EXTENDED ISOLATION STRESS INDUCES EXACERBATION OF ALZHEIMER'S DISEASE-RELATED PATHOLOGY IN 5XFAD MICE

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1. Introduction

In the Unites States alone, it is estimated that 5.7 million people suffer from Alzheimer's disease (AD). Of the six leading causes of death, AD is the only one that increased between the years 2000–2015; there was a 123% increase in deaths from Alzheimer's. Moreover, the annual number of new cases of AD and other dementias is projected to double by 2050, and the total estimated prevalence of the disease by 2050 is expected to reach nearly 14 million. ("2018 Alzheimer's Disease Facts and Figures," 2018). But Alzheimer's disease is most devastating because of its impact on afflicted individuals and those who care for them on a personal level. For example, health care payments for people with AD and other dementias are estimated to be 277 billion dollars in 2018. Close to 4 in 10 people who are providing unpaid care for someone with Alzheimer's report struggling to buy food and have to eat less as a result ("2018 Alzheimer's Disease Facts and Figures," 2018). More than that, the nature of the disease is such that patients with Alzheimer's suffer from cognitive deficits, memory loss, personality changes, and, eventually, death (Huang & Mucke, 2012). The patients decline as their brain undergoes deterioration to a point that is eventually fatal. This process is a devastating one, both for those afflicted with AD as well as for the loved ones forced to witness and care for them during this mental and physical decline.

Despite the pervasiveness of this disease, and the concerted efforts of labs of many disciplines to develop an integrated picture of disease etiology, its causes have yet to be fully elucidated (Selkoe, Mandelkow, & Holtzman 2012). When Alois Alzheimer autopsied the brain of the first patient with what came to be known as Alzheimer's disease, he found decreased brain volume (Alzheimer, Stelzmann, Schnitzlein, & Murtagh, 1995). This was caused, in part, by what are now known as the two pathological hallmarks of AD, an accumulation of Aβ plaques

(Glenner & Wong, 1984a, b) and neurofibrillary tangles (Grundke-Iqbal et al., 1986). Although all AD involves these pathologies, the development of AD comes as two general types: The first, sporadic AD, is diagnosed after the age of 65, is much more common, and develops relatively later in life. Secondly, early-onset Alzheimer's, which is diagnosed before the age of 65, and has genetic causes. Although the genetic origins of early-onset AD have been determined, sporadic AD is much more prevalent. Even so, its etiology is unknown. The increasing pervasiveness of AD combined with the lack of knowledge regarding its pathogenesis present a compelling case for the necessity of further research in this area.

1.2 The role of amyloid-beta in AD

As noted above, scientists have identified the two pathological hallmarks of Alzheimer's disease as amyloid beta (Aβ) plaques (Glenner & Wong, 1984a, b) and the neurofibrillary tangles composed of hyperphosphorylated tau, a microtubule associated protein that helps stabilize the cytoskeletal structure of the neuron (Grundke-Iqbal et al., 1986). Of the two pathologies, the role of Aβ has been explored much more thoroughly by scientists. Aβ is formed when Amyloid Precursor Protein (APP) is cleaved by the proteolytic enzymes β-secretase and γ-secretase. APP is first cleaved by either α-secretase or β-secretase and then the products are both cleaved by γ-secretase. Cleavage of APP by β-secretase results in an N-terminal fragment (sAPP-β) and a C-terminal fragment (CTF) that contains Aβ (β-CTF). β-CTF is then cleaved by γ-secretase resulting in γ-CTF and $A\beta_{1\text{-}42,}$ that is secreted through the cell membrane and subsequently aggregates (Rezai-Zadeh et al., 2005; Haass & Selkoe, 1993). When APP is instead cleaved by α-secretase it results in N-terminal soluble APPα (sAPPα) and α-CTF in the middle of the Aβ domain such that subsequent cleavage by γ-secretase does not form toxic Aβ (Rezai-Zadeh et al., 2005).

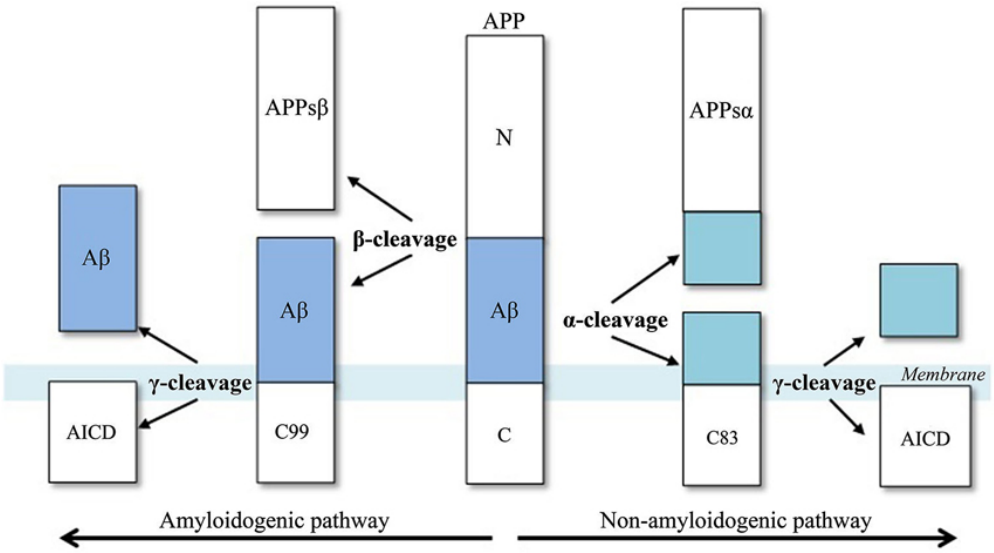

Figure 1. Cleavage of APP into Aβ. (Menting & Claassen, 2014).

In early-onset Alzheimer's, mutations in APP and the presenilin (PS)1 and (PS)2 genes of the γ-secretase complex initiate the amyloid cascade (Hardy & Higgens, 1992; Sherrington et al., 1995). These three genes mutate to alter APP processing, resulting in different ratios of the Aβ peptides, such that more of the toxic $A\beta_{1\text{-}42}$ is produced (Bertram et al., 2010; Huang & Mucke, 2012; Seiffert et al., 2000) and, as a result, cause early onset, autosomal dominant AD, which accounts for fewer than 10% of Alzheimer's disease cases (Campion et al., 1999; Huang & Mucke, 2012).

