron, 20*, 1093-02.

Smith, J. S., & Rajagopal, S. (2016). The Regulators of G Protein-coupled Beta-Arrestins: Multifunctional Receptors. *Journal of Biological Chemistry, 291*(17), 8969-8977.

Soriano, S., Chyung, A., Chen, X., Stokin, G., Lee, V., & Koo, E. (1999). Expression of beta-amyloid precursor protein-CD3 gamma chimeras to demonstrate the selective generation of amyloid beta(1-40) and amyloid beta(1-42) peptides within secretory and endocytic compartments. *J. Biol. Chem.* (274), 32295-32300.

Sparks, D., Scheff, S., Hunsaker 3rd, J., Liu, H., Landers, T., & Gross, D. (1994). Induction of Alzheimer-like b-Amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. *Exp. Neurol.*(126), 88-94.

Spierling, S. R., & Zorrilla, E. P. (2017). Don't stress about CRF: assessing the translational failures of CRF1antagonists. . *Psychopharmacology, 234*(9–10), 1467–1481.

Stenzel-Poore, M. P., Heldwein, K. A., Stenzel, P., Lee, S., & Vale, W. W. (1992). Characterization of the genomic corticotropin-releasing factor (CRF) gene from Xenopus laevis: two members of the CRF family exist in amphibians. *Molecular Endocrinology, 6*(10), 1716–1724.

Swanson, L. W., Sawchenko, P. E., Rivier, J., & Vale, W. W. (1983). Organization of Ovine Corticotropin-Releasing Factor Immunoreactive Cells and Fibers in the Rat Brain: An Immunohistochemical Study. *Neuroendocrinology , 36*(3), 165–186.

Teichmann, A., Rutz, C., Kreuchwig, A., Krause, G., Wiesner, B., & Schülein, R. (2012). The Pseudo signal peptide of the corticotropin-releasing factor receptor type 2A pre- vents receptor oligomerization. *J Biol Chem, 287*, 27265–27274.

Teng, L., Zhao, J., Wang, F., Ma, L., & Pei, G. (2010). A GPCR/secretase complex regulates β- and γ -secretase specificity for Aβ production and contributes to AD pathogenesis . *Cell Res, 20*, 138-153.

Thathiah, A., & De Strooper, B. (2009). Gprotein-coupled receptors, cholinergic dysfunction and Aβ toxicity in Alzheimer's disease . *2*.

Tolar, M., Hey, J., Power, A., & Abushakra, S. (2021). Neurotoxic Soluble Amyloid Oligomers Drive Alzheimer'sPathogenesis and Represent a Clinically Validated Target forSlowing Disease Progression. *International Journal ofMolecular Sciences, 22*(12), 6355.

Tsigos, C., Kyrou, I., Kassi, E., & Chrousos, G. P. (2000). Stress, Endocrine Physiology and Pathophysiology. . Endotext. MDText.com, Inc. .

Vale, W., Spiess, J., Rivier, C., & Rivier, J. (1981). Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science, 213*, 1394-97.

van Echten-Deckert, G., & Walter, J. (2012). Sphingolipids: critical players in Alzheimer's disease. *Prog Lipid Res. , 51*(4), 378-93.

Vassar, R., Bennett, B., Babu-Khan, S., Kahn, S., Mendiaz, E., Denis, P., . . . Citron, M. (1999). Betasecretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. . *Science*(286), 735-741.

Vassar, R., Kovacs, D., Yan, R., & Wong, P. (2009). The beta-secretase enzyme BACE in health and Alzheimer's disease: Regulation, cell biology, function, and therapeutic potential. *J. Neurosci, 29*, 12787-12794.

VAUGHAN, J. M., FISCHER, W. H., HOEGER, C., RIVIER, J., & VALE, W. (1989). Characterization of Melanin-Concentrating Hormone from Rat Hypothalamus. *Endocrinology, 125*(3), 1660–1665. .

Vekrellis, K., Ye, Z., Qiu, W., Walsh, D., Hartley, D., Chesneau, V., . . . Selkoe, D. (2000). Neurons regulate extracellular levels of amyloid beta-protein via proteolysis by insulin-degrading enzyme. *Journal of Neuroscience*(5), 1657-1665.

Venegas, C., & Heneka, M. (2016). Danger-associated molecular patterns in Alzheimer's disease. *Journal of Leukocyte Biology, 101*(1), 87-98.

Walther, C., Ferguson, S. S., & Taylor, J. A. (2013). Arrestins: Role in the Desensitization, Sequestration, and Vesicular Trafficking of G Protein-Coupled Receptors. *Progress in Molecular Biology and Translational Science, 118*, 93–113.

Wang, B., You, Z., & Wise, R. (2009). Reinstatement of cocaine seeking by hypocretin (orexin) in the ventral tegmental area: independence from the local corticotropin-releasing factor network. *Biol Psychiatry, 65*, 857-862.

Wang, J., Dickson, D., Trojanowski, J., & Lee, V. (1999). The levels of soluble versus insoluble brain Abeta distinguish Alzheimer's disease from normal and pathologic aging. *Exp Neurol* (158), 328-337.

Wang, J., Schilling, J. M., Niesman, I. R., Headrick, J. P., Finley, J. C., Kwan, E., & … Patel, H. H. (2014). Cardioprotective Trafficking of Caveolin to Mitochondria Is Gi-protein Dependent. *Anesthesiology, 121*(3), 538–548.

Wang, M.-M., Miao, D., Cao, X.-P., Tan, L., & Tan, L. (2018). Innate immune activation in Alzheimer's disease. *Annals of Translational Medicine, 6*(10), 177.

Wang, X., Su, B., Zheng, L., Perry, G., Smith, M. A., & Zhu, X. (2009). The role of abnormal mitochondrial dynamics in the pathogenesis of Alzheimer's disease . *Journal of Neurochemistry, 109*, 153-159.

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), 1479–85.

Yan, Y., & Wang, C. (2006). Aβ42 is More Rigid than Aβ40 at the C Terminus: Implications for Aβ Aggregation and Toxicity. . *Journal of Molecular Biology, 364*(5), 853–862.

Yao, Y., Chinnici, C., Tang, H., Trojanowski, J., Lee, V., & Practico`, D. (2004). Brain inflammation and oxidative stress in a transgenic mouse model of Alzheimer-like brain amyloidosis. *J. Neuroinflammation*(1), 21.

Yu, C., Nwabuisi-Heath, E., Laxton, K., & LaDu, M. J. (2010). Endocytic pathways mediating oligomeric Aβ42 neurotoxicity. *Molecular neurodegeneration, 5*(1), 19.

Züchner, T., Perez-Polo, J. R., & Schliebs, R. (2004). β-secretase BACE1 is differentially controlled through muscarinic acetylcholine receptor signaling. *J. Neurosci. Res., 77*, 250-257.

Zeitlin, R., Patel, S., Burgess, S., Arendash, G. W., & Echeverria, V. (2011). Caffeine induces beneficial changes in PKA signaling and JNK and ERK activities in the striatum and cortex of Alzheimer's transgenic mice. *Brain Res., 1417*, 127-136.

Zhang, C., & Rissman, R. A. (2017). Corticotropin-releasing factor receptor-1 modulates biomarkers of DNA oxidation in Alzheimer ' s disease mice. *PLoS ONE, 12*(7), 1–12.

Zhang, C., Kuo, C., Moghadam, S. H., Monte, L., Campbell, S. N., Rice, K. C., . . . 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 & Dementia, 12*(5), 527–537.

Zhang, L. F., Shi, L., Liu, H., Meng, F. T., Liu, Y. J., Wu, H. M., . . . Zhou, J. N. (2012). Increased hippocampal tau phosphorylation and axonal mitochondrial transport in a mouse model of chronic stress. . *International Journal of Neuropsychopharmacology, 15*(3), 337-348.

Zhang, L., Shi, L., Liu, H., Meng, F., Liu, Y., & Wu, H. (2012). Increased hippocampal tau phosphorylation and axonal mitochondrial transport in a mouse model of chronic stress. *International Journal of Neuropsychopharmacology, 15*, 337–348.

Zhao, J., Deng, Y., Jiang, Z., & Qing, H. (2016). G Protein-Coupled Receptors (GPCRs) in Alzheimer ' s Disease : A Focus on BACE1 Related GPCRs. *Frontiers in Aging Neuroscience, 8*, 1-15.

Figures

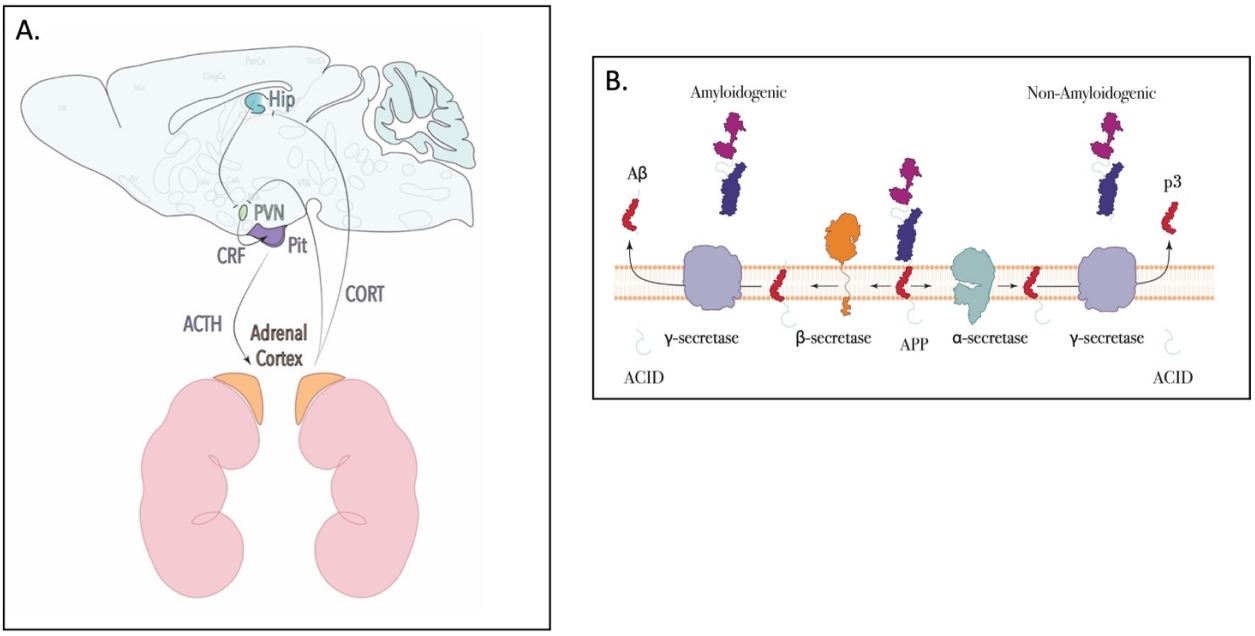

Figure 2. HPA axis and Amyloid Precursor Protein (APP) processing.

