EXTINCTION LEARNING DEFICITS DEVELOP PRIOR TO CONTEXTUAL

ACQUISITION DEFICITS IN MICE EXPRESSING

ALZHEIMER'S PATHOLOGIES

by

Charles Edgar

Submitted in partial fulfillment of the

requirements for Departmental Honors in

the Department of Biology

Texas Christian University

Fort Worth, Texas

May 4, 2020

EXTINCTION LEARNING DEFICITS DEVELOP PRIOR TO CONTEXTUAL

ACQUISITION DEFICITS IN MICE EXPRESSING

ALZHEIMER'S PATHOLOGIES

Project Approved:

Supervising Professor: Michael Chumley, PhD

Department of Biology

Mikaela Stewart, PhD

Department of Biology

Gary Boehm, PhD

Department of Psychology

ABSTRACT

Alzheimer's Disease (AD) is a progressive neurodegenerative disease that currently affects roughly 5.8 million Americans and has no cure and few treatments of limited efficacy. Although the etiology of AD has yet to be fully understood, the production of neurotoxic oligomers and plaques of Amyloid-β (Aβ) is thought to play a critical role in the progression of the disease. AD presents in one of two distinct forms. Familial AD, which presents before the age of 65 and accounts for less than 6% of all clinical cases of AD, arises from mutations in the Presenilin-1 (PS1 or PSEN-1) or Amyloid precursor protein (APP) genes, leading to production of amyloid plaques beginning in early life. Sporadic AD, the more common form, arises from a multitude of genetic and environmental factors, including neuroinflammatory events. The progressive nature of the disease, coupled with the limitations of current treatments in preventing advancement of the illness, necessitates early identification of AD pathology in both laboratory and clinical settings. Extinction learning, or the acquisition and retention of information that supersedes previously-learned information, has not been assessed in the 5xFAD (FAD) mouse model, a transgenic model expressing multiple mutations in the PSEN-1 and APP genes leading to accumulation of plaques in early life. At the same time, changes in extinction learning have not been assessed in non-transgenic C57BL/6J (BL/6) mice following repeated inflammatory insults, which previous studies in Chumley-Boehm Lab have shown to induce amyloid plaque pathologies and associated AD-associated cognitive deficits. Thus, the current study seeks to determine whether extinction behavioral testing could be useful in identifying AD symptoms prior to symptom presentation via other behavioral assessments, which generally begin around six months of age in FAD mice. To assess this, FAD subjects were subjected to behavioral testing via the contextual fear conditioning (CFC) paradigm to assess changes in freezing

behavior over several days following initial exposure to an adverse stimulus, with results compared to non-transgenic controls. Concurrently, BL/6 subjects were subjected to repeated injections of lipopolysaccharide (LPS, a bacterial mimetic and known inflammatory stimulus) as previously established by Weintraub et al. (2012) to induce Aβ production prior to testing. Changes in freezing behavior in these subjects were then compared to that observed in saline-injected controls. Repeated contextual fear testing revealed deficits in extinction learning behavior approaching significance as indicated by reductions in freezing time in FAD subjects compared with nontransgenic subjects beginning at three months of age, with significant deficits observed at four months of age. Additionally, significant deficits in extinction of contextual fear learning was observed in LPS-injected BL/6 subjects compared to controls. These results imply that assessing extinction of contextual learning can be a far more sensitive tool than assessing acquisition of contextual learning.

ACKNOWLEDGMENTS

I can begin no other way than to express the deepest gratitude possible to my PI and my mentor, Dr. Michael Chumley. Dr. Chumley is everything I could have hoped for in a PI. He is kind, encouraging, maintains a friendly and energetic lab atmosphere, and takes an active interest in the development of his students. My time in Chumley Lab has easily been one of the most impactful and formative experiences of my life. Working in the lab with some of the brightest minds at TCU gave me my first and greatest insight into the process of advancing scientific knowledge. I fell in love with the work of our lab and through it came to understand my personal need to pursue biomedical research as a career. Without these experiences and the learning I have had through Chumley Lab, my life and career trajectory would be dramatically different. I have written and rewritten this paragraph a few times and can never think of the proper way to express the depth of my gratitude to Dr. Chumley. Thank you for believing in me and giving me what became the opportunity of a lifetime.