1.3 Neurodegenerative effects of amyloid-beta

While Aβ fibrils aggregate to form plaques, soluble Aβ dimers, trimers, and oligomers also have pathogenic effects (Huang & Mucke, 2012). Though researchers have long since been investigating the role of Aβ plaques in AD, recent focus has shifted toward examining the role of the Aβ oligomers, as humans with high plaque burden sometimes lack cognitive symptomology (Erten-Lyons et al., 2009). Transgenic mice with plaques and a reduced number of oligomers demonstrate no memory impairment during a period of accelerated plaque formation that results

in fewer oligomers, indicating the detrimental role of oligomers may supersede the role of plaques (Lesné, Kotilinek, & Ashe, 2008). These Aβ oligomers can cause aberrant signaling as well as synaptic depression, mainly through triggering excitotoxicity in neurons utilizing glutamate, and simultaneously impairing the function of inhibitory interneurons (Palop & Mucke, 2010). Ultimately, sixty percent of excitatory synapses are lost in the presence of Aβ oligomers (Mucke & Selkoe, 2012). The most affected are hippocampal and neocortical cholinergic, glutamatergic, and serotonergic synapses (Grutzendler & Morris, 2001). As a result, learning and memory are drastically impacted by this Aβ-induced synapse loss and neuronal death and subsequent deficits such as short-term memory impairment are often the first seen in AD patients (Masters & Selkoe, 2012; Mayeux & Stern, 2012).

One of the substantial deficits to memory caused by Aβ has been demonstrated through the research on the effects of long-term potentiation (LTP) in neuronal slice cultures. LTP occurs when the high-frequency firing of action potentials causes physiological changes to the postsynaptic neuron that strengthen the synapse and create more synapses. This takes place through the phosphorylation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) channels and the insertion of new ones in the membrane (Mucke & Selkoe, 2012). However, Aβ oligomers inhibit LTP while increasing long term depression (LTD) and synapse loss (Ittner & Götz, 2011; Mucke & Selkoe, 2012; Snyder et al., 2005). Specifically, Aβ promotes endocytosis of N-methyl-D-aspartate (NMDA) receptors and a decrease in signaling to cAMP response element binding protein (CREB), a transcription factor neurons need to survive (Snyder et al., 2005; Ittner & Götz, 2011).

1.4 Mouse models of AD

Several transgenic mouse models of AD have been developed to study the pathology of familial, early onset AD. Mouse models of familial AD overexpress one or more human genes that often contain mutations that are commonly found in humans with early-onset AD, APP, PS1, or PS2. Mutations of these genes result in dramatically increased Aβ production and plaque formation not typically found in rodents, and these transgenic mice with human APP, PS1, or PS2 mutations also display cognitive impairment (Duff et al., 1996). Additionally, Tg2576 mice have a genetic mutation such that engenders overexpression of APP, elevated $A\beta_{1-42}$, plaques, and impaired cognition by 9−10 months of age (Elder, Sosa, & Gasperi, 2010). The 5xFAD mouse model consists of a combination of 3 APP and 2 PS1 mutations that results in substantially increased $A\beta_{1-42}$ production and plaque load that manifest early in life (Elder et al., 2010). In fact, the 5xFAD mice demonstrate intraneuronal Aβ accumulation and plaque formation by as early as 2 months of age (Oakley et al., 2006). Additionally, the 5xFAD mice already display deficits in a contextual fear conditioning paradigm by 6 months of age (Lison et al., 2014).

1.5 Stress and its impact on AD

1.5a Stress

One of the primary routes through which the body responds to stress, in order to attempt a return to homeostasis, is through the hypothalamic-pituitary-adrenal (HPA) axis (Berton and Nestler, 2006; Chrousos, 2009). The HPA axis response to stress involves the release of corticotropin-releasing factor (CRF) from the hypothalamus (Rivier & Vale, 1983), which causes the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary that results in the adrenal cortex producing glucocorticoids (Smith & Vale, 2006; Kloet & Derijk, 2004). Also

involved is sympathetic nervous system (SNS) activation and the release of epinephrine from the adrenal medulla (Cannon, Britton, Lewis, & Groeneveld, 1927; Selye, 1946). There also exists a negative feedback loop to enable the body to recover from stress, where glucocorticoid receptors in the hippocampus decrease further glucocorticoid release (Keller-Wood & Dallman, 1984; Smith & Vale, 2006; Jacobson & Sapolsky 1991). The effects of stress on the body differ depending on the nature and duration of the stressor, but stress affects numerous body systems, including inflammatory processes (Zlatković, & Filipović, 2013; Black, 1994). Characteristic of the body's response to an acute stressor is gliosis, inflammation through high mobilty group box-1 protein (HMGB-1)-mediated priming of the nucleotide-binding domain, leucine-rich repeat, pyrin domain containing protein 3 (NLRP3) inflammasome and increased production of pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6 (Walker, Nilsson, & Jones, 2013; Weber et al., 2015). Alternatively, in response to chronic stress, immune suppression results from a decrease in immue cell number, reduced natural killer (NK) cell cytotoxicity, and an increase in regulatory T cells (Dhabhar, 2009; Dhabhar & McEwen, 1997; Irwin et al., 1990). Additionally, the effects of stress differ from person to person as the environment plays a large role in the body's response to stress. For example, the extent to which rat pups are groomed by their mothers before they are weaned impacts their development, impacting their adulthood behavioral and endocrine response to stress (Liu et al., 1997; Caldji et al., 1998). Pups groomed more by their mothers display decreased plasma ACTH and corticosterone in response to stress as well as a more sensitive glucocorticoid feedback loop (Liu et al., 1997).

