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Impact of CRFR1 Ablation on Amyloid- β Production and Accumulation in a Mouse Model of Alzheimer's Disease

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Abstract

Stress exposure and the corticotropin-releasing factor (CRF) system have been implicated as mechanistically involved in both Alzheimer's disease (AD) and associated rodent models. In particular, the major stress receptor, CRF receptor type 1 (CRFR1), modulates cellular activity in many AD-relevant brain areas, and has been demonstrated to impact both tau phosphorylation and amyloid- β (A β) pathways. The overarching goal of our laboratory is to develop and characterize agents that impact the CRF signaling system as disease-modifying treatments for AD. In the present study, we developed a novel transgenic mouse to determine whether partial or complete ablation of CRFR1 was feasible in an AD transgenic model and whether this type of treatment could impact A β pathology. Double transgenic AD mice (PSAPP) were crossed to mice null for CRFR1; resultant CRFR1 heterozygous (PSAPP-R1^{+/-}) and homozygous (PSAPP-R1^{-/-}) female offspring were used at 12 months of age to examine the impact of CRFR1 disruption on the severity of AD A β levels and pathology. We found that both PSAPP-R1^{+/-} and PSAPP-R1^{-/-} had significantly reduced A β burden in the hippocampus, insular, rhinal, and retrosplenial cortices. Accordingly, we observed dramatic reductions in A β peptides and A β PP-CTFs, providing support for a direct relationship between CRFR1 and A β production pathways. In summary, our results suggest that interference of CRFR1 in an AD model is tolerable and is efficacious in impacting A β neuropathology.

Keywords

Alzheimer's disease; amyloid- β ; corticotropin-releasing factor; corticotropin-releasing factor receptor 1; hippocampus; hypothalamic-pituitary adrenal axis; stress; triple transgenic

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia, characterized neuropathologically by the accumulation of senile plaques and neurofibrillary tangles. Amyloid- β ($A\beta$), a cleavage product of the amyloid- β protein precursor ($A\beta$ PP), aggregates in extracellular spaces as senile plaques. Tangles are formed by the fibrillization of hyperphosphorylated tau (tau-P). Less than 2% of AD cases are linked to known genetic mutations, with the vast majority of AD cases considered sporadic and possibly resulting from an interaction between other genetic and environmental risk factors that increase susceptibility [1, 2]. Stress is a risk factor highly correlated with AD pathology in rodents and humans [3, 4]. Individuals predisposed to experience anxiety are more likely to develop AD, and the prevalence of the disease is increased among patients diagnosed with early-onset anxiety disorders [5, 6].

The physiological impact of stress is mediated by the hypothalamic-pituitary-adrenal axis. In response to stress, neurons of the hypothalamic paraventricular nucleus secrete corticotropin releasing factor (CRF), which binds to CRF receptor 1 (CRFR1) on the pituitary gland. Although CRFR1 is the pituitary receptor that mediates the neuroendocrine stress response [7], it is also expressed widely in brain, including AD-relevant regions such as the isocortex, hippocampus, and amygdala [8]. CRF signaling through CRFR1 in brain acts as a neuromodulator that modulates cellular activity [9, 10]. Supporting a role of CRF in AD neuropathology, work from our laboratory has demonstrated that both CRF overexpression and acute and repeated restraint stress induced hippocampal tau-P, a process that is dependent on CRFR1 ([11–13] versus [14]). While tau-P resulting from acute stress is transient and potentially involved in stress-related neuroplasticity, tau-P induced by repeated stress or overexpression of CRF is present for extended periods after stress exposure, has a definable structure and is localized to detergent-soluble cellular fractions [12, 13].

Mounting evidence has accumulated showing that CRF signaling is involved in the pathophysiology of AD (e.g., [15–17]) and a substantial number of recent studies demonstrate a role for CRF and CRFR1 in AD-related endpoints [12–14, 17–23]. In the present study, we expanded our investigation of the role of CRFR1 in AD to determine whether chronic interruption of CRFR1 was feasible in an AD mouse model and could impact $A\beta$ pathology. We used double transgenic mice that express mutant forms of the human genes for $A\beta$ PP and presenilin-1 (PSAPP), and reliably produce $A\beta$ plaques beginning at 3–4 months of age [24]. $A\beta$ accumulation in these animals can be exacerbated by behavioral and physiological stress [19]. To examine the impact of chronic loss of CRFR1 on development of $A\beta$ pathology, we developed a novel transgenic model by crossing the PSAPP line to mice null for CRFR1 [25]. Resultant female offspring were used at 12 months of age to examine the impact of CRFR1 disruption on $A\beta$ peptide levels and severity of $A\beta$ plaque pathology.

MATERIALS AND METHODS

Generation of PSAPP-CRFR1^{-/-} mice

CRFR1 knockout mice (CRFR1^{-/-}) were bred from heterozygote breeder pairs of established lines backcrossed to founder mice to achieve a pure C57BL/6 background and genotyped using established PCR protocols [25]. Pregnant females used for generating CRFR1-deficient mice received drinking water supplemented with corticosterone (10 µg/ml; Sigma-Aldrich, St. Louis, MO) from embryonic day 12 to postnatal day 14 to prevent early mortality as a result of pulmonary dysplasia [25]. Female CRFR1^{-/-} mice were crossed with AD transgenic male offspring of Borchelt PSAPP mouse line 85 (*APP-Swe^{K595N,M596L}* and *PS1^{ΔE9}*; Jackson Laboratories, Bar Harbor, ME) [24]. Offspring of this cross were determined by PCR genotyping and named based on genotype status: PSAPP, PSAPP-CRFR1^{+/-}, PSAPP-CRFR1^{-/-}. PSAPP CRFR mutant mice were maintained on corticosterone-supplemented drinking water during testing to allow the normal nocturnal bias in appetitive behavior to approximate the circadian fluctuation in circulating hormone levels as described previously [11, 12]. To assess effectiveness of the replacement regimen, plasma corticosterone levels were determined by radioimmunoassay from blood samples collected at sacrifice. The UCSD Institutional Animal Care and Use Committee approved all experimental protocols.