An illustration describing the HPA axis (A). The HPA axis integrates stress-processing fibers from the limbic and mid-hindbrain at the hypothalamic paraventricular nucleus (PVN) parvocellular neurons. This can trigger the neurons in the hypothalamic median eminence to release corticotropin-releasing factor (CRF) and vasopressin (AVP) into the pituitary portal vessel's capillaries, resulting in effects at the anterior pituitary. CRF can drive these pituitary neurons to produce and release adrenocorticotrophic hormone (ACTH, also known as corticotropin) in the bloodstream. CRF can promote additional alterations to the body once released into the bloodstream. ACTH is shuttled through the blood to the adrenocortical cells of the adrenal gland zona fasciculata, where it can bind to melanocortin type 2 receptors (MC2R) generating the production and release of glucocorticoids (CORTs) into the bloodstream. CORTs provide feedback inhibition on both the PVN and the anterior pituitary, reducing the production of respective hormones *(Baumeister & Pariante, 2015)*. An illustration of APP processing described in the text above (B).

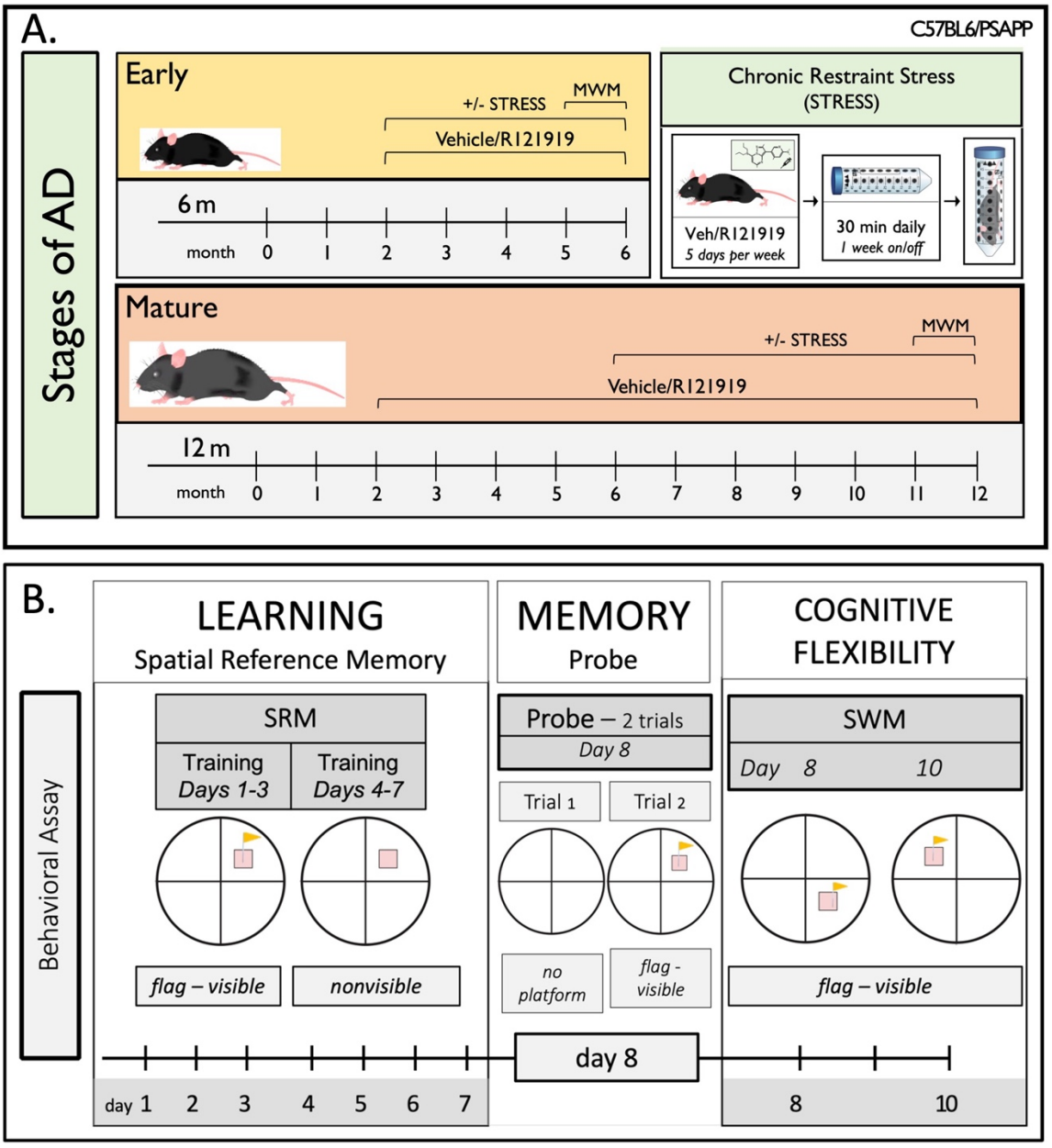

Figure 3. Timeline of experiment and behavior.

Outline of the timeline of stress and injections for 6- and 12- month cohorts (A top left and bottom, respectively), chronic restraint stress paradigm (A top right), and behavioral Morris Water Maze assay (B).

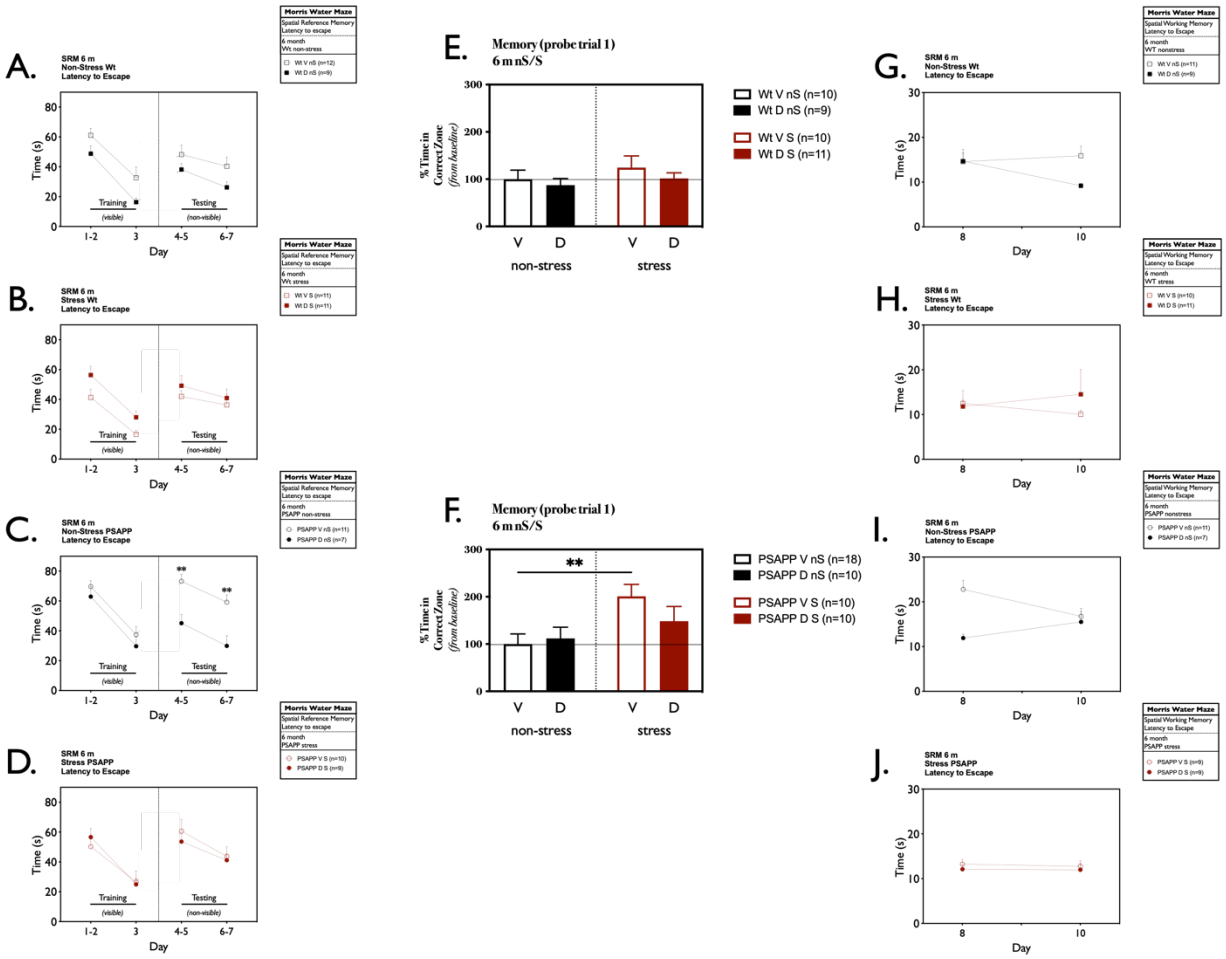

Figure 4. Morris Water Maze Behavior of early cohorts.

Behavioral data for early (6- month) (A-J) cohorts. The results for Morris Water Maze spatial working memory learning task's latency to escape for Tw (A), stress-exposed Wt (B), PSAPP (C), and stress-exposed PSAPP groups (D) display that CRFR1-antagonism significantly improves early PSAPP learning ability. The memory task for spatial working memory task shows that all subjects were able to find the platform (trial 2 not shown). Memory (trial 2 E & F) was not significantly improved with CRFR1-antagonism in both Wt (E) and PSAPP (F) groups, yet stress significantly increased the percent time PSAPP vehicle-treated cohorts spent in the correct zone (F) while CRFR1-antagonism lowered this stress effect to nonsignificant levels. Spatial working memory was not significantly affected by CRFR1-treatment or stress in both Wt (non-stress (G) and stress (H)) and PSAPP (non-stress (I) and stress (J)) early cohorts. *$p<0.05$, **$p<0.01$, ***$p<0.001$. All values are expressed as ± SEM, Wt V nS (n= 12), Wt D nS (n=9), Wt V S (n=11), Wt D S (n=11), PSAPP V nS (n=11), PSAPP D nS (n=7), PSAPP V S (n=10), PSAPP D S (n=9).