Additionally, I would like to thank Julia Peterman, who guided my development as a researcher through this project and several others. The rigor and care with which Julia approaches her projects and assists with those in the lab has been and will continue to be an inspiration to me. Julia has been a valuable advocate for me in my time in Chumley Lab and was incredibly encouraging and supportive of my development as a student researcher. For all the time mentoring, consulting, editing, and helping me in countless ways over the past three years, I cannot thank her enough.

I also want to extend my sincerest thanks to Dr. Gary Boehm. I am beyond fortunate as a member of Chumley-Boehm Lab to have two mentors as an undergraduate researcher, and Dr. Boehm contributed greatly to my development as a thinker and learner. For his encouragement,

insight, and his willingness to serve as a member of my Honors committee, I cannot thank Dr. Boehm enough.

I would like to thank the graduate students with whom I have worked at varying points in my time with Chumley Lab. Kelly Brice, Paige Braden, Chris Hagen, and Jordon White have all immeasurably shaped me as a researcher and encouraged my development in a litany of ways. I cannot imagine a more supportive group of graduate students, and I am truly sad to be leaving this lab. I would also like to thank every undergraduate student in Chumley Lab, both those who mentored me (in particular Alexis Calcagno and Meg Cooksey) and the younger students I have had the privilege of mentoring to varying degrees these last two years; I will miss you all greatly. Finally, I would like to thank Dr. Mikaela Stewart, who graciously agreed to serve on my Honors committee and whose biochemistry course immeasurably improved my scientific literacy, as well as Dr. Akkaraju, the Chair of our department and whose Cell Biology section was among the most enjoyable experiences of my academic career.

TABLE OF CONTENTS

INTRODUCTION

Alzheimer's Disease (AD) is a neurodegenerative disorder marked by loss of memory, cognitive processing, and behavioral changes. The most common form of dementia, AD currently affects roughly 5.8 million Americans, a number that is projected to triple by 2050, and AD-associated afflictions represent the sixth-leading cause of death in America today (1). At the microscopic level, the cardinal features of AD are the presence of insoluble Amyloid-β (Aβ) plaques and Aβ oligomers (thought to be the most neurotoxic form of the protein) in the extracellular matrix (2), along with the development of intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated Tau (3). Ultimately, AD-afflicted neurons experience a loss of synaptic communication, leading to gradual cellular death and atrophy of neural tissue, particularly focused in the hippocampus (4); this area is associated with learning and cognitive processing, both of which are reduced or lost over the course of AD progression. Clinically, AD is generally separated into two distinct modes of development and progression. Familial AD, despite its rarity, has been the basis of every means of assessing AD progression using transgenic mouse models, due in part to the relative ease of development and also due to their usefulness in assessing individual components of amyloidogenesis and AD pathology, such as mutations in the Amyloid Precursor Protein (APP) and Presenilin-1 (PS-1 or PSEN-1) (4). The more common form of AD, sporadic AD (which constitutes roughly 99.5% of cases), has historically been far more difficult to simulate, due in part to the multitude of genetic and environmental factors that can contribute to the development of AD (4).

Neuroinflammatory processes have been a major focus of study in recent years as potential targets for treatment of AD. Inflammation occurs in tissues as a response to the presence of pathogens, but also as a means of clearing debris. The brains of AD sufferers have

been found to display elevated activation of microglia, which act as macrophages of the CSF, in areas with high levels of Aβ deposition (5). Microglial activation is thought to be induced as a protective mechanism against the accumulation of neurotoxic Aβ (6). However, binding of Aβ to Toll-like receptors and NOD-like receptors in microglia induces substantial increases in production of the pro-inflammatory cytokines TNF-α, IL-1β, and C1q (7). Local release of these cytokines in high levels is sufficient on its own to induce damage in neuronal cells. However, these cytokines also serve to induce local astrocytes to an A1 reactive state, causing them to increase deposition of complement at neural synapses, contributing to loss of synaptic communication (8). Furthermore, phagocytosis of Aβ peptide oligomers by microglia leads to a local increase in reactive oxidative species, leading to DNA damage to local neuronal cells that can, in the absence of a proper DNA damage response, induce apoptosis of the neuron (9). Given the role of neuroinflammation in the etiology and progression of AD, studies of novel methods of therapy often focus on counteracting neuroinflammation or oxidative stress. While such studies have shown promise *in vivo* in mouse models of AD, their success in a laboratory setting has yet to be replicated in clinical trials.