Aβ ELISA

Whole brains from 12-month-old female PSAPP, PSAPP-R1^{+/-}, and PSAPP-R1^{-/-} (*n* = 4 each) mice were homogenized in 5 M guanidine HCL buffer (4 ml per 1.5 ml of whole brain extract lysate previously homogenized in a PBS/5mM EDTA homogenization buffer), sonicated for 15–30 s, and incubated for 3–4 h at room temperature. 500 µl of the guanidine extract was added to an equal volume of PBS-T, and the samples were spun at 4°C for 20 min at 16,000 × *g*, and levels of Aβ₄₀ and Aβ₄₂ were analyzed by enzyme-linked immunosorbent assay (ELISA; Life Technologies) according to the manufacturer instructions. Plates were read using a BioRad iMark Microplate Reader. 12-month-old female wild-type (WT) and CRFR1^{-/-} animals (*n* = 4 each) were used as controls.

Western analysis

Groups of 12-month-old female WT (*n* = 5), PSAPP (*n* = 4), PSAPP-R1^{+/-} (*n* = 4), PSAPP-R1^{-/-} (*n* = 4), and CRFR1^{-/-} (*n* = 4) mice were killed by cervical dislocation, decapitated and the hippocampus was rapidly dissected and frozen on dry ice. Tissues were homogenized in RIPA buffer containing protease inhibitors and prepared for western analysis as described previously [11, 12]. 6 µg of protein was loaded and electrophoretically separated on a polyacrylamide gel. Samples for CT-20 analysis were prepared in tricine sample buffer and separated on 10–20% tricine gel. Background subtraction was performed and quantitative band intensity readings were obtained using NIH ImageJ software as described previously [11, 12, 14]. 22C11 (Millipore, 1:5000) was used to detect total levels of AβPP, insulin-degrading enzyme (IDE, EMD Millipore, 1:1000) was used to detect total levels of insulin degrading enzyme, and CT-20, provided by Dr. M. P. Murphy (University of Kentucky, 1:1000), was used to detect C-terminal fragments of AβPP. β-actin (Sigma, 1:1000) was used as a loading control on all blots.

Fluorescent amyloid plaque localization

Groups of 12-month-old female PSAPP, PSAPP-R1^{+/-}, and PSAPP-R1^{-/-} ($n = 5$, each) mice were killed by cervical dislocation, decapitated and the brains were drop fixed in 4% paraformaldehyde for 3 h and then cryprotected overnight in 2% sucrose-PBS. The next morning, 30 μ m-thick sections were cut on a freezing-sliding microtome and stored at -20°C in cryoprotectant solution (20% glycerol and 30% ethylene glycol in 0.1 M phosphate buffer). Sections were mounted on Fisherbrand Superfrost®/Plus Microscope Slides and treated with 70% formic acid before immunohistochemical staining for A β . Sections were immunolabeled for A β using the N-terminal specific anti-human A β antibody 82E1 (1:500 from 50 μ g/ml stock solution; IBL, Hamburg, Germany) and visualized with a goat anti-mouse secondary antibody conjugated to Alexa Fluor® 568 fluorescent dye (1:575; Invitrogen, Carlsbad, CA). Slides were coverslipped using Fluoromount-G™ mounting medium (Southern Biotech, Birmingham, AL). WT and CRFR1^{-/-} mice were used as controls ($n = 5$, each).

A β quantification

The extent of the brain occupied by A β plaques was assayed from fluorescently labeled sections, with regions identified according to anatomical landmarks local to each region of interest (ROI) as depicted in the Franklin and Paxinos atlas. Images were acquired with a Leica DFC310FX camera on a Leica DM5500B microscope at 5 $\times$ optical magnification using Leica Application Suite software. Individual, adjacent images were stitched together in ImageJ using a plug-in developed by S. Preibisch. Fourteen grayscale, 12-bit images of coronal sections, no less than 200 μ m apart, were collected from each animal and analyzed using Metamorph image analysis software licensed to Leica Microsystems. No two sections taken from the same mouse were less than 200 μ m apart. Equivalent brain regions between subjects were identified according to anatomical landmarks established in the third edition of Franklin and Paxinos' atlas, *The Mouse Brain*. The piriform, as well as the rostral-most areas of the amygdala and hippocampus were quantified from equivalent sections between ~3mm rostral to the interaural plane to ~2.6mm rostral to the interaural plane. The amygdala and the hippocampus from the most caudal sections continued to be quantified distinctly. However, between ~2.6mm rostral to the interaural plane to the interaural plane itself, other regions ventral to the rhinal fissure: the ectorhinal, perirhinal, and entorhinal cortices, were included with quantification of the piriform. Sections corresponded between subjects, beginning at approximately +1.10mm (relative to bregma), where the decussation of the corpus callosum is first apparent, and ending at approximately -3.88 mm, where the hilar region of the hippocampus can still be seen. ROIs were outlined with a light pen on a Wacom Cintiq 12WX interactive LCD monitor.

Statistics

Optical density readings from ELISA and western blots were analyzed by two-way ANOVA using SPS software. Individual group means were compared using Tukey's multiple-comparison test. Bar graphs were made using Prism 6 software. Data are presented as mean $\pm$ SEM. For plaque quantification, to adjust plaque with amyloid expressed as a percentage of the area of the sampling region. Density readings from ROI were analyzed by two-way

ANOVA (genotype $\times$ coronal level) for genotypic differences in plaque load across serial coronal levels, with coronal level used as the within subjects variable. Likewise, total plaque load area for each brain region was estimated by summing the thresholded area of each region from every section it was sampled within, and expressing it as a percentage of the total region area across sections.