1.5b Isolation stress and health

Although temporary activation of the HPA axis allows the body to respond to a stressor and then return to homeostasis, extended activation of the HPA axis has been shown to be

maladaptive (McEwen, & Gianaros, 2010). One such chronic stressor that afflicts a considerable portion of the world's population is social isolation (Cacioppo & Hawkley, 2003). In addition to predicting mortality (House, Landis, & Umberson, 1988), social isolation produces effects similar to those of cardiovascular disease (CVD) and stroke (Friedler, Crapser, & McCullough, 2015). Moreover, isolation stress is a risk factor for CVD and ischemic strokes. It also increases the likelihood of re-infarction and mortality in patients while controlling for all other risk factors (Friedler et al., 2015). General emotional stress has been shown to increase one's risk of developing type II diabetes (Pouwer, Kupper, & Adriaanse, 2010) in addition to making the disease worse (Moran et al., 2015). In turn, type II diabetes, like CVD, results in an increased risk of developing sporadic AD (Haan, 2006). Further, depression also is made worse by stress and, in turn, makes AD worse (Rothman & Mattson, 2010; Kloet et al., 2004).

1.5c Stress and AD

Clinical data have shown that stress can be a risk factor for AD (Wilson et al., 2005; Friedler et al., 2015). Aging animals and humans already have decreased HPA axis regulation and hypersecrete glucocorticoids, which potentiate hippocampal damage due to Aβ (Friedler et al., 2015). Moreover, rats that have undergone maternal separation, a form of early life stress where mice are separated from their mothers, display increased plasma corticosterone and decrease hippocampal glucocorticoid receptor density, indicating depressed HPA axis feedback inhibition, in addition to cognitive deficits and increased amyloidogenic processing of APP (Solas et al., 2010). Catania et al. (2009) found that four weeks of chronic unpredictable stress, a paradigm in which mice experience a variety of stressors, increased hippocampal β-CTF and BACE protein levels, indicating increased amyloidgenic cleavage of APP by β and γ secretases. Stress also exacerbates Aβ production in transgenic Alzheimer's disease models (Devi et al.,

2010; Lee et al., 2009; Dong et al., 2004). For example, Dong et al. (2014) found that Tg2576 transgenic mice isolated from 21 days of age to ten months of age demonstrated increases in tissue $A\beta_{1\text{-}40}$, $A\beta_{1\text{-}42}$ levels, and plaques in addition to increased anxiety-like behaviors in elevated plus and spontaneous alteration behavioral paradigms. Hsiao et al. (2011) found that APP/PS1 transgenic mice that were isolated from 3 months of age to 7 months of age demonstrated impaired spatial working memory and a significantly increased $A\beta_{40}/A\beta_{42}$ ratio without plaque formation. This effect is not uniform, however, as Devi et al. (2010) administered 5 days of restraint stress, in which mice are immobilized, to 5xFAD transgenic mice and found increased Aβ in the hippocampus, but not neocortex, of female mice only. Further, illustrating the importance of the nature and duration of the stressor, restraint stress in TgCRND8 transgenic mice from 1 to 3 or 4 to 6 months of age did not significantly increase the number of Aβ plaques (Yuan et al., 2013).

The mechanisms behind stress-induced increases in Aβ are still not fully understood. Nor is the interaction between age and duration of stress on AD-related pathology, though studies have indicated stress-induced neurodegeneration and fear conditioning deficits are dependent on corticotropin releasing factor 1 (CRF1) and corticotropin releasing factor receptor 1($CRFR_1$) (Carroll et al., 2011; Dong et al., 2014) and result in increases in beta-site APP cleaving enzyme 1 (BACE1) expression and decreased methylation of 5'—C′—phosphate'—G'—3' (CpGs) in the BACE1 promoter region (Cordner & Tamishiro, 2016). This indicates that BACE1 expression may be epigenetically altered by glucocorticoids as hippocampal expression of BACE1 was correlated with adrenal weight (Cordner & Tamishiro, 2016). Additionally, glucocorticoid treatment *in vitro* increased CTF-β, the precursor to Aβ resulting from BACE1 cleavage, as did glucocorticoid administration *in vivo* (Green et al., 2006). Further, Zlatkovic and Filipović

(2013) demonstrated that chronic stress led to nuclear factor kappa-B (NF-κB) activation as well as upregulated inducible nitric oxide synthase (iNOS) expression and the antioxidant N-acetylcysteine prevented isolation stress-induced increases in γ secretase and Aβ, indicating a role for oxidative stress in these processes (Hsiao, Kuo, Chen, & Gean, 2012). Another possibility is that acute stress induced-gliosis results in an increase in pro-inflammatory cytokines due to microglia, leading to increases in Aβ that remain through chronic stress (Walker et al., 2013; Weber et al., 2015).

1.6 Hypotheses

We hypothesized that a social stressor, isolation stress, would significantly exacerbate soluble amyloid beta production as well as plaque load in 5xFAD+ transgenic mice in comparison to group-housed control transgenic animals and wild-type mice. More specifically, we hypothesized that there will be a significant increase in amyloid beta in 5xFAD+ animals isolated for 2 or 3 months over wild-type animals. Further, it was hypothesized that isolated, 5xFAD+ animals would perform worse in contextual fear conditioning (CFC), a hippocampus-dependent learning and memory task, than group-housed, 5xFAD+ or isolated wild-type animals, and that the longer the isolation the worse the performance. Freezing in CFC is indicative of learning of the context shock association, and animals that remain active display a contextual learning deficit. Pilot data from our lab demonstrated there is not yet a deficit in contextual fear conditioning in 3 month-old 5xFAD+ animals, but that it is present by 6 months of age (see Figure 1). It is hypothesized that isolation stress will worsen performance in CFC and that there will be a deficit in the 4- and 5-month-old animals isolated for 2 or 3 months.