As stated above, AD presents in two forms, familial AD (associated with relatively rare genetic mutations) and sporadic AD. A number of transgenic animal models exist to simulate familial AD, including the 5XFAD mouse model, which expresses a total of five familial AD-associated mutations—the Swedish (K670N/M671L), London (V717I), and Florida (I716V) APP mutations as well as M146L and L286V mutations in PS1—resulting in rapid Aβ plaque deposition beginning as early as three months of age (10). Meanwhile, previous studies in our lab and others have shown that repeated exposure to the lipopolysaccharide (LPS) endotoxin induces an elevation in central Aβ with an associated decline in hippocampus-associated cognitive

function (11, 12). Based on these observations, our lab has developed a seven-day LPS injection protocol to induce sporadic AD-like pathologies in non-transgenic C57BL6-J mice (11).

In the clinical setting, AD can be divided into three temporal stages: an early asymptomatic, preclinical stage in which amyloidosis and other pathologies are developing (13); a symptomatic pre-dementia phase, marked by noticeable impairment in at least one cognitive domain (14); and a symptomatic dementia phase associated with significant deficits in multiple areas of cognition and working memory, coupled with progressive loss of independent function (15). In both a clinical and laboratory setting, early identification of AD symptoms is critical in order to better treat and characterize the disease, respectively. In the laboratory, developing novel means of identifying AD symptoms (especially via behavioral testing) is critical in evaluating the efficacy of potentially therapeutic compounds in either protecting against AD onset or ameliorating symptoms, helping to better identify candidates for eventual treatments of the disease. Meanwhile, as no cure exists for AD, and current therapies such as acetylcholinesterase treatments are aimed at slowing progression of the disease, the early identification of symptoms in suspected sufferers of the illness can significantly impact both the overall prognosis of the sufferer and the length of time they can expect to live without major impairment.

Cognitive behavioral testing for Alzheimer's symptoms in a laboratory setting takes a number of forms, but can largely be considered to examine three types of learning tasks, subdivided based on the type of memory being tested—spatial, contextual, or working. Spatial learning tests include the Morris water maze, which measures acquisition of spatial cues as a means of identifying and swimming to a shallow platform in a circular tub of water, and the radial arm maze, which measures acquisition of spatial cues to identify the arms of a multiple-arm setup which contain food or water (16). Working memory tests include the Y-maze, which

measures spontaneous alternation between arms without repetition, and the novel object recognition test, which evaluates a mouse's memory of a previously-introduced object by examining the amount of time spent examining such an object compared to a previously-unseen object (16). Finally, contextual memory tests include the contextual fear conditioning paradigm, which tests associative learning by evaluating the extent of a mouse's freezing response—that is, total immobility apart from breathing—in response to exposure to an environmental context (an audible tone coupled with idiosyncratic wall patterns) in which the mouse had previously received a mild adverse stimulus (16). Evaluation of the fear response is particularly useful, as many transgenic strains present with impairments in amygdala-associated functions such as fear and anxiety (16).

Extinction learning is a form of classical conditioning that occurs when the acquisition of novel information contradicts and replaces previously acquired information, associated with the gradual decrease in response to a conditioned stimulus that is not reinforced over multiple re-exposures (17). Deficits in extinction learning have been shown to predate deficits in acquisition in contextual learning in studies of the APP/PS1 transgenic AD mouse model (18, 19), indicating that behavioral analyses measuring extinction learning could be useful in assessing the earliest symptoms of AD, in better characterizing early disease progression, and in evaluating the usefulness of various compounds at different stages of intervention. This project sought to evaluate the potential usefulness of assessing changes in extinction learning as a means of identifying AD-associated learning deficits at an earlier timepoint than could otherwise be detected in 5XFAD mice, which carry three more AD-associated mutations than APP/PS1 mice and develop amyloid pathologies at an earlier timepoint (around three months of age). Furthermore, this project sought to identify whether multiple exposures to LPS could induce

alterations in extinction learning in non-transgenic C57BL6J control mice. Extinction learning was quantified by measuring changes in contextual fear response over several days following initial acquisition of contextual learning.