RESULTS

Impact of CRFR1 ablation on A β plaque load at 12 months of age

As found in previous studies with AD models, our initial trials with antibodies 4G8 and 6E10 yielded heavy A β PP and/or intracellular A β labeling (e.g., [26]), which was problematic for developing adequate threshold programming to solely identify A β plaques using our Leica Metamorph software (data not shown). For this reason, we chose antibody 82E1 for detection of A β plaques because our tests suggest it provided the most favorable detection of plaques with virtually no cellular labeling (Fig. 1).

Plaque load in cortical regions surrounding the medial longitudinal and rhinal fissures of each animal was measured at fourteen coronal levels, as described in Materials and Methods. We found large reductions in plaque accumulation through hilar region of the hippocampus (both $p < 0.05$) and the insular, retrosplenial, ectorhinal, and perirhinal cortices ($p < 0.05$, $p < 0.01$, $p < 0.01$, respectively) in both PSAPP-R1^{+/-} and PSAPP-R1^{-/-} mice compared to PSAPP cohorts (Fig. 1). Percent differences in total plaque area percentages per region are shown in Table 1.

Impact of CRFR1 ablation on A β peptide levels in brain

To determine whether levels of A β were reduced with CRFR1 ablation, we examined total brain levels of A β ₄₀ and A β ₄₂ in whole brain lysates by ELISA in PSAPP mice as a function of CRFR1 status at 12 months of age ($n = 4/\text{group}$) (Fig. 2). We found that both PSAPP-R1^{+/-} and PSAPP-R1^{-/-} mice had significantly reduced levels of both A β ₄₀ and A β ₄₂, compared to PSAPP counterparts (for A β ₄₀: PSAPP-R1^{-/-} and PSAPP-R1^{+/-} versus PSAPP, both $p < 0.001$; for A β ₄₂: PSAPP-R1^{-/-} and PSAPP-R1^{+/-} versus PSAPP, both $p < 0.05$) (Fig. 2). Importantly, levels of both A β ₄₀ and A β ₄₂ in PSAPP-R1^{-/-} and PSAPP-R1^{+/-} were not significantly different from that seen in WT or CRFR1 knockout mice (all $p > 0.05$).

Upstream mechanisms

Although our data suggest an important interaction between CRFR1 signaling and A β , the mechanisms underlying this relationship are uncertain. To obtain a preliminary assessment of players involved, we used western blot analysis to assess whether overall levels of A β PP were altered by total or partial loss of CRFR1. We found no change in transgenic overexpression of A β PP in our CRFR1 mutant cohorts (PSAPP-R1^{-/-} and PSAPP-R1^{+/-} versus PSAPP, all $p > 0.05$) (Fig. 3). Control mice (WT, R1^{-/-} lanes) had expected basal levels of A β PP. In terms of A β production pathways, we observed dramatic reduction in A β PP CTFs in PSAPP-R1 mutant mice. In particular, a significant reduction in CTF- β in PSAPP-R1^{-/-} and PSAPP-R1^{+/-} (both $p < 0.0001$ compared to PSAPP) was observed but

not CTF- α (both $p > 0.05$ compared to PSAPP), suggesting a CRF-dependent mechanism of A β production. CTFs are generated by secretase cleavage of A β PP. To probe whether CRFR1 is involved in modulation of degradation pathways of A β , we tested whether the expression of IDE, involved in the degradation of A β , was altered in PSAPP mice lacking CRFR1 (PSAPP versus PSAPP-R1^{-/-} and PSAPP-R1^{+/-} compared to PSAPP cohorts, both $p < 0.01$) (Fig. 3). Collectively, our data demonstrate that PSAPP-R1 mutant mice have reduced IDE and A β PP CTFs, suggesting the mechanisms underlying the observed change may involve A β production rather than clearance.

DISCUSSION

In this study, we developed a novel triple transgenic model to determine whether ablation of the major receptor involved in stress response, CRFR1, was feasible in an AD mouse model and determined the impact of this ablation on A β neuropathology. Our anatomical and biochemical findings demonstrate that accumulation of A β is significantly reduced in many important regions involved in learning and memory in animals lacking CRFR1. Importantly, our data provides an important mechanistic link between stress signaling intermediates in the CNS and the neuropathology of AD; CRFR1 ablation leads to significant and selective reduction of CTF β , which suggests that the reduction in A β observed may involve modulation of secretase activity or trafficking. In terms of A β degradation, although confirmation with enzyme assays are needed, the reduction in resting levels of IDE signal that is observed with CRFR1 ablation may be due to reduced activity as a result of reduced presence of A β .

The role of CRF signaling in AD

Stress sensitivity and disorders are very common in AD patients, which are thought to mechanistically involve alterations in the hypothalamic-pituitary-adrenal axis (reviewed in [27]). Furthermore, anatomical and biochemical data indicate the involvement of CRF in the development of AD, with reduced cortical CRF immunoreactivity (in the face of increased hypothalamic expression) being a prominent neurochemical change [15, 28], a process which occurs early in disease progression in areas vulnerable to AD neuropathology [29–34]. CRF-positive dystrophic neurites have been found associated with A β plaques [30] and marked increases in CRF binding have been described in specific cortical regions of AD patients, suggesting upregulation of CRFRs in impacted areas [16]. It has been hypothesized that these changes result from changes in a specific CRF binding protein [35], which is expressed in brain [36] and can reversibly neutralize CRF bioactivity. Furthermore, studies in rodent models demonstrate that CRF overexpression can lead to tau phosphorylation and aggregation, brain atrophy, and cognitive impairment [13, 20–22].

In addition to stress and CRF, considerable attention has been focused on effectors of the stress cascade, such as glucocorticoids, as mediators of neuronal vulnerability in AD. Glucocorticoids are dominant stress hormones, whose increased levels with age have been linked to an enhanced neuronal vulnerability, notably in the hippocampus [37], a key learning and memory-related structure and a major target of both A β and tau pathology in AD [38]. Glucocorticoid (cortisol) levels in AD patients are elevated relative to age-matched

controls, and several lines of evidence support a role of stress steroids in A β regulation (reviewed in [39]). Although glucocorticoids would appear to be an important target in AD, administration of steroids in human AD trials does not alter cognition (ADCS Prednisone Trial [40]) and results of studies from AD rodent models have been mixed [17, 23, 37, 41–46].