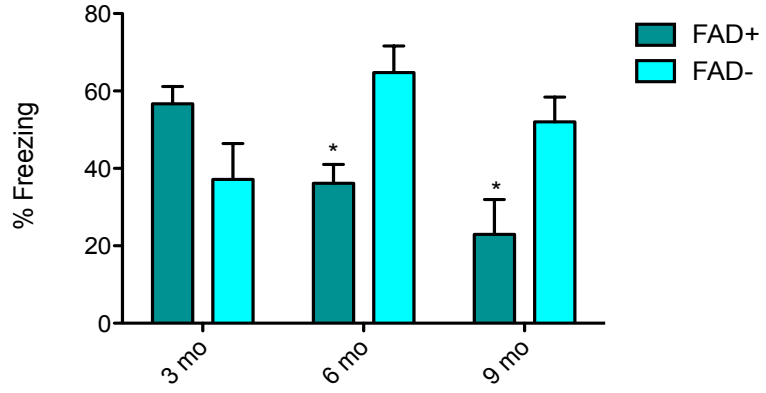

Figure 2. 5xFAD mice demonstrate impaired cognition by 6 months of age. 5xFAD mice freeze significantly less than wild type mice in a contextual fear conditioning paradigm at 6 and 9 but not 3 months of age. Bars represent mean ± SEM. Significance differences ($p<0.05$) are represented by *.

2. Methods

2.1 Subjects

Animals used in experiments were male 5xFAD mice bred in the TCU vivarium, isolated or group-housed at the age of 2 months. All animals were housed and treated in accordance with protocols approved by Texas Christian University's Institutional Animal Care and Use Committee (IACUC) and with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). All subjects were isolated or housed in groups of 2 to 4 in standard polycarbonate cages (12.5cm x 15cm x 25cm). Food and water was available *ad libitum* and animals were maintained on a consistent light/dark schedule, with lights on at 0700 and lights off at 1900.

2.2 Treatment conditions

At 2 months of age, experimental subjects were isolated or remained group housed for 2 or 3 months, at which point they underwent training and then, 24 hours later, testing in contextual fear conditioning. Tissue collection occurred 24 hours after testing.

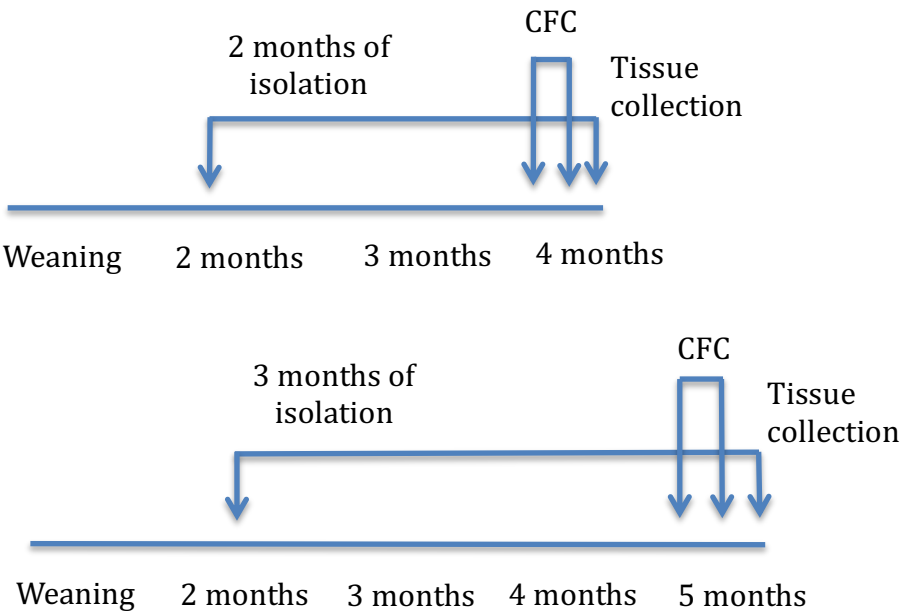

Figure 3. Experimental timeline. At 2 months of age, experimental subjects were isolated or remained group housed for 2 or 3 months, at which point they underwent contextual fear conditioning.

2.3 Behavioral Paradigm

After extended isolation or group housing, animals underwent contextual fear conditioning. This protocol includes a training session (day 1) and a testing session (day 2) 24 hours later. The training session consists of a 120s acclimation period followed by a 2s foot shock at 0.5mA, 60s with no shock, and then an additional 2s 0.5mA shock. Animals remain in the chamber for an additional 60s following the conclusion of the aversive stimulus. On testing day, animals are placed back into the chamber and freezing is monitored for 180s, but no shocks are delivered. Contextual fear conditioning is conducted in chambers (Coulbourn Instruments, Whitehall, PA, 7Wx7Dx12H) with an electrified grid floor through which the aversive stimulus is delivered. The delivery of the stimulus and the resulting freezing behavior is monitored and calculated using FreezeFrame™ software (ActiMetrics Software, Wilmette, IL).

Previous research from our laboratory has demonstrated that the incorporation of an olfactory cue (peppermint oil 1:10 in water) and a highly salient wall design (black polka dots) into the context increases the mouse's percent freezing. We interpret this increase in freezing behavior as better learning of the context-shock pairing (Phillips & Ledoux, 1992; Kahn et al., 2012; Kranjac et al., 2012). The time freezing was analyzed using analysis of variance (ANOVA) procedures (SPSS 22.0, IBM, Armonk, NY), in which Genotype (5xFAD+/-) and Treatment (isolated/group housed) were used as independent variables. All statistical analyses were conducted using an alpha level of ≤ 0.05 to determine significant group differences. Following a significant omnibus F value, post-hoc comparisons utilized Fisher's PLSD.

2.4 Tissue preparation and ELISA

2.4a Tissue extraction

Animals were anesthetized with ketamine (120mg/kg) and xylazine (16mg/kg) and perfused for 5 minutes with saline adjusted to a pH of 7.4. The brain was then removed and one hemisphere was fixed in 4% paraformaldehyde (PFA) in 2x phosphate buffered saline (PBS) while the hippocampus was excised from the other hemisphere and the tissue homogenized with PRO-PREP (Bora Scientific, Boca Raton, FL). The samples were immediately frozen on dry ice and stored overnight at -80 °C. The lysate was centrifuged at 10,000 rpm the next day for 40 minutes, before the lysate was removed and a *DC* Protein Assay (Bio-Rad Laboratories, Hercules, CA.) conducted.