Materials and Methods

Subjects: Animal subjects used in the first experiment were male transgenic 5xFAD mice and wild-type controls of 3 and 4 months of age at time of behavioral analysis. All animals were bred in the Texas Christian University vivarium from a stock obtained from The Jackson Laboratory (Bar Harbor, ME). The animals used in the second experiment were 4–7-month-old adult male C57BL/6J mice bred in the Texas Christian University vivarium, which were also derived from a breeding stock from the Jackson Laboratory (Bar Harbor, ME). C57BL/6J mice are a non-transgenic inbred strain that finds use in our lab in studies of sporadic AD-like pathology. This strain of mice was previously used by Khan et al. (2012), following the LPS treatment schedule outlined below, to induce peripheral and central production of Aβ. All animals were housed in the Texas Christian University vivarium in groups of two to four subjects under a 12-hour light/dark cycle with food and water access ad libitum. All animal handling and housing was in accordance with animal care and safety standards established by the Institutional Animal Care and Use Committee (IACUC) of Texas Christian University.

Confirmation of 5xFAD genotype: 5xFAD genotype was confirmed via polymerase chain reaction using the Jackson Laboratory Standard PCR Assay - Tg(APP-Sw-Fl-Lon, PSEN1*M146L*L286V)6799Vas-Chr3 Protocol (Protocol No. 31769). The modifications made were as follows: (to be added). Animals from 5xFAD breeding pairs that tested negative for 5xFAD mutations were used as genetic background controls (C57BL6/J) for the 5xFAD study.

Treatment conditions: Animals were subjected to initial acquisition (training) in the adverse context outlined below. Single intraperitoneal injections of 250 µg/kg of LPS (Escherichia coli serotype: 055:B5; SigmaAldrich, St Louis, MO), or equivalent volume of saline, were then administered to C57BL6-J mice daily for seven consecutive days immediately prior to beginning behavioral testing period. This staggering of acquisition and testing was done to ensure that CFC testing exclusively assessed deficits in extinction learning rather than acquisition due to LPS administration, which could otherwise have confounded results.

Behavioral testing: Animal extinction learning for both experiments was assessed via a contextual fear conditioning (CFC) paradigm using were fully automated fear conditioning units (Coulbourn Instruments, Whitehall) to house animals and administer mild adverse stimuli and FreezeFrameTM software (ActiMetricsSoftware, Wilmette, IL) to track subjects' movements continuously throughout each trial. The acquisition training period began with a 180s acclimation period followed by a single 2s, 0.5mA foot shock. The same electrical stimulus was administered following a brief 60s interlude. After an additional 60s of monitoring, animals were returned to their home cages overnight before being returned to the chambers at the same time the following day for testing, where freezing behavior indicative of contextual fear learning was monitored for 180s. In order to evaluate extinction learning, CFC testing was repeated daily until a significant difference in extinction learning was observed between FAD^+ mice and their FAD^- control counterparts and between LPS-treated C57BL6-J mice and their saline-treated counterparts. In experiment 1, testing took place 24 hours after training. In experiment 2, training occurred prior to the first administration of LPS and testing took place 24 hours after the last LPS injection, for the reasons enumerated above.

Results

5xFAD mice do not display a deficit in acquisition learning of CFC at three months of age

A Student's t-test was used to examine differences across condition (5xFAD or WT mice) in acquisition learning in a contextual fear conditioning paradigm. Analyses revealed no significant differences between 5xFAD and WT mice on test day 1 (*NS;* Figure 1A), indicating no difference in contextual fear acquisition between the two groups.