The role of stress CRF signaling regulation on A β production and processing pathways

Expression of β -secretase has been shown to be upregulated by several forms of cell stress such as hypoxia, ischemia, impaired glucose metabolism, and oxidative stress [47]. Such forms of physiologic stressors can be induced *in vivo* by exposure to chronic behavioral stressors. For example, increases in lipid peroxidation have been demonstrated in TG2576 mice that have endured repeated restraint [42]. These mice also exhibited enhanced aggregation of A β , an effect that could be blocked with acute administration of a CRFR1 antagonist [42].

A β is derived from A β PP, by the action of two aspartyl proteases, β - and γ -secretases [47–50]. Resultant fragments of A β PP are left behind in the membrane after proteolysis, called C-terminal fragments (CTFs). β -secretase (BACE-1) cleaves A β PP, generating the C99 fragment, which is also called CTF- β and comprises the N-terminus of A β [48, 50]. When γ -secretase cleaves CTF- β , A β is released and is found in several forms, the most abundant of which consist of 38, 40, or 42 amino acids [50]. An alternative, non-amyloidogenic pathway also exists and involves a third protease, α -secretase. Unlike BACE-1, α -secretase cuts within the A β domain of A β PP, generating the CTF- α fragment (aka C83). γ -secretase cleavage of CTF- α does not lead to A μ generation [48, 50]. In terms of upstream mechanisms, we found a dramatic reduction in CTF- β but not CTF- α seen in PSAPP-R1 mutant mice, which implicates CRFR1 as a mediator of β or γ -secretase activity, expression and/or trafficking. Our future/ongoing studies are poised to address these issues. In particular, knowing whether CRFR1 is involved in modulation of β -secretase trafficking, such that it would no longer be localized to the locus of γ -secretase in endosomes, is of primary interest [51, 52].

CRFR1 regulation of both tau and A β pathways?

As summarized in the introduction, much of our work has been focused on stress-induced tau-P, its mechanistic regulation and relevance to AD [11, 12, 14]. Still unsettled is the precise role of stress-induced tau-P and its relevance to tau pathology in AD; studies propose that stress-induced tau-P is a functional component of neuroplasticity, conferring adaptation to stressful stimuli [53, 54], while our group and others suggest that the relationship between stress and tau-P may also define a potential means by which chronic stress exposure may translate into neuropathology [11, 55–58]. We hypothesize that stress-induced tau-P is an essential process required for stress adaptation that becomes dysfunctional with advancing age and/or with chronic overstimulation. Although the phenomenology may be distinct, a similar case can be made for toxicity associated with A β . A β is produced in the brain of throughout life, but with advancing age perhaps the ability to clear or process A β may become altered or lost, which causes accumulation. Although the upstream mechanisms involved remain to be precisely isolated, our data suggest that CRFR1

interruption impacts production pathways involved in A β , which may facilitate the development of new therapeutic strategies for AD.

Implications

In animal models, and in postmortem analysis of brain tissue from human patients, synaptic loss, apoptosis, and tau pathology reliably coincide with cognitive impairment and premature death [59–61]. A prevailing hypothesis in the field postulates that overall plaque burden may not correlate highly with memory loss associated with AD due to the accumulation of A β occurring early in the preclinical (asymptomatic) period. In support of this hypothesis, analysis of total A β (soluble oligomers and insoluble aggregates) in human brains has revealed that severity of A β -burden in the rhinal cortex correlates with severity of synaptic loss in AD [62]. Furthermore, data from studies employing commonly used fluorescent dyes that bind to β -pleated sheets in A β plaques are limited by their lack of specificity to A β itself and to non-neuritic plaques. The use of antibodies directed against A β , which recognize both dense core plaques and diffuse plaques, such as 82E1 used in our study, have revealed correlations between plaque load and the behavioral sequelae of AD [63]. This suggests that diffuse or non-fibrillar aggregates of A β , which predominate in the areas of the brain where we found genotypic changes in plaque load, may be more toxic than neuritic plaques.

Although the hippocampus is well recognized as an area of learning and memory, the retrosplenial and rhinal cortices are also involved in learning and memory processes. Certain models of memory are negatively impacted by damage to areas of the rhinal cortex and the perirhinal cortex has shown to be important in novel-object recognition tasks (for review, [64]). The perirhinal cortex is also among the first cortical regions affected in AD [38, 65]. Similar pathology is found in other regions of the medial temporal lobe that are intimately connected with the perirhinal cortex, such as the entorhinal cortex [38], which sends projections to the hippocampus along the perforant pathway, which is known to be severely impacted by A β . The degree of severity of both A β and NFT lesions in this area correlate well with one another, as well as with cognitive decline [38, 66]. Some tests of memory may be more sensitive than others to the impact of A β along the rhinal and longitudinal fissures. For example, stress insults to the perirhinal cortex have been shown to impact tests of novel object recognition [67, 68]. Likewise, conditioned place aversion has been shown to be dependent on the insular and cingulate cortices [69, 70].

Future directions

Our future studies aim to determine the upstream mechanisms behind the CRFR1-A β relationship and the development of pharmacologic treatment for AD that targets central CRF pathways. Of particular interest here is our observation that the impact on A β did not differ significantly (i.e., no gene dose-dependent effect) between animals null for one or both alleles of CRFR1. These data suggest that perhaps total blockade of CRFR1 is not needed to achieve efficacy in this context, which is an important point to consider for future pharmacological testing. In terms of upstream mechanisms, we find the dramatic reduction in CTF- β but not CTF- α seen in PSAPP-R1 mutant mice particularly striking and consider a determination of whether CRFR1 is a mediator of β or γ -secretase activity, expression,

and/or trafficking a priority. As mentioned earlier, the data presented here leave open the possibility of multiple upstream steps being involved, with the activity and trafficking of secretase enzymes being important to determine. Lastly, with our study demonstrating that CRFR1 ablation was feasible (i.e. not lethal), our upcoming studies will focus on the testing of small molecule selective antagonists to CRFR1 in the AD model.