2.4b *DC* Protein Assay

DC Protein Assays utilize a working reagent that is used with detergent-based buffers. The protein standard curve consists of dilutions from 0.2–1.5 mg/ml of γ-globulin, made in the same buffer as the lysates. Five µl of standards and 5 µl of sample were pipetted into a 96 well plate

with 25 μl of reagent A' and 200 μl of reagent B. After 15 min, the plate was put into the plate reader (BMG LabTech FLUOstar Omega, Cary, NC), and the optical density of the samples was read at 750 nm. The results were then used to standardize general protein content for the ELISA.

2.4c Aβ ELISA procedure

The BetaMark $A\beta_{x-42}$ ELISA (BioLegend, San Diego, CA) was performed according to manufacturer instructions. In brief, the samples were diluted with working incubation buffer including the HRP- labeled detection antibody, and the dilutions of the standard were plated in duplicate. 5xFAD+ samples were diluted 1:8 and 5xFAD- samples were diluted 1:4. The plate was incubated overnight at 2–8 °C. The next day, wells were washed five times with wash buffer and the TMB substrate was added to each well. The plate was then incubated for 45 min at room temperature and well optical density read at 620 nm (BMG LabTech FLUOstar Omega, Cary, NC).

2.5 Histochemistry for analysis of plaque counts

The hemispheres fixed in PFA were washed with PBS, embedded in 3% agarose and cut into 40 μm sagittal sections with a Leica VT1000 S Vibratome. Sections were stored in a 1% PFA and 0.03% azide solution. A middle to late hippocampal section from each animal was washed 3 times for 10 minutes in Millipore water, stained with Thioflavin-T for 5 minutes, washed for 2 minutes in 70% ethanol, washed twice for 2 minutes in 50% ethanol, and then washed twice for 2 minutes in Millipore water. The sections were then mounted and examined utilizing confocal microscopy. ImageJ (NIH, Bethesda, MD) was utilized to count plaques in the hippocampus and subiculum. The average counts were obtained from three counters, using one section containing the area of interest per animal.

3.0 Results

3.1 Experiment 1A: Effects of Two Months of Isolation on Cognition

Two months of isolation stress leads to impaired freezing in contextual fear conditioning

A two-way analysis of variance (ANOVA) was used to determine whether two months of isolation stress impacted cognition in a contextual fear conditioning paradigm. There was a main effect of Condition (Isolation or Group Housing; $F(1, 72) = 7.589$, $p < .01$) on freezing, such that isolated animals froze significantly less than group housed animals. There was also a main effect of Genotype (FAD+ or FAD-; $F(1, 72)$ 9.624, $p < .01$) on freezing, such that FAD+ animals froze significantly more than FAD- animals. There was not a significant Condition x Genotype interaction ($F(1, 72) = 4.317$, *NS*); Figure 3). Overall, two months of isolation resulted in a cognitive deficit in contextual fear conditioning.

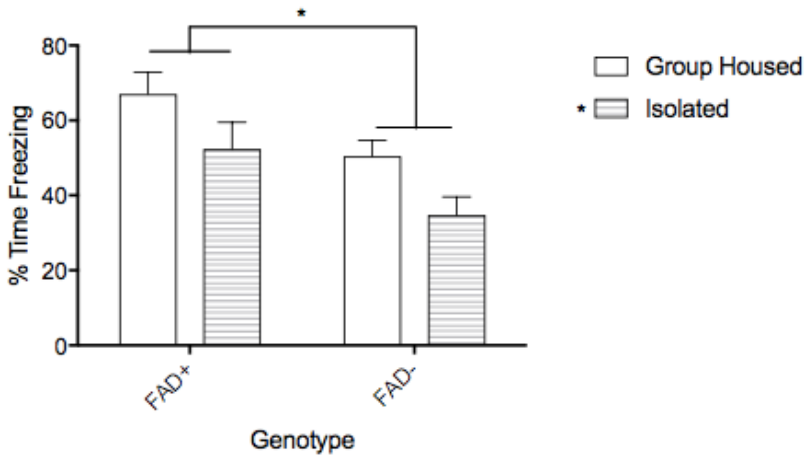

Figure 4. Two months of isolation stress leads to impaired cognition. A 2x2 ANOVA revealed a main effect of condition such that isolated animals froze significantly less than group housed animals. There was also a main effect of genotype such that FAD+ animals froze significantly more than FAD- animals. Bars represent mean ± SEM. Significance differences ($p<0.05$) are designated by *.

3.2 Experiment 1B: Effects of Two Months of Isolation on Aβ

Two months of isolation stress did not significantly impact hippocampal Aβ levels

A 2x2 ANOVA was used to determine if two months of isolation stress impacted hippocampal Aβ levels. Because the Levene's test of equality of error variances revealed significant heterogeneity of variance, F= 24.909, an ANOVA was conducted on the natural log of raw scores. Analyses revealed a main effect of Genotype (FAD+ or FAD-; $F(1, 26)$ 239.138, $p < .001$) such that FAD+ animals had significantly more hippocampal Aβ than FAD- animals. There was not a significant main effect of Condition (Isolation or Group Housing; $F(1, 26)$= .225, *NS*) or a significant Condition x Genotype interaction ($F(1, 26)$= .843, *NS*); Figure 4). Overall, two months of isolation did not significantly increase hippocampal Aβ levels.

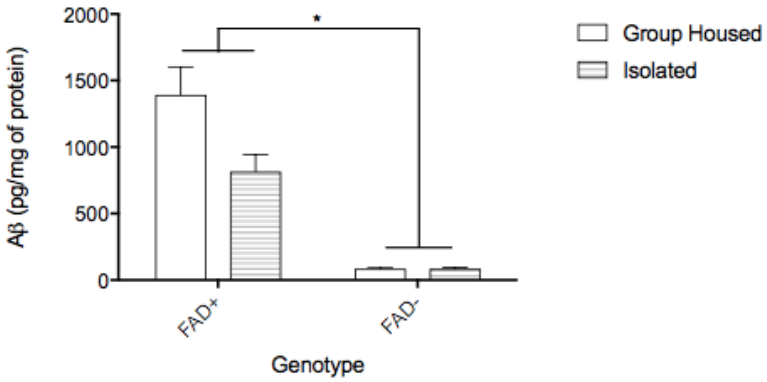

Figure 5. Two months of isolation stress did not significantly impact soluble Aβ levels. A 2x2 ANOVA revealed a main effect of genotype such that FAD+ animals had significantly more hippocampal Aβ than FAD- animals regardless of condition. Significance differences ($p<0.05$) are designated by *.