5xFAD mice experience marginally significant impairments in extinction learning of CFC at three months of age

A repeated-measures analysis of variance (ANOVA) was used to examine differences across condition (5xFAD or WT) and time (Testing day 1, 2, 3, 4, 5). Results revealed a significant main effect of time ($p \leq 0.001$; Figure 1B), where animals froze less on subsequent testing days. There was a marginally significant test-genotype interaction effect ($p = 0.066$). There were also significant linear contrast effects of both test ($p \leq 0.001$) and genotype-test interaction ($p = 0.04$) at the same time point, indicating significant differences in rate of extinction between 5xFAD and WT mice at three months of age.

5xFAD mice do not display a deficit in acquisition learning of CFC at four months of age

A Student's t-test was used to examine differences across condition (5xFAD or WT mice) in acquisition learning in a contextual fear conditioning paradigm. Analyses revealed no significant difference between 5xFAD and WT mice on test day 1 (*NS;* Figure 1C), indicating no difference in contextual fear acquisition between the two groups.

5xFAD mice experience significant impairments in extinction learning of CFC at four months of age

A repeated-measures analysis of variance (ANOVA) was used to examine differences across condition (5xFAD or WT) and time (Testing day 1, 2, 3, 4, 5). Results revealed a significant main effect of time ($p \leq 0.001$; Figure 1D), where animals froze less on subsequent testing days. There was a significant test-genotype interaction effect ($p=0.04$). There were also significant linear contrast effects of test ($p \leq 0.001$) and test-genotype interaction ($p=0.02$) by test day five, indicating an impairment in extinction learning in 5xFAD animals at 4 months of age.
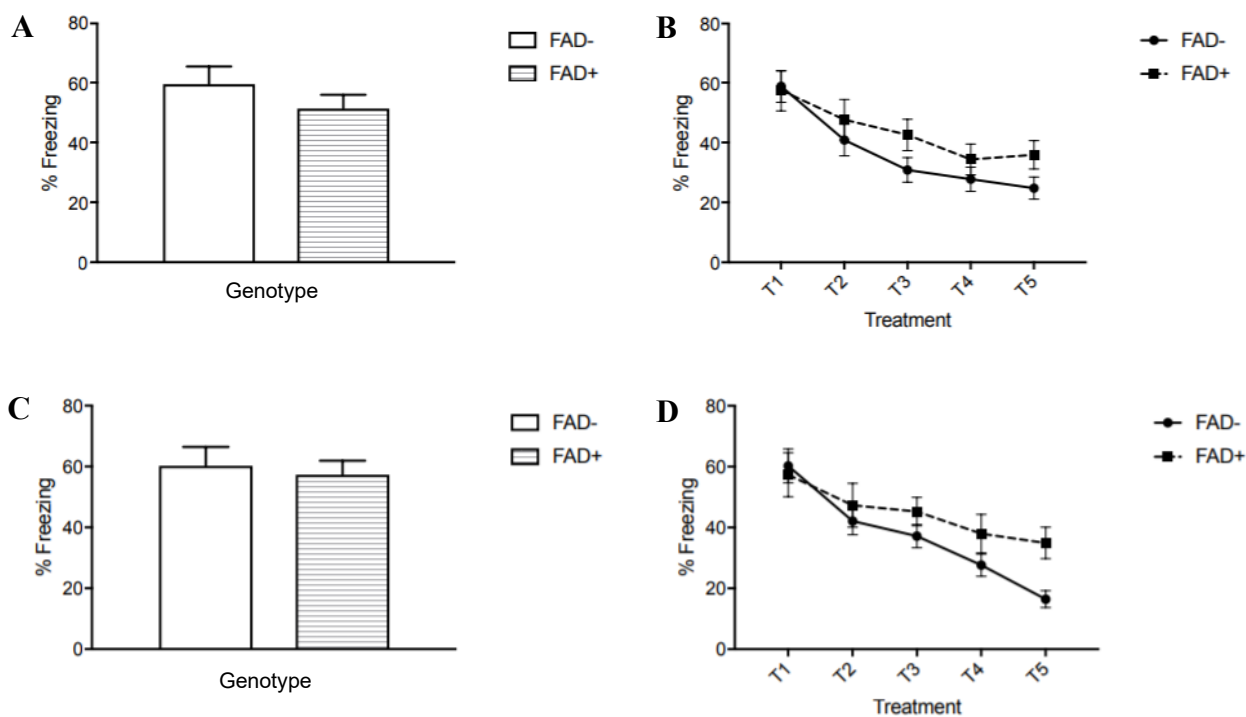