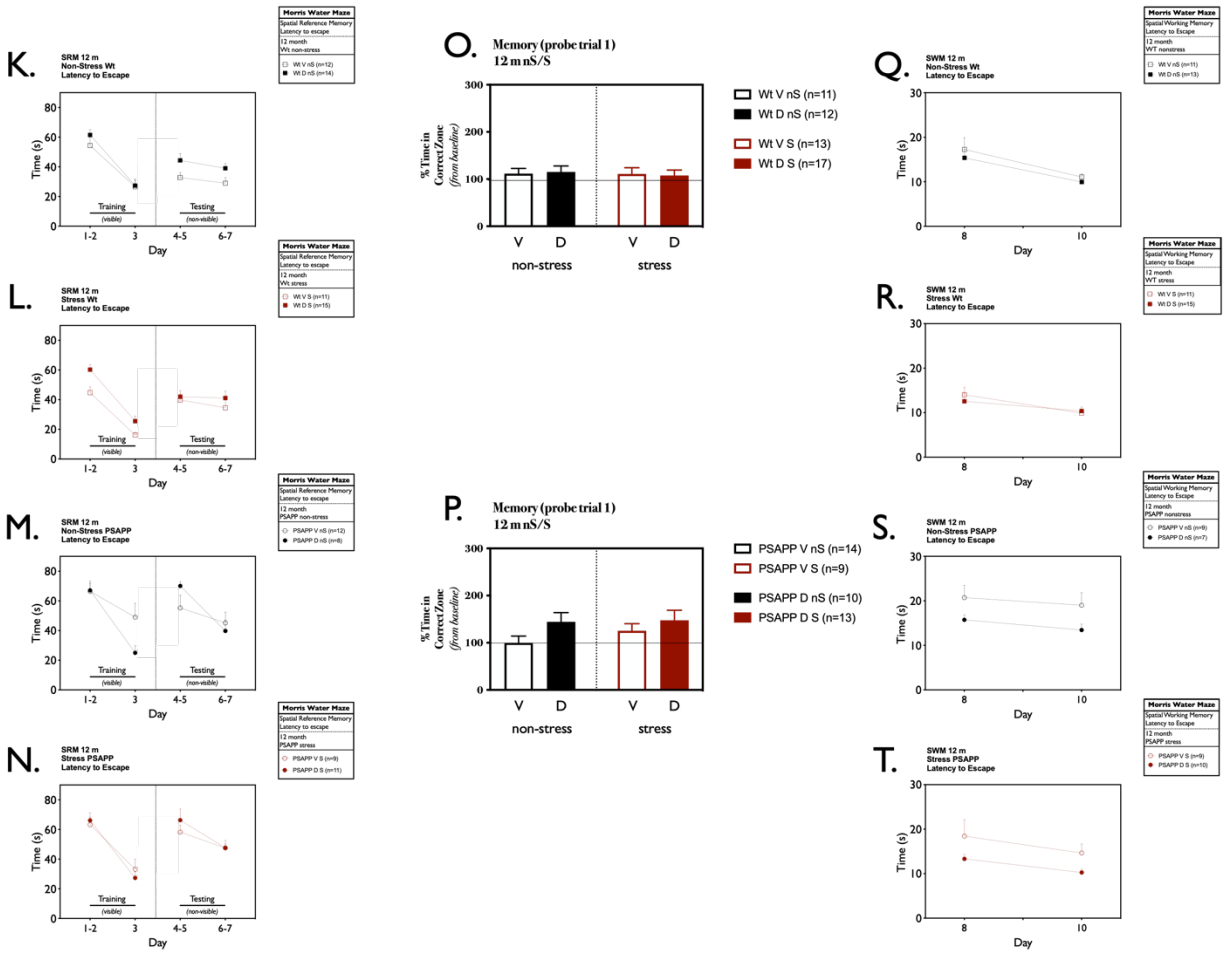

Figure 4 continued. Morris Water Maze Behavior of mature cohorts.

Treatment with CRFR1-antagonist and/or stress did not alter mature cohorts' behavior in Morris Water Maze tasks. The results for Morris Water Maze spatial working memory learning task's latency to escape for Wt (K), stress exposed Wt (L), PSAPP (M), and stress exposed PSAPP groups (N) display that CRFR1-antagonism did not alter mature cohorts' learning ability. The memory task for spatial working memory shows that all subjects were able to find the platform (trial 2 not shown). Memory (trial 2 O & P) was not significantly altered with CRFR1-antagonism and/or stress in both Wt (O) and PSAPP (P) groups. Spatial working memory was not significantly affected by CRFR1-treatment or stress in both Wt (non-stress (Q) and stress (R)) and PSAPP (non-stress (S) and stress (T)) mature cohorts. *p<0.05, **p<0.01, ***p<0.001. All values are expressed as ± SEM, Wt V nS (n= 12), Wt D nS (n=14), Wt V S (n=11), Wt D S (n=15), PSAPP V nS (n=12), PSAPP D nS (n=8), PSAPP V S (n=9), PSAPP D S (n=11).

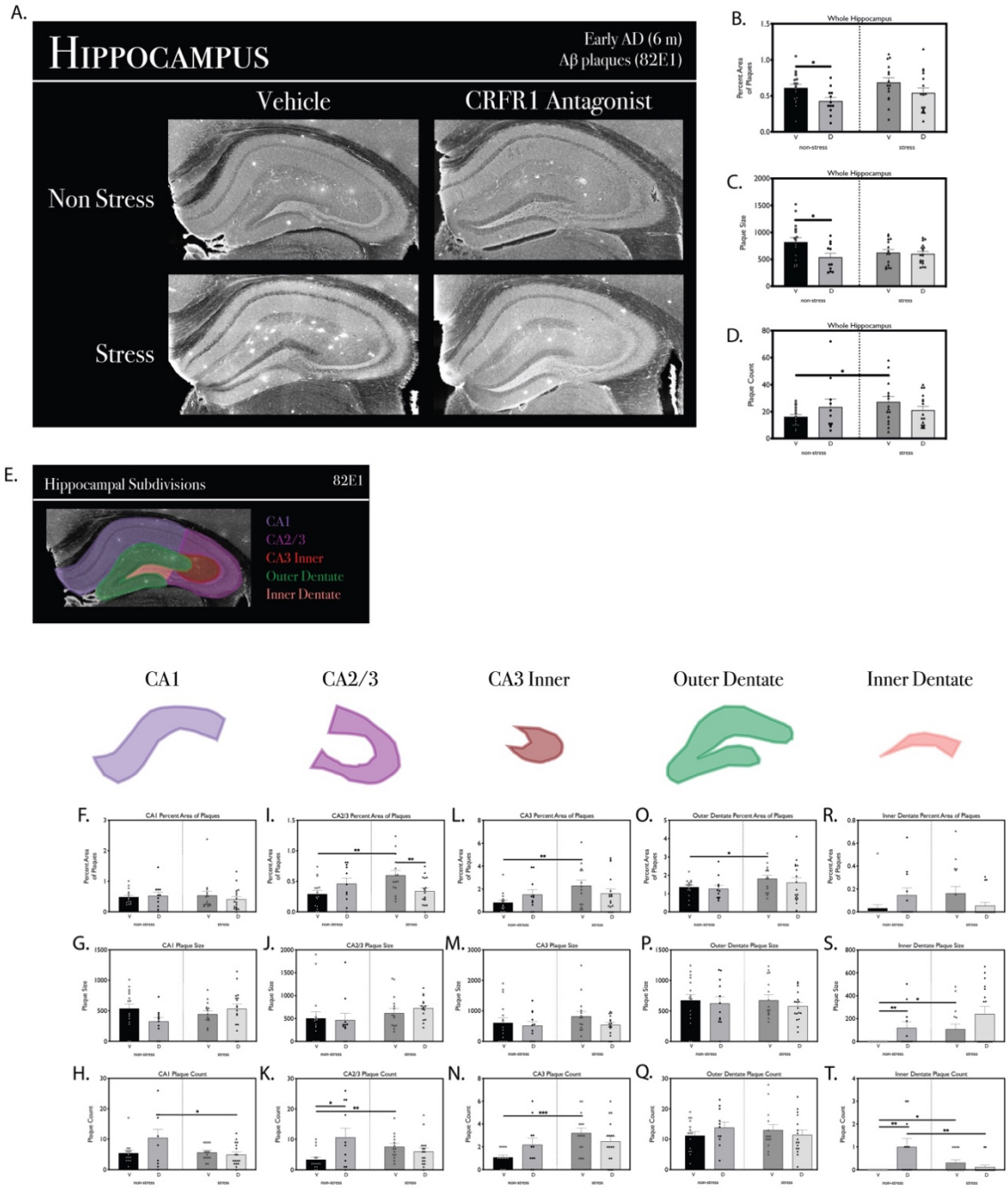

Figure 5. Early AD hippocampal plaque immunohistochemistry.

Example comparative images of early AD hippocampal plaque analysis (A) and delineation of hippocampal subregions quantified (E). Quantification of young whole hippocampal (B-D), CA1 (F-H), CA2/3 (I-K), CA3 inner (L-N), outer dentate (O-Q), and inner dentate (R-T) 82E1-stained plaque deposition. Percent area (top row of graphs), plaque size (middle row of graphs), and plaque number (bottom row of graphs) were quantified in coronal brain sections of the hippocampus. *p<0.05, **p<0.01, ***p<0.001. All values are expressed as ± SEM, PSAPP V nS (n=17), PSAPP D nS (n=10), PSAPP V S (n=10), PSAPP D S (n=10).