3.3 Experiment 1C: Effects of Two Months of Isolation on Plaques

Two months of isolation stress leads to increased hippocampal plaques in FAD+ mice

A Student's t-test was used to examine differences across Condition (Isolation or Group Housing) in hippocampal plaque number of FAD+ mice. Analyses revealed significant differences in hippocampal plaque counts between isolated and group housed animals ($t(1, 23)$=

2.214, $p < .05$); Figure 5). Overall, animals isolated for two months had more hippocampal Aβ plaques than did group housed controls.

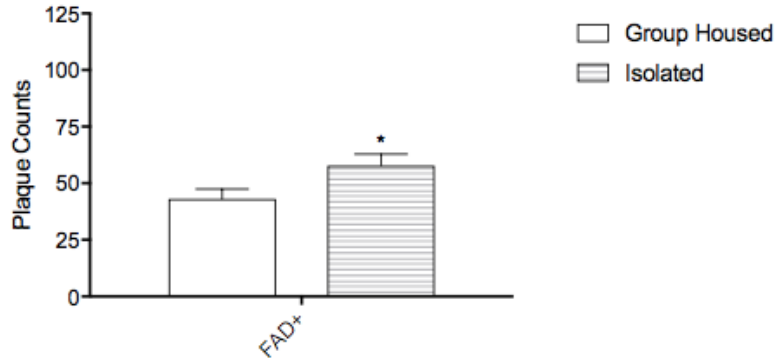

Figure 6. Two months of isolation stress leads to increased plaques in FAD+ mice. Student's t-tests revealed significant differences in hippocampal plaque counts between isolated and group housed animals. Bars represent mean ± SEM. Significance differences ($p<0.05$) are designated by *.

3.4 Experiment 2A: Effects of Three Months of Isolation on Cognition

Three months of isolation stress leads to impaired freezing in contextual fear conditioning

A two-way ANOVA was used to determine whether three months of isolation stress impacted cognition in a contextual fear conditioning paradigm. There was a main effect of Condition (Isolation or Group Housing; $F(1, 31)= 9.968$, $p < .01$) on freezing, such that isolated animals froze significantly less than group housed animals. There was not a significant main effect of Genotype (FAD+ or FAD-; $F(1, 31)= .277$, *NS*) or a significant Condition x Genotype interaction ($F(1, 31)= .074$, *NS*; Figure 6). Overall, three months of isolation resulted in a cognitive deficit in contextual fear conditioning.

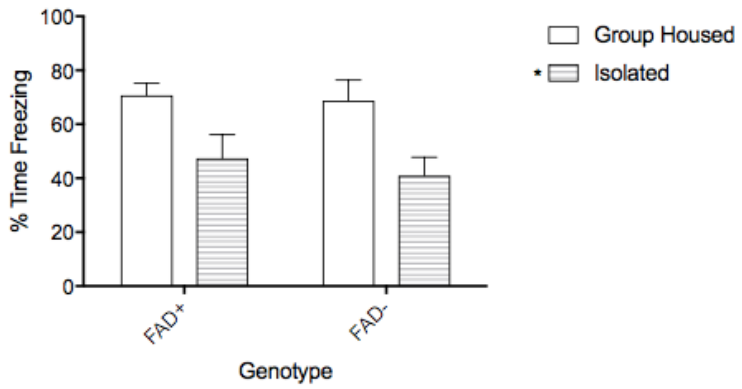

Figure 7. Three months of isolation stress leads to impaired cognition. A 2x2 ANOVA revealed a main effect of condition, such that isolated animals froze significantly less than group housed animals regardless of genotype. Bars represent mean ± SEM. Significance differences (p<0.05) are designated by *.

3.4 Experiment 2B: Effects of Three Months of Isolation on Aβ

Three months of isolation stress did not significantly impact hippocampal Aβ levels

A 2x2 ANOVA was used to determine whether three months of isolation stress impacted hippocampal Aβ levels. Because the Levene's test of equality of error variances revealed significant heterogeneity of variance, F= 14.605, an ANOVA was conducted on the natural log of raw scores. Analyses revealed a main effect of Genotype (FAD+ or FAD-; F(1, 32)= 95.377, p < .001) such that FAD+ animals had significantly more hippocampal Aβ than FAD- animals. There was not a significant main effect of Condition (Isolation or Group Housing; F(1, 32)= .433, *NS*) or a significant Condition x Genotype interaction (F(1, 32)= .244, *NS*; Figure 7). Overall, three months of isolation did not significantly alter hippocampal Aβ levels.

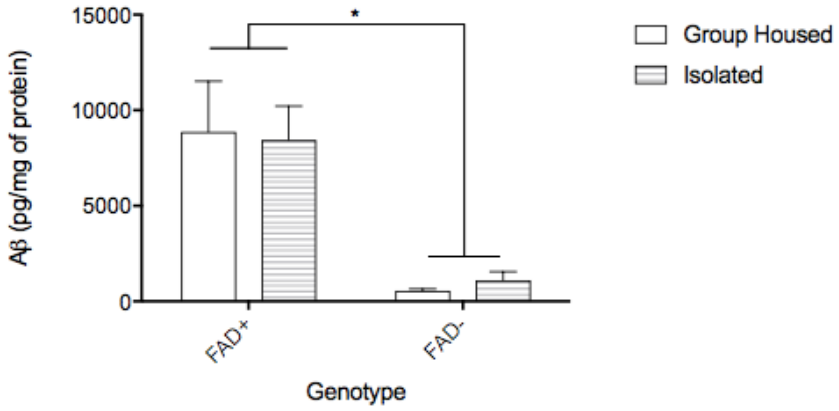

Figure 8. Three months of isolation stress did not significantly impact soluble Aβ levels. A 2x2 ANOVA revealed a main effect of genotype such that FAD+ animals had significantly more hippocampal Aβ than FAD- animals regardless of condition. Significance differences (p<0.05) are designated by *.