Figure 1: Results of contextual fear studies in 5xFAD mice. (**A**) Comparison of mean freezing response time on testing day one (T1) following initial contextual fear conditioning reveals no significant difference in acquisition between 5xFAD and WT at three months of age. (**B**) Comparison of changes in freezing time over five days following acquisition reveals significant effect of time and a marginally significant test*genotype interaction, as well as a significant linear contrast effect of both test and genotype-test interaction at three months of age. (**C**) Comparison of mean freezing response time on test day one reveals no significant difference in acquisition between genotypes at four months of age. (**D**) Comparison of changes in freezing time over five-day trial reveals significant effects of time and of test-genotype interaction as well as a significant linear contrast effect of test and test-genotype interaction at four months of age.

LPS-treated C57BL/6J animals do not display impairments in retention of learning in CFC

A Student's t-test was used to examine differences across treatment (LPS or saline) in retention of acquisition learning in a contextual fear conditioning paradigm. Analyses revealed no significant differences between LPS and saline treated animals on test day 1 (*NS;* Figure 2), indicating no difference in retention of contextual fear learning between the two groups, which took place prior to repeated administration of the inflammatory stimulus.

LPS-treated C57BL/6J animals display impairments in extinction learning of CFC

A repeated-measures analysis of variance (ANOVA) was used to examine differences across treatment (LPS or saline) and time (Testing day 1, 2). Results revealed that, on the second day of testing, LPS-treated animals displayed significantly ($p<0.05$; Figure 2) higher percent freezing times than their saline-treated counterparts, indicating a significant deficit in extinction learning in LPS-treated animals.
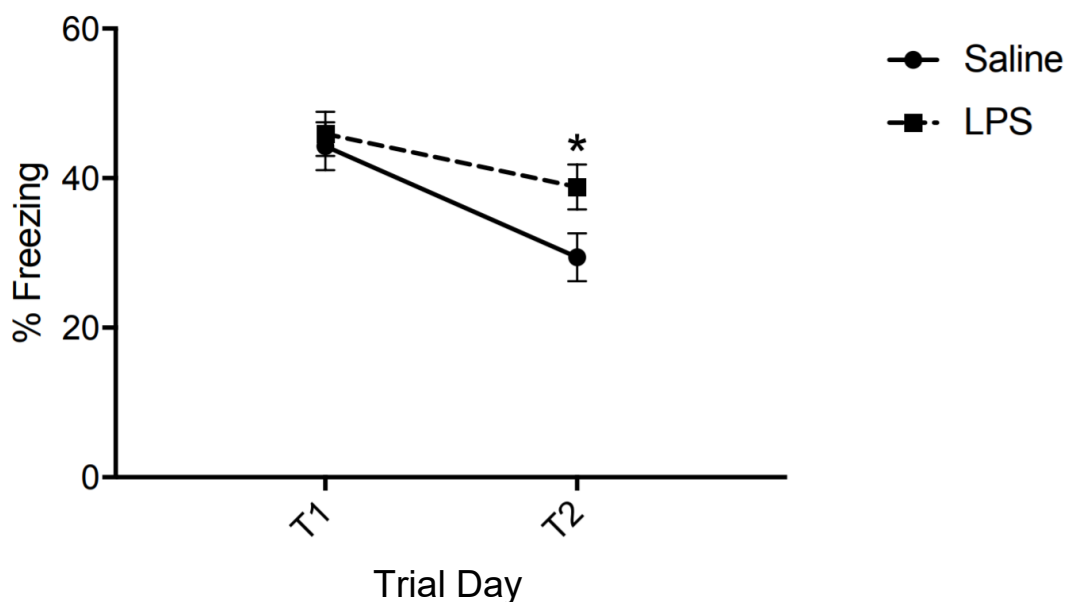

Figure. 2*: No significant difference was seen in freezing between LPS and saline-treated mice at test day one. LPS mice displayed significantly higher percent freezing than their saline-treated counterparts on test day two.