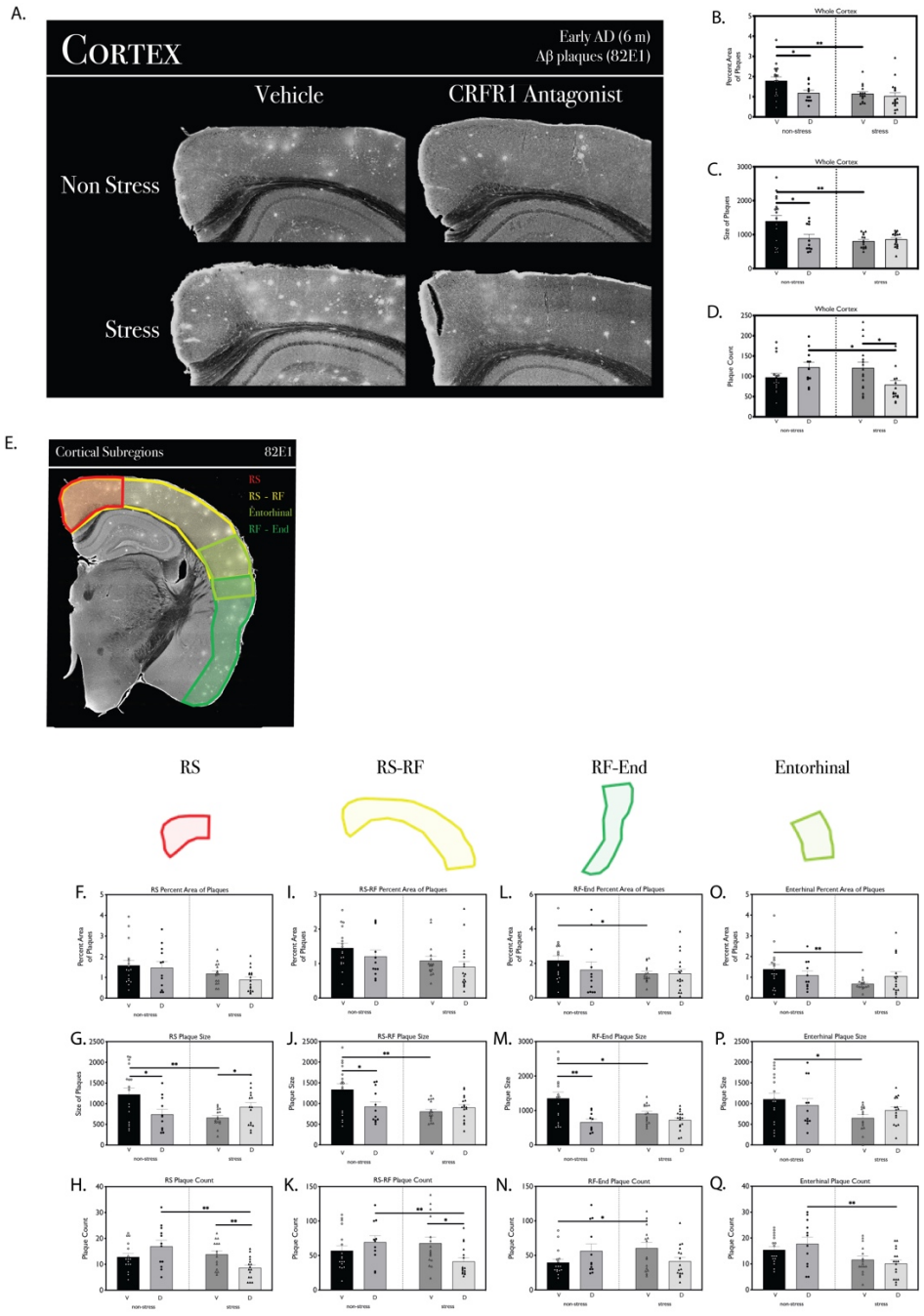

Figure 6. Early AD cortical plaque immunohistochemistry.

Example comparative images of early AD cortical plaque analysis (A) and delineation of cortical subregions quantified (E). Quantification of young whole cortical (B-D), RS (F-H), RS-RF (I-K), RF-End (L-N), and Entorhinal (O-Q) 82E1-stained plaque deposition. Percent area (top row of graphs), plaque size (middle row of graphs), and plaque number (bottom row of graphs) were quantified in coronal brain sections of the cortex. *p<0.05, **p<0.01, ***p<0.001. All values are expressed as ± SEM, PSAPP V nS (n=17), PSAPP D nS (n=10), PSAPP V S (n=10), PSAPP D S (n=10).

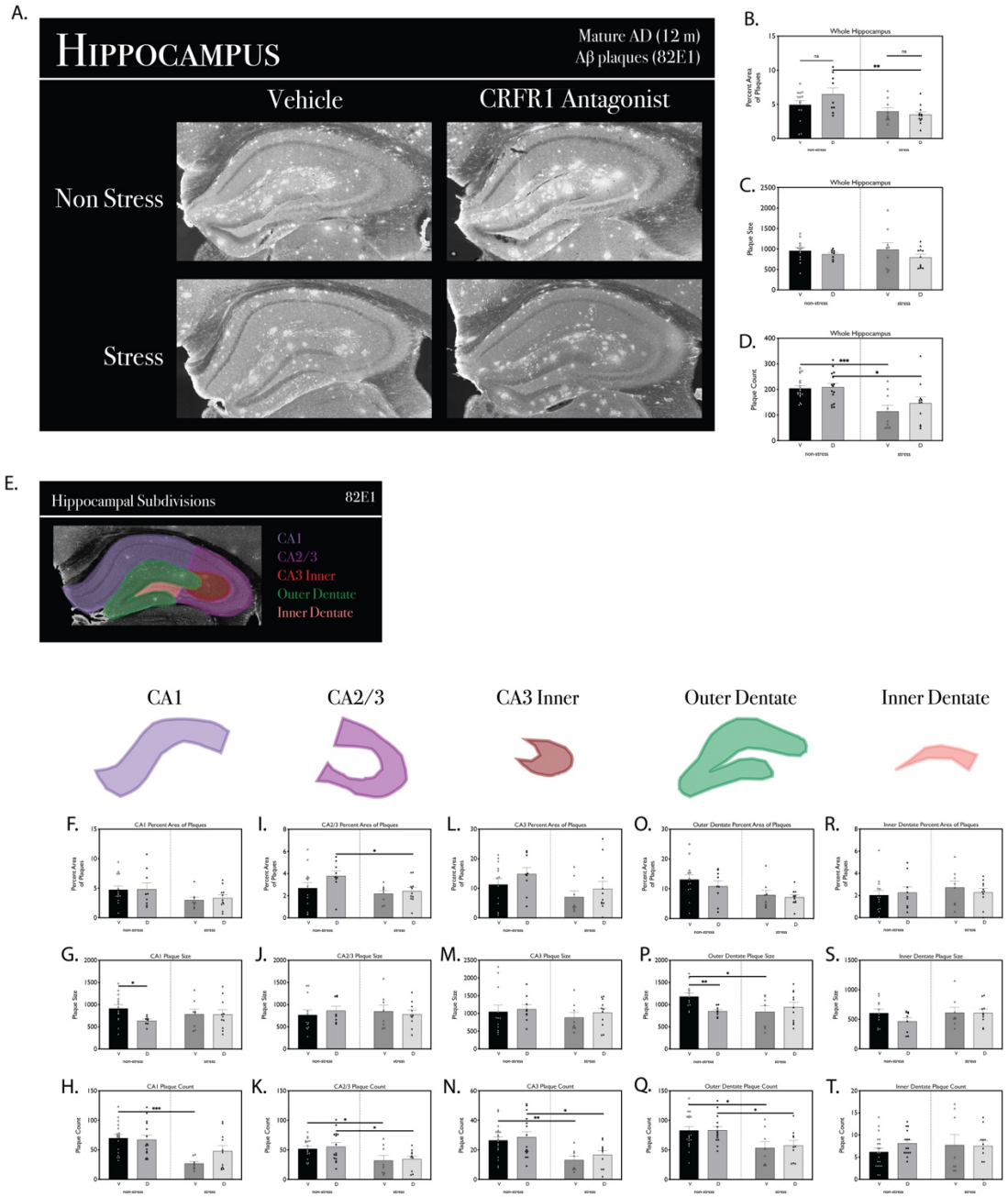

Figure 7. Mature hippocampal plaque immunohistochemistry.

Example comparative images of mature hippocampal plaque analysis (A) and delineation of hippocampal subregions quantified (E). Quantification of mature whole hippocampal (B-D), CA1 (F-H), CA2/3 (I-K), CA3 inner (L-N), outer dentate (O-Q), and inner dentate (R-T) 82E1-stained plaque deposition. Percent area (top row of graphs), plaque size (middle row of graphs), and plaque number (bottom row of graphs) were quantified in coronal brain sections of the hippocampus. *p<0.05, **p<0.01, ***p<0.001. All values are expressed as ± SEM, PSAPP V nS (n=12), PSAPP D nS (n=12), PSAPP V S (n=9), PSAPP D S (n=12).

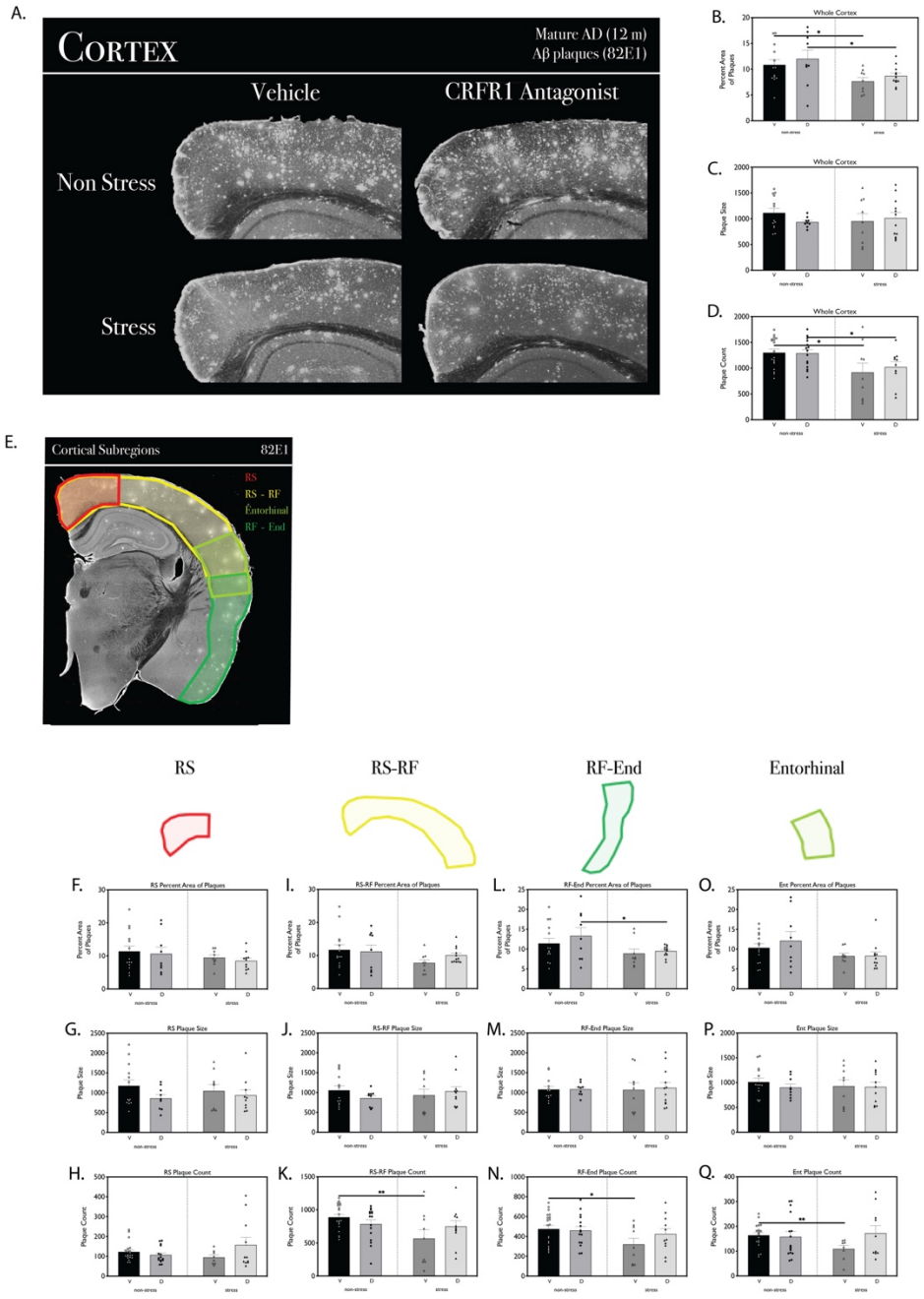

Figure 8. Mature cortical plaque immunohistochemistry.