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REFERENCES

1. Kivipelto M, Rovio S, Ngandu T, Kareholt I, Eskelinen M, Winblad B, Hachinski V, Cedazo-Minguez A, Soininen H, Tuomilehto J, Nissinen A. Apolipoprotein E epsilon4 magnifies lifestyle risks for dementia: A population-based study. *J Cell Mol Med*. 2008; 12:2762–2771. [PubMed: 18318693]
2. Lee DC, Rizer J, Selenica ML, Reid P, Kraft C, Johnson A, Blair L, Gordon MN, Dickey CA, Morgan D. LPS-induced inflammation exacerbates phospho-tau pathology in rTg4510 mice. *J Neuroinflammation*. 2010; 7:56. [PubMed: 20846376]
3. Almeida JR, Runquist D, Sanchez i Nogue V, Liden G, Gorwa-Grauslund MF. Stress-related challenges in pentose fermentation to ethanol by the yeast *Saccharomyces cerevisiae*. *Biotechnol J*. 2011; 6:286–299. [PubMed: 21305697]
4. Pardon MC. Therapeutic potential of some stress mediators in early Alzheimer's disease. *Exp Gerontol*. 2011; 46:170–173. [PubMed: 20870017]
5. Ownby RL, Crocco E, Acevedo A, John V, Loewenstein D. Depression and risk for Alzheimer disease: Systematic review, meta-analysis, and meta-regression analysis. *Arch Gen Psychiatry*. 2006; 63:530–538. [PubMed: 16651510]
6. Wilson RS, Schneider JA, Bienias JL, Arnold SE, Evans DA, Bennett DA. Depressive symptoms, clinical AD, and cortical plaques and tangles in older persons. *Neurology*. 2003; 61:1102–1107. [PubMed: 14581672]
7. Chen R, Lewis KA, Perrin MH, Vale WW. Expression cloning of a human corticotropin-releasing-factor receptor. *Proc Natl Acad Sci U S A*. 1993; 90:8967–8971. [PubMed: 7692441]
8. Lewis K, Li C, Perrin MH, Blount A, Kunitake K, Donaldson C, Vaughan J, Reyes TM, Gulyas J, Fischer W, Bilezikjian L, Rivier J, Sawchenko PE, Vale WW. Identification of urocortin III, an additional member of the corticotropin-releasing factor (CRF) family with high affinity for the CRF2 receptor. *Proc Natl Acad Sci U S A*. 2001; 98:7570–7575. [PubMed: 11416224]
9. Van Pett K, Viau V, Bittencourt JC, Chan RKW, Li H-Y, Arias C, Prins GS, Perrin M, Vale W, Sawchenko PE. Distribution of mRNAs encoding CRF receptors in brain and pituitary of rat and mouse. *J Comp Neurol*. 2000; 428:191–212. [PubMed: 11064361]
10. Justice NJ, Yuan ZF, Sawchenko PE, Vale W. Type 1 corticotropin-releasing factor receptor expression reported in BAC transgenic mice: Implications for reconciling ligand-receptor mismatch in the central CRF system. *J Comp Neurol*. 2008; 511:479–496. [PubMed: 18853426]
11. Rissman RA, Lee KF, Vale W, Sawchenko PE. Corticotropin-releasing factor receptors differentially regulate stress-induced tau phosphorylation. *J Neurosci*. 2007; 27:6552–6562. [PubMed: 17567816]