3.4 Experiment 2C: Effects of Three Months of Isolation on Plaques

Three months of isolation stress leads to increased hippocampal plaques in FAD+ mice

A Student's t-test was used to examine differences across Condition (Isolation or Group Housing) in hippocampal plaque number of FAD+ mice. Analyses revealed significant differences in hippocampal plaque counts between isolated and group housed animals (t(1, 9)= 2.582, p < .05); Figure 8). This is shown in Figure 9. Overall, FAD+ animals isolated for three months had more hippocampal Aβ plaques than did group housed controls.

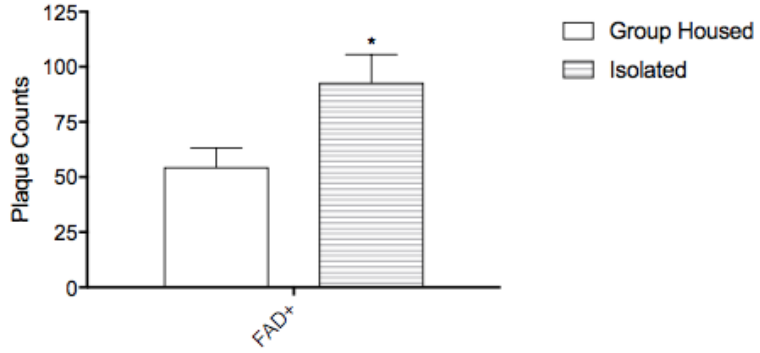

Figure 9. Three months of isolation stress leads to increased plaques in FAD+ mice. Student's t-tests revealed significant differences in hippocampal plaque counts between isolated and group housed animals. Bars represent mean ± SEM. Significance differences (p<0.05) are designated by *.

A. B.

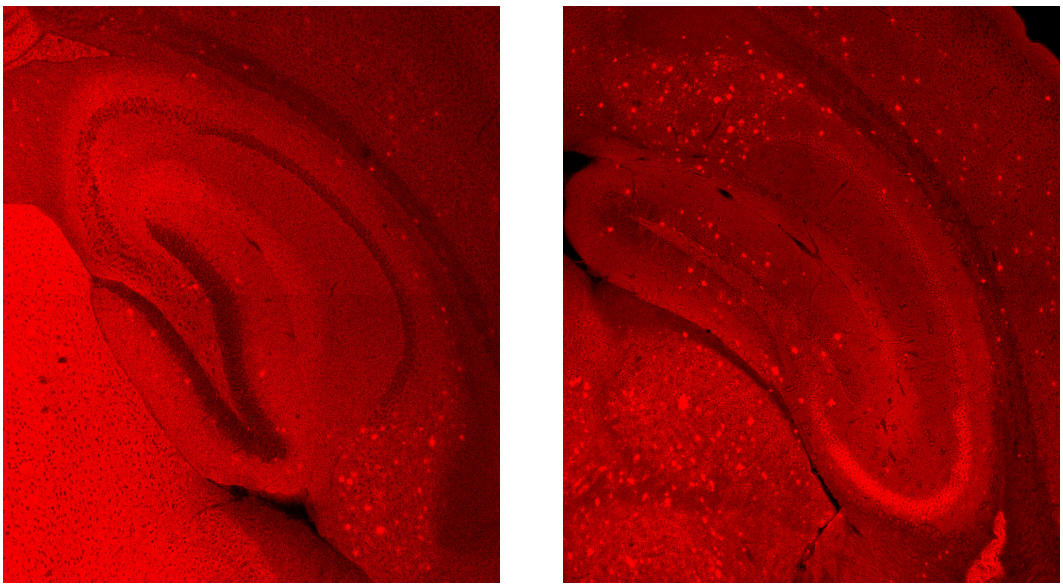

Figure 10. Thioflavin-T fluorescence. Representative images of plaques in the hippocampus and subiculum of 5xFAD+ animals group housed (A) and isolated for 3 months (B).

4.0 Discussion

These experiments aimed to determine whether isolation stress increases hippocampal amyloid beta and results in cognitive deficits in 5xFAD transgenic mice. Stress has been shown to exacerbate Aβ production in transgenic mouse models of the disease (Devi et al., 2010; Lee et al., 2009; Dong et al., 2004). However, these effects are not uniform and the parameters of the stress, such as type of stressor, duration, the animals used, and others, have all been shown to play a central role in the effect of the stress on AD pathology (Hsiao et al., 2011; Devi et al., 2010; Yuan et al., 2013). The present studies sought to test the effects of 2 and 3 months of social isolation on Aβ plaques, soluble hippocampal Aβ, and cognition. The hypotheses were mainly supported. In experiment one, contextual fear conditioning data revealed that group housed animals froze significantly more than did animals that were isolated for two months, indicating that isolation impaired the animals' ability to associate the context shock pairing.

Additionally, 5xFAD+ animals isolated for 2 months had a significantly increased number of Aβ plaques in the hippocampus. They did not, however, differ significantly from group housed animals in hippocampal $A\beta_{x-42}$ levels. This could indicate isolation impacted plaque production itself, without altering overall Aβ production.

As hypothesized, after 2 months of isolation the isolated animals displayed a cognitive deficit in CFC. In addition, FAD+ animals froze more than FAD- animals. We have previously shown that FAD+ mice display a deficit in CFC by 6 months of age but at 3 months of age they seem to do better than the group housed cohort (see Figure 1). This could be consistent with a more recent trend in the Alzheimer's literature, the idea that plaques bind up the more toxic Aβ oligomers that would be disrupting synapses (Taneja, Verma, & Vats, 2015), as studies have shown that $A\beta_{x-40}$ and $A\beta_{x-42}$ are correlated with cognitive deficits (Näslund, 2000; Oakley, 2006). Additionally, evidence suggests that small amounts of Aβ may even potentiate memory formation (Garcia-Osta & Alberini, 2009). This could explain why FAD+ mice perform slightly better than FAD- mice in CFC at a young age, and why this pattern is quickly reversed as animals age.