Example comparative images of mature cortical plaque analysis (A) and delineation of cortical subregions quantified (E). Quantification of mature whole cortical (B-D), RS (F-H), RS-RF (I-K), RF-End (L-N), and Entorhinal (O-Q) 82E1-stained plaque deposition. Percent area (top row of graphs), plaque size (middle row of graphs), and plaque number (bottom row of graphs) were quantified in coronal brain sections of the cortex. *p<0.05, **p<0.01, ***p<0.001. All values are expressed as ± SEM, PSAPP V nS (n=12), PSAPP D nS (n=12), PSAPP V S (n=9), PSAPP D S (n=12).

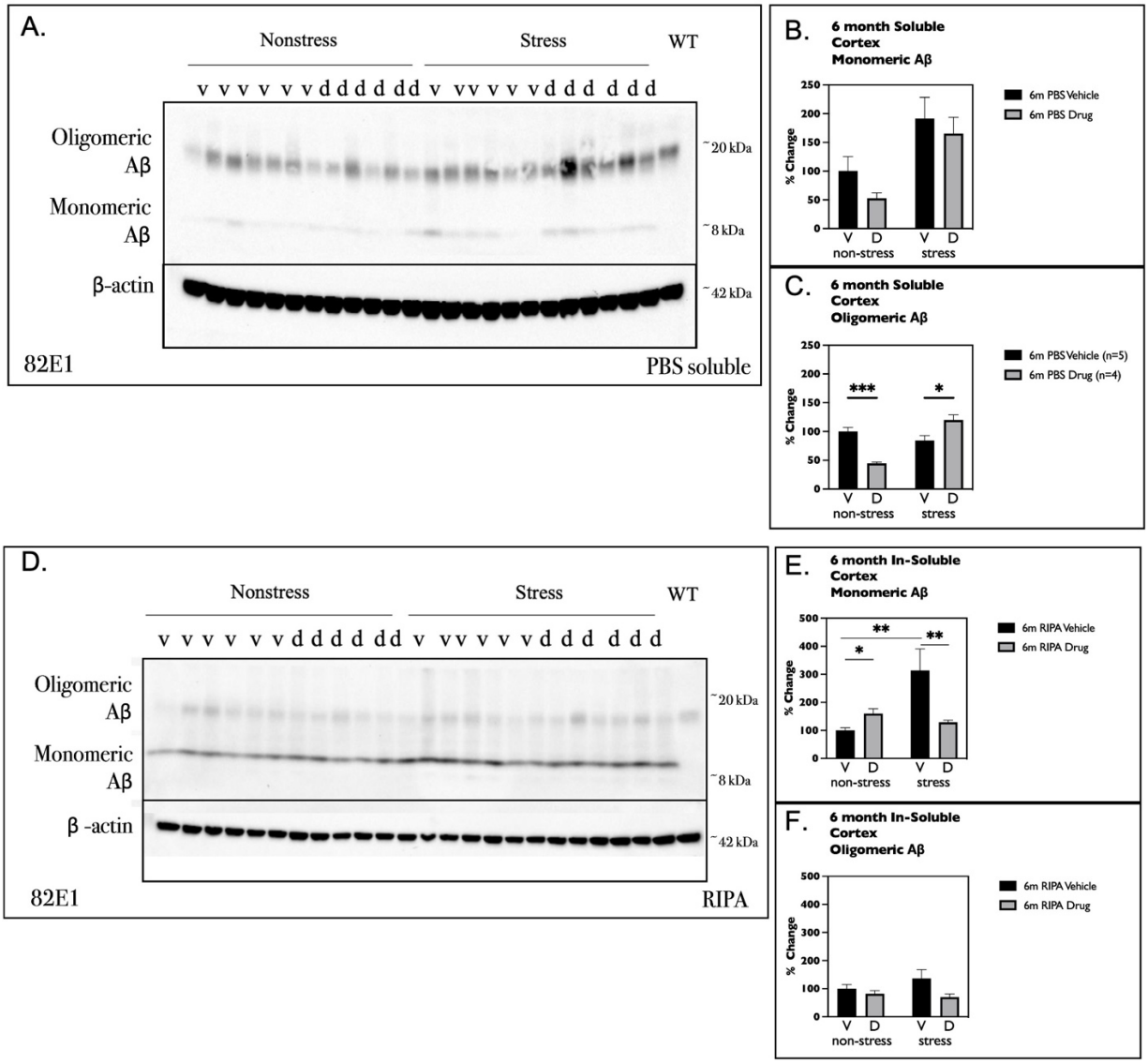

Figure 9. Western Blot biochemical analysis of plaque species in early AD cohorts.

Effects of R121919 and/or stress treatment on soluble (example blot (A), graph of monomeric Aβ (B) and of oligomeric Aβ (C)) and insoluble (example blot (D), graph of monomeric Aβ (E) and of oligomeric Aβ (F)) fractions of young and AD cortices. For soluble fractions, the cortex from each subject was homogenized and sonicated in PBS buffer. For in-soluble fractions (RIPA soluble), PBS homogenate was sonicated in RIPA buffer. Then each fraction was separated using a 10% Criterion gel and transferred to a membrane. Aβ was detected using 82E1 and the loading control was probed using β-actin, as the primary antibody. * $p<0.05$, ** $P<0.01$, *** $P<0.001$. All values are expressed as mean ± SEM, n=6 PSAPP mice/group. V = vehicle; D = drug.

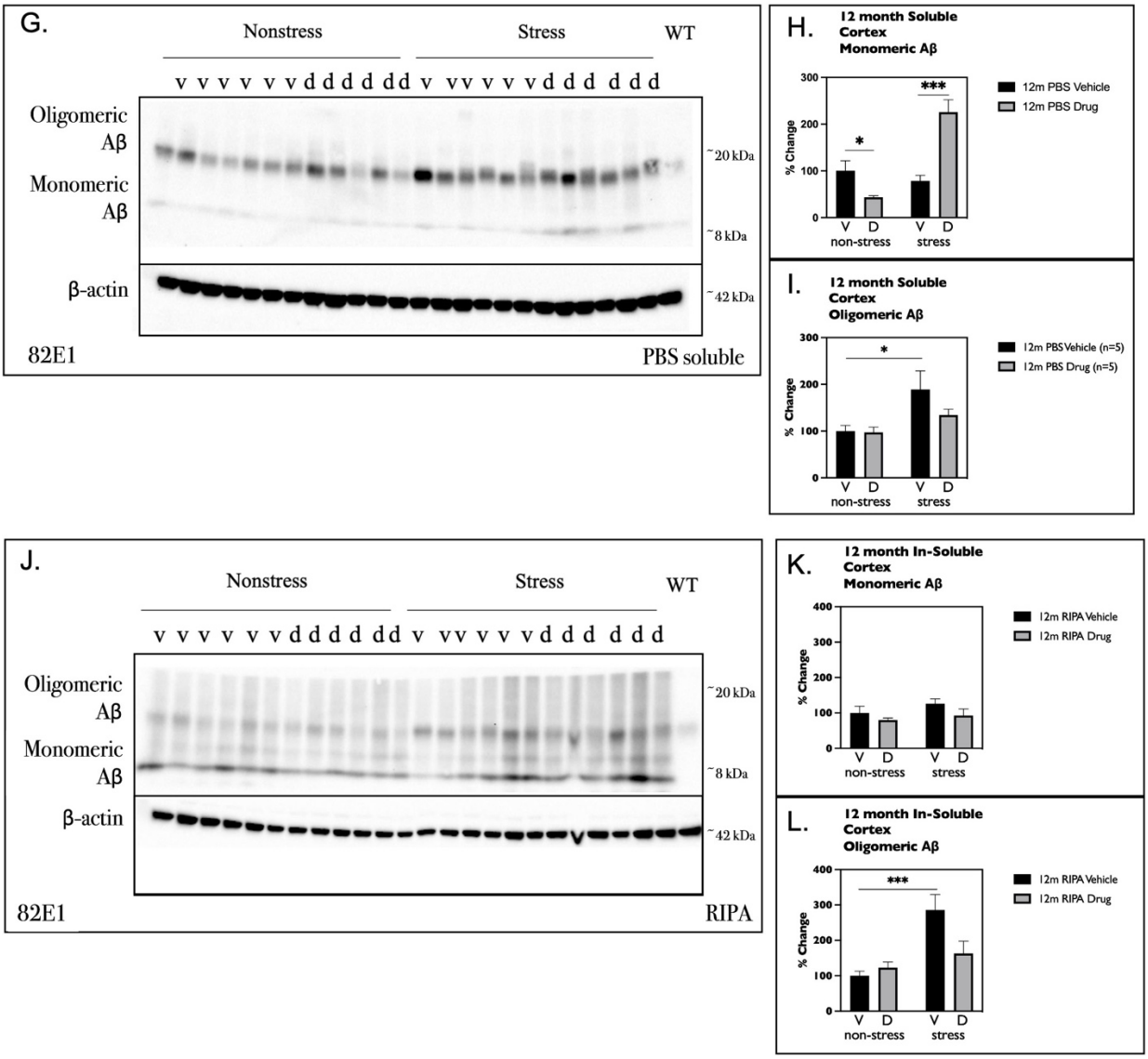

Figure 9 continued. Western Blot biochemical analysis of plaque species.