12. Rissman RA, Staup MA, Lee AR, Justice NJ, Rice KC, Vale W, Sawchenko PE. Corticotropin-releasing factor receptor-dependent effects of repeated stress on tau phosphorylation, solubility, and aggregation. *Proc Natl Acad Sci U S A*. 2012; 109:6277–6282. [PubMed: 22451915]
13. Campbell SN, Zheng C, Monte L, Roe AD, Rice KC, Tache Y, Masliah E, Rissman RA. Increased tau phosphorylation and aggregation in mice overexpressing corticotropin-releasing factor. *J Alzheimers Dis*. 2015; 43:967–976. [PubMed: 25125464]
14. Roe AD, Staup MA, Serrats J, Sawchenko PE, Rissman RA. Lipopolysaccharide-induced tau phosphorylation and kinase activity–modulation, but not mediation, by corticotropin-releasing factor receptors. *Eur J Neurosci*. 2011; 34:448–456. [PubMed: 21722209]
15. Davis KL, Mohs RC, Marin DB, Purohit DP, Perl DP, Lantz M, Austin G, Haroutunian V. Neuropeptide abnormalities in patients with early Alzheimer disease. *Arch Gen Psychiatry*. 1999; 56:981–987. [PubMed: 10565496]
16. De Souza EB, Whitehouse PJ, Kuhar MJ, Price DL, Vale WW. Reciprocal changes in corticotropin-releasing factor (CRF)-like immunoreactivity and CRF receptors in cerebral cortex of Alzheimer's disease. *Nature*. 1986; 319:593–595. [PubMed: 3003585]
17. Kang JE, Cirrito JR, Dong H, Csernansky JG, Holtzman DM. Acute stress increases interstitial fluid amyloid-beta via corticotropin-releasing factor and neuronal activity. *Proc Natl Acad Sci U S A*. 2007; 104:10673–10678. [PubMed: 17551018]
18. Dong H, Csernansky JG. Effects of stress and stress hormones on amyloid-beta protein and plaque deposition. *J Alzheimers Dis*. 2009; 18:459–469. [PubMed: 19584430]
19. Dong H, Goico B, Martin M, Csernansky CA, Bertchume A, Csernansky JG. Modulation of hippocampal cell proliferation, memory, and amyloid plaque deposition in APPsw (Tg2576) mutant mice by isolation stress. *Neuroscience*. 2004; 127:601–609. [PubMed: 15283960]
20. Goebel M, Fleming SM, Million M, Stengel A, Tache Y, Wang L. Mice overexpressing corticotropin-releasing factor show brain atrophy and motor dysfunctions. *Neurosci Lett*. 2010; 473:11–15. [PubMed: 20132869]
21. Carroll JC, Iba M, Bangasser DA, Valentino RJ, James MJ, Brunden KR, Lee VM, Trojanowski JQ. Chronic stress exacerbates tau pathology, neurodegeneration, and cognitive performance through a corticotropin-releasing factor receptor-dependent mechanism in a transgenic mouse model of tauopathy. *J Neurosci*. 2011; 31:14436–14449. [PubMed: 21976528]
22. Dong H, Murphy KM, Meng L, Montalvo-Ortiz J, Zeng Z, Kolber BJ, Zhang S, Muglia LJ, Csernansky JG. Corticotrophin releasing factor accelerates neuropathology and cognitive decline in a mouse model of Alzheimer's disease. *J Alzheimers Dis*. 2012; 28:579–592. [PubMed: 22045495]
23. Dong H, Wang S, Zeng Z, Li F, Montalvo-Ortiz J, Tucker C, Akhtar S, Shi J, Meltzer HY, Rice KC, Csernansky JG. Effects of corticotrophin-releasing factor receptor 1 antagonists on amyloid-beta and behavior in Tg2576 mice. *Psychopharmacology (Berl)*. 2014; 231:4711–4722. [PubMed: 24862368]
24. Jankowsky JL, Slunt HH, Ratovitski T, Jenkins NA, Copeland NG, Borchelt DR. Co-expression of multiple transgenes in mouse CNS: A comparison of strategies. *Biomol Eng*. 2001; 17:157–165. [PubMed: 11337275]
25. Smith GW, Aubry JM, Dellu F, Contarino A, Bilezikjian LM, Gold LH, Chen R, Marchuk Y, Hauser C, Bentley CA, Sawchenko PE, Koob GF, Vale W, Lee KF. Corticotropin releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. *Neuron*. 1998; 20:1093–1102. [PubMed: 9655498]
26. Winton MJ, Lee EB, Sun E, Wong MM, Leight S, Zhang B, Trojanowski JQ, Lee VM. Intraneuronal APP, not free Abeta peptides in 3xTg-AD mice: Implications for tau versus Abeta-mediated Alzheimer neurodegeneration. *J Neurosci*. 2011; 31:7691–7699. [PubMed: 21613482]
27. Chi S, Yu JT, Tan MS, Tan L. Depression in Alzheimer's disease: Epidemiology, mechanisms, and management. *J Alzheimers Dis*. 2014; 42:739–755. [PubMed: 24946876]
28. Whitehouse PJ, Vale WW, Zweig RM, Singer HS, Mayeux R, Kuhar MJ, Price DL, De Souza EB. Reductions in corticotropin releasing factor-like immunoreactivity in cerebral cortex in Alzheimer's disease, Parkinson's disease, and progressive supranuclear palsy. *Neurology*. 1987; 37:905–909. [PubMed: 3495748]

29. Nemeroff CB, Kizer JS, Reynolds GP, Bissette G. Neuropeptides in Alzheimer's disease: Apostomortem study. *Regul Pept.* 1989; 25:123–130. [PubMed: 2566190]
30. Powers RE, Walker LC, DeSouza EB, Vale WW, Struble RG, Whitehouse PJ, Price DL. Immunohistochemical study of neurons containing corticotropin-releasing factor in Alzheimer's disease. *Synapse.* 1987; 1:405–410. [PubMed: 3509803]
31. Raadsheer FC, van Heerikhuize JJ, Lucassen PJ, Hoogendijk WJ, Tilders FJ, Swaab DF. Corticotropin-releasing hormone mRNA levels in the paraventricular nucleus of patients with Alzheimer's disease and depression. *Am J Psychiatry.* 1995; 152:1372–1376. [PubMed: 7653697]
32. Pedersen WA, Wan R, Zhang P, Mattson MP. Urocortin, but not urocortin II, protects cultured hippocampal neurons from oxidative and excitotoxic cell death via corticotropin-releasing hormone receptor type I. *J Neurosci.* 2002; 22:404–412. [PubMed: 11784785]
33. Rehman HU. Role of CRH in the pathogenesis of dementia of Alzheimer's type and other dementias. *Curr Opin Investig Drugs.* 2002; 3:1637–1642.
34. Swaab DF, Bao AM, Lucassen PJ. The stress system in the human brain in depression and neurodegeneration. *Ageing Res Rev.* 2005; 4:141–194. [PubMed: 15996533]
35. Behan DP, Heinrichs SC, Troncoso JC, Liu XJ, Kawas CH, Ling N, De Souza EB. Displacement of corticotropin releasing factor from its binding protein as a possible treatment for Alzheimer's disease. *Nature.* 1995; 378:284–287. [PubMed: 7477348]
36. Potter E, Behan DP, Linton EA, Lowry PJ, Sawchenko PE, Vale WW. The central distribution of a corticotropin-releasing factor (CRF)-binding protein predicts multiple sites and modes of interaction with CRF. *Proc Natl Acad Sci U S A.* 1992; 89:4192–4196. [PubMed: 1315056]
37. Sapolsky RM, Krey LC, McEwen BS. Prolonged glucocorticoid exposure reduces hippocampal neuron number: Implications for aging. *J Neurosci.* 1985; 5:1222–1227. [PubMed: 3998818]
38. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol (Berl).* 1991; 82:239–259. [PubMed: 1759558]
39. Landfield PW, Blalock EM, Chen KC, Porter NM. A new glucocorticoid hypothesis of brain aging: Implications for Alzheimer's disease. *Curr Alzheimer Res.* 2007; 4:205–212. [PubMed: 17430248]
40. Aisen PS, Davis KL, Berg JD, Schafer K, Campbell K, Thomas RG, Weiner MF, Farlow MR, Sano M, Grundman M, Thal LJ. A randomized controlled trial of prednisone in Alzheimer's disease. Alzheimer's Disease Cooperative Study. *Neurology.* 2000; 54:588–593. [PubMed: 10680787]
41. Csernansky JG, Dong H, Fagan AM, Wang L, Xiong C, Holtzman DM, Morris JC. Plasma cortisol and progression of dementia in subjects with Alzheimer-type dementia. *Am J Psychiatry.* 2006; 163:2164–2169. [PubMed: 17151169]
42. Lee KW, Kim JB, Seo JS, Kim TK, Im JY, Baek IS, Kim KS, Lee JK, Han PL. Behavioral stress accelerates plaque pathogenesis in the brain of Tg2576 mice via generation of metabolic oxidative stress. *J Neurochem.* 2009; 108:165–175. [PubMed: 19012747]
43. Guo Q, Zheng H, Justice NJ. Central CRF system perturbation in an Alzheimer's disease knockin mouse model. *Neurobiol Aging.* 2012; 33:2678–2691. [PubMed: 22336193]
44. Baglietto-Vargas D, Medeiros R, Martinez-Coria H, LaFerla FM, Green KN. Mifepristone alters amyloid precursor protein processing to preclude amyloid beta and also reduces tau pathology. *Biol Psychiatry.* 2013; 74:357–366. [PubMed: 23312564]
45. Sapolsky RM, Krey LC, McEwen BS. The neuroendocrinology of stress and aging: The glucocorticoid cascade hypothesis. *Endocr Rev.* 1986; 7:284–301. [PubMed: 3527687]
46. Green KN, Billings LM, Roozendaal B, McGaugh JL, LaFerla FM. Glucocorticoids increase amyloid-beta and tau pathology in a mouse model of Alzheimer's disease. *J Neurosci.* 2006; 26:9047–9056. [PubMed: 16943563]
47. Vassar R, Kovacs DM, Yan R, Wong PC. The beta-secretase enzyme BACE in health and Alzheimer's disease: Regulation, cell biology, function, and therapeutic potential. *J Neurosci.* 2009; 29:12787–12794. [PubMed: 19828790]
48. Haas C, Hung AY, Citron M, Teplow DB, Selkoe DJ. beta-Amyloid, protein processing and Alzheimer's disease. *Arzneimittelforschung.* 1995; 45:398–402. [PubMed: 7763333]

49. Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S, Amarante P, Loeloff R, Luo Y, Fisher S, Fuller J, Edenson S, Lile J, Jarosinski MA, Biere AL, Curran E, Burgess T, Louis JC, Collins F, Treanor J, Rogers G, Citron M. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science*. 1999; 286:735–741. [PubMed: 10531052]
50. Selkoe DJ. Alzheimer's disease: Genes, proteins, and therapy. *Physiol Rev*. 2001; 81:741–766. [PubMed: 11274343]
51. Das U, Scott DA, Ganguly A, Koo EH, Tang Y, Roy S. Activity-induced convergence of APP and BACE-1 in acidic microdomains via an endocytosis-dependent pathway. *Neuron*. 2013; 79:447–460. [PubMed: 23931995]
52. Meckler X, Checler F. Visualization of specific gamma-secretase complexes using bimolecular fluorescence complementation. *J Alzheimers Dis*. 2014; 40:161–176. [PubMed: 24413617]
53. Arendt T, Stielor J, Strijkstra AM, Hut RA, Rudiger J, Van der Zee EA, Harkany T, Holzer M, Hartig W. Reversible paired helical filament-like phosphorylation of tau is an adaptive process associated with neuronal plasticity in hibernating animals. *J Neurosci*. 2003; 23:6972–6981. [PubMed: 12904458]
54. Hartig W, Stielor J, Boerema AS, Wolf J, Schmidt U, Weissfuss J, Bullmann T, Strijkstra AM, Arendt T. Hibernation model of tau phosphorylation in hamsters: Selective vulnerability of cholinergic basal forebrain neurons - implications for Alzheimer's disease. *Eur J Neurosci*. 2007; 25:69–80. [PubMed: 17241268]
55. Okawa Y, Ishiguro K, Fujita SC. Stress-induced hyperphosphorylation of tau in the mouse brain. *FEBS Lett*. 2003; 535:183–189. [PubMed: 12560101]
56. Planel E, Miyasaka T, Launey T, Chui DH, Tanemura K, Sato S, Murayama O, Ishiguro K, Tatebayashi Y, Takashima A. Alterations in glucose metabolism induce hypothermia leading to tau hyperphosphorylation through differential inhibition of kinase and phosphatase activities: Implications for Alzheimer's disease. *J Neurosci*. 2004; 24:2401–2411. [PubMed: 15014115]
57. Feng Q, Cheng B, Yang R, Sun FY, Zhu CQ. Dynamic changes of phosphorylated tau in mouse hippocampus after cold water stress. *Neurosci Lett*. 2005; 388:13–16. [PubMed: 16005567]
58. Ikeda Y, Ishiguro K, Fujita SC. Ether stress-induced Alzheimer-like tau phosphorylation in the normal mouse brain. *FEBS Lett*. 2007; 581:891–897. [PubMed: 17289030]
59. Guillozet AL, Weintraub S, Mash DC, Mesulam MM. Neurofibrillary tangles, amyloid, and memory in aging and mild cognitive impairment. *Arch Neurol*. 2003; 60:729–736. [PubMed: 12756137]
60. Moechars D, Dewachter I, Lorent K, Reverse D, Baekelandt V, Naidu A, Tesseur I, Spittaels K, Haute CV, Checler F, Godaux E, Cordell B, Van Leuven F. Early phenotypic changes in transgenic mice that overexpress different mutants of amyloid precursor protein in brain. *J Biol Chem*. 1999; 274:6483–6492. [PubMed: 10037741]
61. Moechars D, Lorent K, Van Leuven F. Premature death in transgenic mice that overexpress a mutant amyloid precursor protein is preceded by severe neurodegeneration and apoptosis. *Neuroscience*. 1999; 91:819–830. [PubMed: 10391465]
62. Lue LF, Kuo YM, Roher AE, Brachova L, Shen Y, Sue L, Beach T, Kurth JH, Rydel RE, Rogers J. Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. *Am J Pathol*. 1999; 155:853–862. [PubMed: 10487842]
63. Howlett DR, Richardson JC, Austin A, Parsons AA, Bate ST, Davies DC, Gonzalez MI. Cognitive correlates of Abeta deposition in male and female mice bearing amyloid precursor protein and presenilin-1 mutant transgenes. *Brain Res*. 2004; 1017:130–136. [PubMed: 15261108]
64. Dere E, Huston JP, De Souza Silva MA. The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. *Neurosci Biobehav Rev*. 2007; 31:673–704. [PubMed: 17368764]
65. Thangavel R, Van Hoesen GW, Zaheer A. The abnormally phosphorylated tau lesion of early Alzheimer's disease. *Neurochem Res*. 2009; 34:118–123. [PubMed: 18437565]
66. Naslund J, Haroutunian V, Mohs R, Davis KL, Davies P, Greengard P, Buxbaum JD. Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. *JAMA*. 2000; 283:1571–1577. [PubMed: 10735393]