As in experiment one, it was also hypothesized in experiment two that isolation stress would increase hippocampal amyloid beta in 5xFAD mice and that isolation would lead to cognitive deficits in a contextual fear conditioning paradigm. These hypotheses were also mainly supported. The results from contextual fear conditioning revealed group housed animals froze significantly more than did animals that were isolated for three months, indicating isolation impaired the animals' ability to associate the context shock pairing. Additionally, 5xFAD+ animals isolated for 3 months had a significant increase in number of Aβ plaques in the hippocampus. They did not, however, differ significantly from group housed animals in

hippocampal $A\beta_{x-42}$ levels. This again could indicate isolation impacted plaque production without altering overall Aβ production.

These results demonstrate significant isolation-induced elevations in Aβ plaques and a concomitant cognitive deficit for the mice isolated for 2 and 3 months over group-housed animals. As this is the first study to examine isolation stress in the 5xFAD transgenic mouse model, it contributes to our understanding of what factors impact AD pathology. As the mutations in the 5xFAD lead to more severe pathology than the APP/PS1 or 3xTg mouse models, it enables us to study early-onset, or familial AD, and predict whether social isolation plays a role in the pathogenesis of humans with familial AD. Because the pathology is so severe in this model, evidence that social isolation can still influence it is important. Future research will explore the mechanisms behind the isolation-induced exacerbation of pathology and cognitive deficits. In order to investigate potential mechanisms, extensions of this research will examine the role of HPA axis hyperactivity and dysregulated secretase activity, via investigating BACE1 and β-CTF levels, as research has demonstrated decreased HPA axis regulation can potentiate hippocampal damage due to Aβ (Friedler et al., 2015) and stress can play a role in this (Solas et al., 2010). Moreover, the link between gliosis and inflammation will be explored by examining microglial activation and pro-inflammatory cytokine expression, as it is possible that stress induced-gliosis results in an increase in microglial production of pro-inflammatory cytokines which can contribute toward the exacerbation of Aβ (Walker et al., 2013; Weber et al., 2015). Also, because research has indicated a role for oxidative stress in stress induced exacerbation of AD like pathology (Zlatkovic & Filipović, 2013; Hsiao et al., 2012), markers of oxidative stress will also be explored by examining changes in iNOS and NF-κB protein levels, along with indicators of cellular oxidative damage.

Importantly, research examining ways to ameliorate stress-induced deficits has demonstrated the utility of exercise (Kiuchi, Lee, & Mikami, 2012), enriched environment (Jeong et al., 2011), antioxidants (Hsiao et al., 2012), and β_2 adrenergic receptor antagonists (Ni et al., 2007) as interventions. A follow-up study to this research will examine if the social isolation induced increase in Aβ plaques and cognitive deficit in the 5xFAD mice can be prevented with exercise alone or exercise and an enriched environment during the 3 months of social isolation. Because the elderly, the primary population suffering from Alzheimer's disease, are also routinely socially isolated, research into how to prevent isolation stress from exacerbating AD pathology becomes more critical with each passing year.

In summary, our findings were consistent with the literature demonstrating that stress can adversely impact Alzheimer's disease pathogenesis. We extended prior research in showing that 2 or 3 months of social isolation significantly increases the number of Aβ plaques in the hippocampus of male 5xFAD transgenic mice without significantly altering hippocampal $A\beta_{x\text{-}42}$ levels. Two and 3 months of isolation stress also resulted in a cognitive deficit in contextual fear conditioning. These findings demonstrate the need for further research exploring how stress can impact patients suffering from Alzheimer's disease, along with finding ways to prevent these deficits. As the aging population increases and the prevalence of Alzheimer's disease grows the answers to these questions will only become increasingly vital.

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VITA

PERSONAL BACKGROUND

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EDUCATION

Diploma, Ursuline Academy of Dallas, Dallas, Texas, 2011
Bachelor of Science, Psychology, Xavier University, Cincinnati, Ohio, 2015
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EXPERIENCE

Supplemental Instructor in Anatomy and Physiology, Learning Assistance Center, Xavier University, 2013-2015
Tutor in Anatomy and Physiology, Psychology, and History, Learning Assistance Center, Xavier University, 2013-2015
Research Assistant, Department of Psychology, Xavier University, 2013-2014
Research Assistantship, Department of History, Xavier University, 2014
Teaching Assistantship, Texas Christian University, 2015-present

VOLUNTEER WORK

Site Leader for Xavier University volunteers to Evanston Academy Elementary School, 2012, 2014-2015

PROFESSIONAL MEMBERSHIPS

Golden Key Honors Society, 2017-present
Psychoneuroimmunology Research Society, 2017-present
Society for Neuroscience, 2015-present
Phi Beta Kappa Honors Society, 2015-present
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ABSTRACT

EXTENDED ISOLATION STRESS INDUCES EXACERBATION OF ALZHEIMER'S DISEASE-RELATED PATHOLOGY IN 5XFAD MICE

By Julia Lauren Peterman
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Thesis Advisor: Gary W. Boehm, Professor of Psychology

The prevalence of Alzheimer's Disease, a neurodegenerative disease characterized by the pathological hallmarks of amyloid beta (Aβ) plaques and neurofibrillary tangles, is increasing while its causes are unknown. Interestingly, stress can exacerbate Aβ production in transgenic mouse models of the Alzheimer's. We hypothesized that a social stressor, isolation stress, would exacerbate Aβ production in 5xFAD+ transgenic mice in comparison to group-housed control animals and 5xFAD- mice. Further, it was hypothesized that isolated, 5xFAD+ animals would freeze less in a contextual fear-conditioning (CFC) paradigm, a hippocampus-dependent memory task, than group housed, 5xFAD+ or isolated 5xFAD- animals. After extended isolation or group housing, animals underwent CFC, following which freezing behavior was monitored during testing 24 hours later. Twenty-four hours after testing, animals were perfused and a brain hemisphere was collected for sectioning and staining for plaques, while the hippocampus was removed from the other hemisphere and Aβ was quantified by an $A\beta_{x-42}$ ELISA. Two and three months of isolation stress increased the number of Aβ plaques in the hippocampus of 5xFAD+ mice without significantly altering soluble Aβ levels. Animals isolated for 2 and 3 months also displayed a cognitive deficit in CFC.