Effects of R121919 and/or stress treatment on soluble (example blot (G), graph of monomeric Aβ (H) and of oligomeric Aβ (I)) and insoluble (example blot (J), graph of monomeric Aβ (K) and of oligomeric Aβ (L)) fractions of young and mature AD cortices. For soluble fractions, the cortex from each subject was homogenized and sonicated in PBS buffer. For in-soluble fractions, PBS homogenate was sonicated in RIPA buffer. Then each fraction was separated using a 10% Criterion gel and transferred to a membrane. Aβ was detected using 82E1 and the loading control was probed using β-actin, as the primary antibody. * $p<0.05$, ** $P<0.01$, *** $P<0.001$. All values are expressed as mean ± SEM, n=6 PSAPP mice/group. V = vehicle; D = drug.

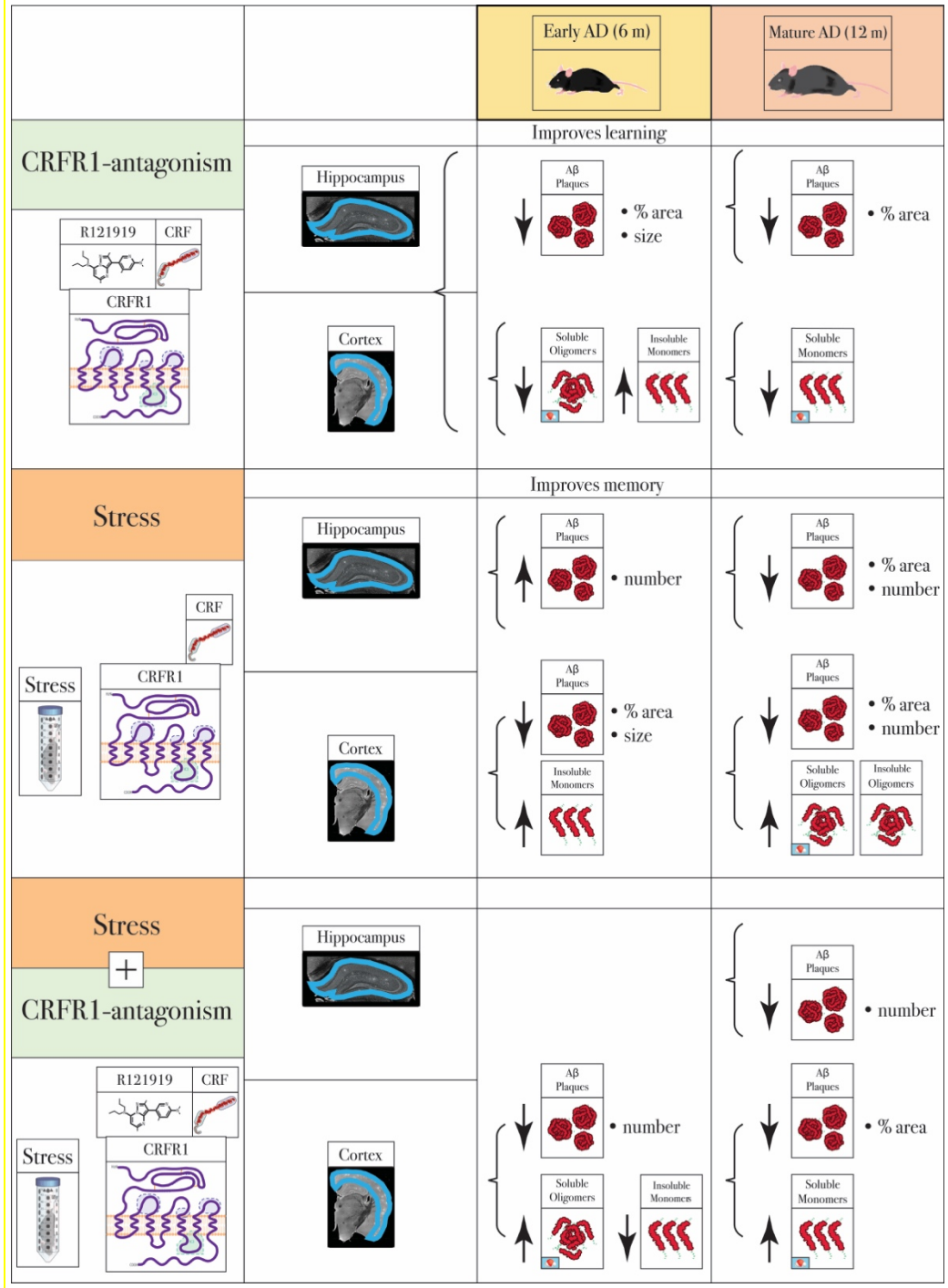

Figure 10. Summary of findings.

Supplement.

Tables

Table 1. Cholesterol regulation of secretase processing of APP.

Cholesterol	**High**	**Low**
α-secretase	↓	↑
β-secretase	↑	↓
Amyloidogenic?	↑	↓

Table 2. GPCR signaling pathway proteins that mediate APP processing.

Mechanism	AD related changes	Notes	Source
MAPK/ERK	Increase BACE1 expression	M1 mAChR	(Jiang, et al., 2012), (Züchner, Perez-Polo, & Schliebs, 2004)
PKA	cRaf-1 inhibition (p-serine259) → decrease NFkB activity → decrease in BACE1 expression (controlled by NFkB)	Caffeine	(Arendash, et al., 2009), (Zeitlin, Patel, Burgess, Arendash, & Echeverria, 2011)
ARF6	Modulates BACE1 sorting into early endosomes	GTPase	(Sannerud, et al., 2011)
Rab11	Modulates BACE1 localization to axons from soma	GTPase	(Buggia-Prévot, et al., 2014)
Rheb	Regulates stability and activity of BACE1	GTPase	(Shahani, et al., 2014)
p60TRP gene	APP- dephosphorylation → inhibits BACE1 cleavage	Also affects PSEN1/2	(Mishra & Heese, 2011)

Table 3. GPCR-related changes in AD-related processing. Adapted from Zhao et al., (2016).

GPCRs	Family	Relation to AD
CRFR1	B	(Aβ↓), (Justice, et al., 2015); (tau hyperphosphorylation↓), (Carroll, et al., 2011) and (Rissman, et al., 2012); (hippocampal synaptophysin level↑), (Scullion, Hewitt, & Pardon, 2013)
PACR1	B	(sAPPα↑), Kojro et al. (2006); (cognition↑), Rat et al. (2011) and Yang et al. (2015)
M1 AChR	A	Nitsch et al. (1992), Jones et al. (2008), and Jiang et al. (2012); Buxbaum et al. (1992); (Züchner, Perez-Polo, & Schliebs, 2004); Davis et al. (2010); Caccamo et al. (2006)
M2	A	(Züchner, Perez-Polo, & Schliebs, 2004)
M3	A	Nitsch et al. (1992); (Züchner, Perez-Polo, & Schliebs, 2004)
5-HT2R	A	(sAPP↑, Aβ↓), Nitsch et al. (1996); (Aβ↓), Arjona et al. (2002)
5-HT4R	A	(sAPPα↑, Aβ↓), Robert et al. (2001), Giannoni et al. (2013), Tesseur et al. (2013), and Pimenova et al. (2014)
5-HT5R	A	(Improved cognition and memory), Upton et al. (2008), Maher-Edwards et al. (2010), Rossé and Schaffhauser (2010), and Benhamú et al. (2014)
DOR	A	(Aβ↓), Teng et al. (2010) and Cai and Ratka (2012)
Adrenergic R	A	(Aβ↑), Ni et al. (2006) and Chen et al. (2014); (tau phosphorylation↑), Branca et al. (2014) and Wisely et al. (2014)
ATR	A	(Tau phosphorylation and neurodegeneration↑), (AbdAlla et al., 2009a,b); (memory↓), Ongali et al. (2014)
Adenosine R	A	(Aβ↓), Canas et al. (2009), Espinosa et al. (2013), Giunta et al. (2014), Nagpure and Bian (2014), and Orr et al. (2015); (BACE1↓, Aβ↓), Arendash et al. (2006)
CXCR2	A	(Aβ↑), Bakshi et al. (2008, 2009, 2011)
CXCR3	A	(plaque↑), Krauthausen et al. (2015)
GPR3	A	(Aβ↑), Thathiah et al. (2009) and Nelson and Sheng (2013)
P2Y R	A	(Aβ↑), Ajit et al. (2014) and Erb et al. (2015)

Table 3. continued. GPCR-related changes in AD-related processing. Adapted from Zhao et al., (2016).

CX3CR1	A	(amyloid plaque↓), Lee et al. (2010), Liu et al. (2010), Cho et al. (2011) and Condello et al. (2015)
CCR2	A	(Aβ↓); El Khoury et al. (2007)
mGluR1	C	(C83, C99, Aβ40↑), Kim et al. (2010); (sAPP↑), Lee et al. (1995), Kirazov et al. (1997) and Nitsch et al. (1997)
mGluR2	C	(tau phosphorylation↑), Lee et al. (2009); (C83, C99, Aβ42↑), Kim et al. (2010)
mGluR3	C	(C83, C99, Aβ42↑), Kim et al. (2010)
mGluR5	C	(C83, C99, Aβ40↑), Kim et al. (2010)

Table 4. Studies relating stress to AD pathogenesis.

	Animal model	Method of Stress Induction or area	Aim	Results
Bissette et al., 1985 (Bissette, Reynolds, Kilts, Widerlöv, & Nemeroff, 1985)	X	X	Human-AD CRF →	caudate ↓ CRF-IR
De Souza et al., 1986 (De Souza, Whitehouse, Kuhar, Price, & Vale, 1986)	X	X	Human-AD CRF→	↑ CRF-binding
Powers et al., 1987 (Powers, et al., Immunohistochemical study of neurons containing CRF in Alzheimer's disease, 1987)	X	X	Human-AD CRF→	amygdalar ↓ CRF-IR hypothalamic ↑ PVN CRF-IHC CRF dystrophic neurites loc. Aβ
Raadsheer et al., 1995 (Raadsheer, et al., 1995)	X	X	Human-AD CRF mRNA→	hypothalamic PVN ↑ CRF-mRNA
Davis et al., 1999 (Davis, Mohs, Marin, Purohit, & Perl, 1999)	X	X	Human-AD CRF →	cortical ↓ CRF-IR
Kang et al., 2007 (Kang, Cirrito, Dong, Csernansky, & Holtzman, 2007)	+ Tg2576 4 months	Chronic stress (CS) – Isolation 3 months	Chronic Stress – Aβ?	CS – + ↑ Aβ in interstitial fluid (Aβ -ISF) by 84% + no change in FL-APP, CTFα, & CTFβ + carbonate buffer soluble hippocampal tissue →↑Aβ40 38% ↑Aβ42 59% + No change in IDE, NEP or apoE

Table 4. Continued. Studies relating stress to AD pathogenesis.