67. Albasser MM, Amin E, Iordanova MD, Brown MW, Pearce JM, Aggleton JP. Perirhinal cortex lesions uncover subsidiary systems in the rat for the detection of novel and familiar objects. *Eur J Neurosci.* 2011; 34:331–342. [PubMed: 21707792]
68. Albasser MM, Amin E, Iordanova MD, Brown MW, Pearce JM, Aggleton JP. Separate but interacting recognition memory systems for different senses: The role of the rat perirhinal cortex. *Learn Mem.* 2011; 18:435–443. [PubMed: 21685150]
69. Stehberg J, Simon F. Involvement of the insular cortex in retention of conditioned taste aversion is not time dependent. *Neurobiol Learn Mem.* 2011; 95:14–18. [PubMed: 20955809]
70. Xiao X, Yang Y, Zhang Y, Zhang XM, Zhao ZQ, Zhang YQ. Estrogen in the anterior cingulate cortex contributes to pain-related aversion. *Cereb Cortex.* 2013; 23:2190–2203. [PubMed: 22784608]

Figure 1.

PSAPP mice lacking either both ($CRFR1^{-/-}$) or one ($CRFR1^{+/-}$) allele for CRFR1 exhibited consistently lower mean values of plaque load per μm^2 in all brain areas. Statistically significant reductions were seen in the insular, retrosplenial, ectohippocampus, and perirhinal cortices ($p < 0.05$, $p < 0.01$, $p < 0.01$, $p < 0.05$, respectively). The hilar region of the hippocampus and the molecular layer of the dentate gyrus also showed significant decrease in A β accumulation (both $p < 0.05$ for both $CRFR1^{-/-}$ and $CRFR1^{+/-}$) as a function of CRFR1 ablation. PSAPP mice, panels A, D; PSAPP- $CRFR1^{+/-}$, panels B, E; PSAPP- $CRFR1^{-/-}$, panels C, F. Retrosplenial (RS) cortex; hilar layer of the hippocampus, CA4; the molecular layer of the dentate gyrus (mDG); ectohippocampus (Ect); perirhinal cortex (PRh); corpus callosum (cc); rhinal fissure (rf); lateral nucleus of the amygdala (La). Scale bar 400 μm .

Figure 2.

Changes in Aβ peptides in PSAPP mice with CRFR1 ablation. Analysis of whole brain lysates by ELISA revealed that (A) levels of Aβ₄₀ and (B) Aβ₄₂ were significantly reduced in PSAPP mice null for one (PSAPP-R1^{+/-}) or both (PSAPP-R1^{-/-}) alleles for CRFR1 compared to PSAPP counterparts (for Aβ₄₀, PSAPP-R1^{-/-} and PSAPP-R1^{+/-} versus PSAPP, $p < 0.001$; for Aβ₄₂, PSAPP-R1^{-/-} and PSAPP-R1^{+/-} versus PSAPP, $p < 0.05$). Levels of both Aβ₄₀ and Aβ₄₂ in PSAPP-R1^{+/-} and PSAPP-R1^{-/-} were not significantly different than that of wild-type (wt) animals or CRFR1 knockout mice ($p > 0.05$). All data are expressed as Mean±SEM.

Figure 3.

Upstream mediators of the CRFR1-Aβ relationship. We found no change in transgenic overexpression of AβPP in our CRFR1 mutant cohorts (PSAPP-R1^{-/-} and PSAPP-R1^{+/-} versus PSAPP, all $p > 0.05$), indicating that observed reductions in Aβ in PSAPP-R1 mutant mice are not due to a decrease in transgenic AβPP expression. β-secretase activity on AβPP results in the generation of AβPP C-terminal fragments (CTFs) detected with the CT20 antibody, which are highly present at high levels in PSAPP mice. We found a significant reduction in CTFβ in PSAPP-R1^{-/-} and PSAPP-R1^{+/-} (both $p < 0.0001$ compared to PSAPP) but not CTFα (both $p > 0.05$ compared to PSAPP), suggesting a CRF-dependent mechanism of Aβ production. The Aβ degrading enzyme, IDE, was significantly reduced in PSAPP-R1^{-/-} and PSAPP-R1^{+/-} compared to PSAPP cohorts ($p < 0.01$, each), potentially indicating reduced activity due to lower levels of Aβ.

Table 1Percent of total plaque area per μm^2 in regions showing significant change

	PSAPP	PSAPP-R1^{+/-}	PSAPP-R1^{-/-}	<i>p</i> value versus PSAPP
Insular	3.46	2.15	2.02	<0.05
Retrosplenial	4.87	2.89	2.77	<0.01
Ectorhinal/perirhinal	6.75	4.23	3.82	<0.01
Dentate gyrus mol	6.25	4.16	3.65	<0.05
Hilus	4.72	2.13	1.48	<0.05