Rissman et al., 2007 (Rissman, Lee, Vale, & Sawchenko, 2007)	C57BL/6	Acute restraint stress (AS)- + 50 ml conical tube – 30 min sac. → 0-90 min post	Can acute restraint stress → hippo p-tau (AT8, PHF-1), S^{422}?	Rapid ↑ hippo p-tau (all) w/ robust (2-10-fold) peak @ 30-40 min - transient: normalizes by ~ 90 min post
		AS + Adrenalectomy (ADX)	Is the increase because of glucocorticoids?	No, since no change in stress effect on p-tau.
	CRFR1KO, CRFR2KO, & wt	AS + genotype or Ant	Effects of CRFR1-antagonism or genetic ablation?	→ abolished p-tau with disruption of CRFR1 → exaggerated p-tau in CRFR2KO
		AS	Modulation of p-tau kinases?	Acute Restraint Stress ↑ regulated by CRFR1 – + GSK3β active-pY216 + c-Jun N-terminal protein kinases-JNK46/54 + ERK2-pT202/Y204 + Regulatory protein of cdk5-p35 No effect on – + GSK3β- pS9 or total unphos + ERK1
	C57BL/6	AS – or – Repeated stress (RS) – + 14 consecutive days sac. → 20 min & 24 h post	Insoluble?	RAB/RIPA – AS vs RS – 20m & 24h – - Detergent-soluble- WB- + AT8 & PHF-1 – in RS both times, none in AS

Table 4. Continued. Studies relating stress to AD pathogenesis.

Carroll et al., 2011 (Carroll, et al., 2011)	Tg2576 3-4 months	AS – + Restraint 3 hours Sac. → 14h after initiation of restraint	Acute Stress Aβ?	AS – + ↑ Aβ-ISF by 32% (13h post) + 13 h carbonate-soluble hippo ○ no sig. increase Aβ40 or Aβ42 ○ no change FL-APP& CTFβ ○ ↓ CTFα (17%) No change in IDE, NEP or apoE
		Corticosterone (CORT) (50 mg/kg, IP)	Is Δ in Aβ due to Corticosterone or CRF?	CORT – Aβ -ISF (1-6 h) no change
		CRF → directly in hippo- reverse microdialysis (RMd)	How does direct application of CRF into the hippo affect Aβ-ISF?	CRF – ↑ Aβ-ISF – dose dependent
		AS- α-helical CRF 9-41 (α-h9-41) → hippo- RMd (30 min prior to stress – end)	How does CRFR antagonist effect AS-induced increase in Aβ-ISF?	α-h9-41 – AS – prevented increase- Aβ -ISF (stress – ↑ CRF)
		CRF	Effect of CRF on CA1?	e-phys. CRF + rat hippo pyramidal CA1 → ↑ firing
		AS- TTX (5 uM) → hippo- RMd (30 min prior to stress – end)	Does blockade of sodium channels inhibit AS-induced Aβ-ISF?	TTX +Not stressed ↓ Aβ-ISF (~60%) Blocked stress Aβ inc.
Dong et al., 2011 (Dong, et al., 2011)	Tg2576 Tg2576 (TT) + Tetop-CRF CamKII-tTA	*(CRF-OE mouse model)*	Neuroanatomy? - Aβ? - dendritic branching & spine density? Behavioral phenotypes?	Aβ localization – + cortex & hippo ↑ Aβ plaques Dendritic morphology – + L4 fCtx + CA1 ↓ dendritic branch & spines Behavior – ↓ working & context memory

Table 4. Continued. Studies relating stress to AD pathogenesis.

Rissman et al., 2012 (Rissman, et al., 2012)	C57Bl/6 CRFR1-/- + provided with 10 mg/L CORT on days E12-P14 + 21 d pre-testing CRFR2-/- CRFR1/2KO	AS – + Restraint in 50 mL conical tube – 30 min RS – + 14 consecutive days sac. → 20 min & 24 h post	Does the CRFR regulation of AS-induced hippo p-tau, apply to CS or RS?	p-tau (PHF-1 & AT8) in CRFR1, 2, &1/2 -/- groups -soluble & detergent-soluble hippo fractions--AS or RS- + soluble RS cumulative effect on PHF-1 & AT8 ○ CRFR1-/- **no effect** that sig. greater than cntls ○ CRFR2-/- ↑ effect (A-24, R-20) than cntls + Detergent-soluble ○ CRFR2-/- ↑ effect (R-24 vs 20) - CRFR-dependent AS-induced effects on p-tau and solubility extends to RS.
		Pharm. + Antalarmin + R121919 (20 mg/kg/d IP or 14-d ALZET osmotic minipump) 20 min prior to stress	Can CRFR1-antagonists mitigate RS-induced changes in p-tau (PHF-1 & AT8)?	RS-CRFR1 antagonists Ant – + block AS p-tau + no effect on RS p-tau 20 min or 24 h R121919 – + p-tau (AT8 & PHF-1) ↓ RS & AS

Table 4. Continued. Studies relating stress to AD pathogenesis.

Continued Rissman et al., 2012 (Rissman, et al., 2012)	C57Bl/6 CRFR1-EGFP ♂ 15-22 wk	AS –	Cellular localization of p-tau?	IHC p-tau (PHF-1) hippo AS vs RS AS 20 min → + ↑ perikarya hilar DG + deep granule cell layer + subgranular zone + Ammon's horn – + Pyramidal CA1-3 - Small multipolar cells in dendritic zones (probably interneurons) AS 24 hrs → no big difference (transient) RS 20 min & 24 hrs (vs AS 20 min) → ↓ intens. & dens. (similar staining areas)
		AS –	Colocalization of PHF-1 with CRFR1-EGFP?	+ Hilar neurons of DG (dominant site) – AS

Table 4. Continued. Studies relating stress to AD pathogenesis.

Zhang et al., 2012 (Zhang L. , et al., 2012)	ICR ♂	Neonatal isolation pre-weaning → Chronic mild stress – Social isolation	How does chronic stress effect p-tau & axonal transport of mitochondria in the hippo?	Behavior – ↑ anxiety behaviors Chronic stress effects – ↑ plasma CORT ↑ hypothalamic CRF mRNA ↑ p-tau AT8 ↓ tau-1 ↑ synaptosomal mitochondria -hippo (COX IV)
		+CRFR1 antag. – CP154,526 +CORT R antag. – RU486	Is this effect due to CRFR1 or CORT?	CP154,526 – not RU486 – ↓ p-tau (AT8) & COX IV
	Primary hippo culture	+Lithium (LiCl) ○ Gsk3β inhibitor	Is there a connection between p-tau and mito movement?	→ p-tau inhib. by /LiCl ↓ mitochondrial transport

Table 4. Continued. Studies relating stress to AD pathogenesis.

Baglietto-Vargas et al., 2015 (Baglietto-vargas, et al., 2015)	3xTG-AD mice 5-6 months	Short Multimodal Stress – 5 hrs +Restraint 50 mL tube +5 per cage +Placed on shaker +Brightly lit room +Random loud noise	Does stress cause a change in memory?	Novel object discrimination test → stress decreased wt and 3xTg-AD mice's ability.
	3xTG-AD 5-6 months N2A cells	Short Multimodal Stress – +CRF	Does acute stress affect dendritic spines in AD mice?	CA3 (sr & slm) stereology → significant ↓ in spines
	3xTG-AD mice 5-6 months N2A cells APP/hAβ/PS1; Crfr1$^{-/-}$ ♂	Short Multimodal Stress – Single intense trauma – + 120 min immobilization (tape and cutting board) + bright light (>700 lux) Trigger – Re-apply stressor for 15 min	How is the Aβ pathway effected?	Hippo ↓ dendritic spines (thin/stubby) ↑ Aβ (soluble and insoluble) ↑ CTF (C99 ns, C83 p=0.07) ↑ BACE1 & ADAM17

Table 4. Continued. Studies relating stress to AD pathogenesis.

Continued. Baglietto-Vargas et al., 2015 (Baglietto-vargas, et al., 2015)	3xTG-AD 5-6 months N2A cells	Short Multimodal Stress – +CRF	P-Tau (AT8, HT7, PHF1)?	No significant changes.
	3xTG-AD mice 5-6 months N2A cells APP/hAβ/PS1; Crfr1$^{-/-}$ ♂ Primary neuronal culture – P0	Short Multimodal Stress – Single intense trauma – + 120 min immobilization (tape and cutting board) + bright light (>700 lux) Trigger – + Re-apply stressor for 15 min PVN + Aβ + CRF–cre recombinase transgene (Crhtm1(cre)Zjh) + floxed-stop tdTomato reporter gene [Ai9;B6.Cg-Gt(ROSA) 26Sortm9(CAG-tdTomato)Hze/J]	What stress hormonal changes could be effecting 6 hrs post?	AS- elevates CRF and CRFR1 (anti-CRHR1 1:1000, Everest Biotech, Ramona, CA, USA)
			Does + CRF (cells) alter APP route?	↑ CTF99 &CRT83
			Examine mechanisms of PTSD relation to AD.	Post-trauma: More anxiety Trauma raised CSF Aβ @ 1-2 & 6-12 mo post exposure. CRFR1KO AD mice- no Aβ change from trauma APP/hAβ/PS1;CRFR1KO higher levels of Aβ → AD model animals = more sensitive to trauma Need CRFR1 to induce
			Can Aβ activate CRF neuron?	Apply Aβ = increase in firing in 5 min.

Table 4. Continued. Studies relating stress to AD pathogenesis.

Campbell et al., 2015 (Campbell, et al., 2015)	CRF-OE (6-7 mo) CRF-OE	+ R121919 (20 mg/kg/d) – 30 days +/- R121919	Does ↑ CRF exposure (+ downstream steroids), +/- CRFR1-antagonism with R121919 (30 days), effect hippo p-tau & kinase activity?	CRF-OE (vs WT) → ↑ p-tau (AT8, PHF-1, S262, S422) ↑ GSK-3 pS9, GSK-3 pY216, ERK ½, MAPK p38, CDK5 CRF-OE + D (vs CRF-OE) → ↓ p-tau (AT8 & PHF-1) Did not block at S262 & S422 ↓ p-JNK (active form)
			Localization?	*IHC PHF-1 hippo* – CRF-OE Labeling localized to → + Ammon's horn + perikarya scattered: CA1 & CA2 ○ stratum oriens ○ pyramidale + axonal staining: stratum laconosum-moleculare CRF-OE + D Labeling localized to → + cell/fiber: CA3, hilus, or DG
			EM p-tau presence?	CRF-OE insoluble hippo immuno-gold EM → ↑ negatively stained round/globular aggregates (~50 nm diameter) Not present in CRF-OE+D

Table 4. Continued. Studies relating stress to AD pathogenesis.

Campbell et al., 2015 (Campbell, et al., 2015)	♀ (12 mo) PSAPP PSAPP-R1+/- PSAPP-R1-/- + 10 mg/L CORT on days E12-P14 + CORT for 21 d pre-testing	X	Does genetic CRFR1 disruption effect the severity of AD pathology?	PSAPP-R1+/- & PSAPP-R1-/- (vs PSAPP) → + Aβ → ↓ in hippo, insular, rhinal, & RS (retrosplenial) cortices + Aβ pep & AβPP-CTFs ↓ + IHC 82E1 → ↓ in hilar hippo & Ins, RS, & EctR/Peri cortices + ELISA Aβ40 & Aβ42 → ↓ (but not sig. diff when vs WT or CRFR1-KO) + WB AβPP → no change (22C11) + CTF-β → large ↓ (both) + CTF-α → no change IDE → ↓ expression

Table 4. Continued. Studies relating stress to AD pathogenesis.

Park et al., 2015 (Park H. , et al., 2015)	C57BL6	Acute restraint 3h → 0h or 24h	Acute stress increases Aβ?	Increased Aβ levels → return to baseline 24h post
	Primary neuronal – C57BL6 forebrain	CRF	Does CRF increase Aβ?	↑ Aβ40 34%, Aβ42 52%, and Aβ38 11%
	SH-SY5Y		CRF effects on Aβ?	↑ Aβ40, Aβ42, and Aβ total
	SH-SY5Y–FLAG-CRFR1		CRFR1 endocytosis-modified surface biotinylation?	CRF = ↑ CRFR1 internalization
	HEK293–CRFR1-GFP		Localization of CRFR1?	CRFR1 (on PM) + CRF → 1 hr = CRFR1-GFP in EEA1 expressing intracellular compartments
	N2a cells –	OE γ-secretase components (APH1, NCT, PEN2, PSEN1)	Does CRF affect γ-secretase localization (NCT antibody)?	CRFR1 (on PM) colocalized with NCT + CRF → ↓ cell surface CRFR1 and NCT → seen to redistribute to intracellular vesc. (↑ coloc.)
	HEK293	-	How does CRFR1 associate with γ-secretase?	IP – CRFR` → NCT, PS1, PEN2 IP – PS1 → CRFR1, NCT IP – PEN2 → CRFR1, NCT
	- CRFR1 + D386/IC3 + D386 + D412 + IC3 mutants WT	-	Where in CRFR1 is this interaction?	Co-IP – PS1 → ↓ interaction with CRFR1 D386/1C3 & D386 (compared to D412, IC3 mutants, and WT)

Table 4. Continued. Studies relating stress to AD pathogenesis.

Continued. Park et al., 2015 (Park H. , et al., 2015)	MEF + WT + β-arrestin2 KO + β-arrestin1 KO β-arrestin1 & 2 KO	-	Is this association mediated by β-arrestin2?	Co-IP – CRFR1 → 2KO PS1 ** ↓ compared to WT → 1/2KO PS1 *** ↓ comp WT

Table 4. Continued. Studies relating stress to AD pathogenesis.

Continued. Park et al., 2015 (Park H. , et al., 2015)	SH-SY5Y CRFR1 line	CRF	Lipid rafts?	CRFR1 & PS1 norm in both CRF ↑ distribution of γ-secretase (PS1) into lipid rafts → in WT but not D386/IC3 mutant
	H4 broken cell	CRF + Astressin (Ast) + α-h9-41 + Antalarmin (Ant) NBI-27914	Does CRF directly modulate γ-secretase?	Broken cell assay – γ-secretase + APP CTF + C99 isolated + incubated with CRF → ↑ Aβ40*** & Aβ42* + CRF = ↑ Rate of Aβ production
	H4-APP695wt cells + D386/IC3 + D386 + D412 + IC3 mutants WT		CRFR1 mutant localization of R-induced ↑ Aβ –	Only CRFR1 D386/IC3 didn't show CRF-mediated ↑ in Aβ40 & total
	SH-SY5Y C57BL6 H4 BRI-C99 OE		Pharm effect on Aβ production –	CRFR1-antagonists + CRF or stress + Ast → did not block + α-h9-41 → did not block + Ant → did not block, H4 ↑ Aβ42:Aβ40 NBI-27914 → did not block, H4 ↑ Aβ42:Aβ40

Table 4. Continued. Studies relating stress to AD pathogenesis.

Justice et al., 2015 (Justice, et al., 2015)	Slice e-phys - CRF–cre;loxP–stop–loxP–tdTomato mice (P15–P24)	PVN	Is CRF neuronal excitation from network activity or from cell-autonomous Aβ-CRF neurons? Is Aβ acting through mGluR5 (found to activate through prion protein)?	Blocked w/ APV, CNQX, picrotoxin + 10% Aβ CM → increase AP freq → Aβ direct affects CRF neuron excitability Block mGluR5 w/MPEP + 10% Aβ CM → blocked Aβ-induced excitation → might be working through mGluR5 (also coloc)
Le et al., 2016 (Le, et al., 2016)	Primary hippo neuronal cultures E19.5 C57BL/6J	+acute (0.5 hr) +chronic (2 hrs) CRF	How does treatment effect p-tau? Axon transport? CREB? BDNF? Mitochondria?	C- CRF – ↑ p-tau (w/ globular-deposition of p-tau – dendrites & axon) ↓ (small) mitochondrial velocity; ↑ mitochondrial distance A- CRF – ↑ mitochondrial velocity & distance A- & C- CRF → ↓ pCREB (active) ↓ axonal transport BDNF

Table 4. Continued. Studies relating stress to AD pathogenesis.

Zhang et al., 2016 (Zhang, et al., 2016)	PSAPP	+ R121919 (20 mg/kg/d)	MWM? Aβ?	♀ PSAPP R121919-treated – (v.s. veh.) + Cog def. - ↓ (MWM SRM & SWM) + ↓ Aβ (82E1) hippo & ctx + ↑ MAP2 & synaptophysin staining + CTFα & CTFβ (normalized to APP levels) sig. ↓ hippo (RAB) + BACE-1 activity in hippo (RAB) ↓ + **no sig dif.**- RAB/RIPA cortical extracts' Aβ peptides (MesoScale bioassays) ♂ R121919-treated – (v.s. veh.) + ↓ Aβ (82E1) hippo & ctx + ↑ synaptophysin ctx + CTFα & CTFβ (normalized to APP levels) sig. ↓ hippo (RAB) + **no sig dif.**- RAB/RIPA cortical extracts' Aβ peptides (MesoScale bioassays)

Table 4. Continued. Studies relating stress to AD pathogenesis.

Zhang et al., 2017 (Zhang & Rissman, 2017)	PSAPP (♂ + ♀)	+ R121919 (20 mg/kg/d, sc)	ROS → DNA oxidation → 8-DHdG in urine → 5-mdC (DNA methylation) → antioxidant capacity Do these change with AD treatment with R121919?	Tg-drug ♂ sig. ↓ H2O2 in urine Tg-drug ♂ and ♀ sig. ↓ 8-OHdG in urine Tg-drug ♀ Ctx ↓ total antioxidant capacity
Bangasser et al., 2017 (Bangasser, et al., 2017)	SILAM mouse vs CRF-OE mice: + FOE + MOE	-	Sex-biased CRFR1 dynamics	♀ bias FOE vs MOE → Gs, PKA, GABA, AD-related signaling pathway (APP → Aβ) (MAPT), BACE1 (pSer498) (8x FOE > MOE), 14-3-3, P70S6K, ephrin ♂ bias MOE vs FOE → G ♀ FOE vs FWT → FOE ↑ PHF1 in insoluble fraction (none in ♂)
	Tg2576 vs TT mice (forebrain CRF OE)			pSer498 BACE1 (but not BACE1) ↑ ♀TT but not ♂TT Aβ plaques ♀ TT > all other groups (♂ & ♀ Tg2576, & ♂TT)

Hippo (hippocampus)
Ins (insular)
RS (retrosplenial)
EctR (ectorhinal)
Peri (perirhinal)
AnS (Acute Exposure to Novelty Stress)
RnS (Repeated Exposure to Novelty Stress)

BSTov (lateral dorsal bed nucleus of the stria terminalis)

Table 5. Literature summary on stress effects on secretases.

Stress on Secretases-		**(Kang et al., 2007)** (Kang, Cirrito, Dong, Csernansky, & Holtzman, 2007)	**(Carroll et al., 2011)** (Carroll, et al., 2011)	**(Baglietto-Vargas et al., 2015)** (Baglietto-vargas, et al., 2015)	**(Campbell et al, 2015)** (Campbell, et al., 2015)	**(Zhang et al., 2016)** (Zhang, et al., 2016)
Experiment design		Tg2576 (4 mo) CS-isolation 3 mo	Tg2576 (3-4 mo) AS-restraint 3 hrs → sac. 14 hr after restraint initiation	3xTG mice (5-6 mo) Short multimodal stress – initial restraint + trigger	PSAPP CFRR1-/- or +/- (21 mo)(vs PSAPP)	Female PSAPP + R121919 (vs veh.)
α-secretase	**CTFα**	No change	↓	↑	No change	↓
	ADAM9	X	X	No change	X	X
	ADAM10	X	X	No change	X	X
	ADAM17	X	X	↑	X	X
β-secretase	**CTFβ**	No change	No change	No change	↓	↓
	BACE1	X	X	↑	X	↓
γ-secretase		X	X	X	X	X
		X	X	X	X	X
		X	X	X	X	X
IDE		No change	No change	X	↓	X
NEP		No change	No change	X	X	X
ApoE		No change	No change	X	X	X