Discussion

The progression of Alzheimer's disease is marked by the progressive preclinical development of amyloid pathology and tauopathy without presentation of outwardly noticeable cognitive decline until late-stage disease. Due to this absence of symptoms at crucial timepoints in disease development, by the time Alzheimer's is clinically identified, the disease has invariably advanced to the point that therapeutic intervention is ineffective at alleviating symptomology; at best, it may temporarily slow progression of the illness. The nature of this illness necessitates development of highly sensitive analyses that facilitate evaluation of Alzheimer's progression at the earliest timepoints possible in order to facilitate earlier intervention and improve the chances of therapeutic success. To that end, this study aimed to evaluate whether deficits in extinction learning were present prior to the onset of deficits in acquisition learning in contextual fear conditioning in the 5xFAD mouse model. It further sought to evaluate whether similar deficits could be observed following induction of a peripheral inflammatory response via administration of lipopolysaccharide to non-transgenic C57BL/6J mice.

It was first hypothesized that testing in CFC would reveal deficits in extinction learning in 5xFAD mice before the onset of acquisition learning deficits. This hypothesis was derived from previous research in the APP/PS1 transgenic mouse model of AD, which showed deficits in extinction learning beginning at four months (18)—far earlier than deficits in acquisition learning, which occur as early as 6 months of age in APP/PS1 mice (19). Because of the greater number of mutations harbored, 5xFAD mice begin accumulating plaques earlier in life, yet have been shown to develop acquisition deficits at around the same time as APP/PS1 mice, at 6 months of age (20). As hypothesized, we did not observe acquisition deficits in contextual fear

conditioning at the 3 and 4 month timepoints, consistent with previous research indicating the onset of deficits in acquisition learning at 6 months of age in 5xFAD mice (20). CFC testing revealed marginally significant deficits in extinction learning at 3 months of age. Further, there was a significant genotype-test interaction, indicating differential outcomes between 5xFAD and WT mice at 4 months of age, a finding consistent with those of Bonardi et al (18) demonstrating extinction learning deficits at four months of age in APP/PS1 mice. Taken together, these findings indicate that deficits in extinction learning in CFC are present in 5xFAD mice before deficits in acquisition learning.

It was then hypothesized that testing in CFC of C57BL/6J mice following induction of an inflammatory response and the associated increase in Aβ production would reveal deficits in extinction learning. Extinction deficits (but not deficits in retention of contextual fear learning)were observed in C57BL/6J mice treated with peripherally-administered LPS over seven days to induce production of Aβ. These findings were consistent with previous research in our lab which showed increases in central Aβ burden associated with induction of an inflammatory response (11), as well as with the findings of the APP/PS1 study which found impairments in extinction learning associated with early AD pathology (18). Taken together, these findings reveal that extinction learning is significantly inhibited in animal models of both familial and sporadic AD.

Although behavioral analyses are useful in assessing the extent of cognitive deficit associated with the particular dementia, they are not themselves diagnostic. Additionally, the inducible nature of the multiple-LPS-injection AD model unfortunately precludes any ability to evaluate at what timepoint in sporadic AD progression these changes in behavior might occur, only that such changes would occur in sporadic AD. Meanwhile, the 5xFAD model is

representative of mutations that would lead to early-onset familial Alzheimer's; such cases present clinically at a much younger age, as seen in the presence of amyloid plaques in 5xFAD mice as early as 3 months of age (10). While useful in assessing some of the dysfunctions in APP or PSEN-1 that can lead to development of sporadic AD, it cannot fully account for the heterogeneity of sporadic AD pathogenesis and progression on its own.

The results of this study indicate that extinction learning represents a highly sensitive means of assessing cognitive changes brought on by Alzheimer's disease in a laboratory setting. Future studies analyzing the efficacy of various treatments in arresting the progression of AD in 5xFAD, LPS-treated C57BL/6J, or the previously-established APP-PS1 model can thus employ this method of assessing extinction of previously-acquired contextual fear stimuli as a means of identifying early cognitive deficits. This method may be particularly useful in longitudinal settings assessing the effects of prenatal or early childhood stressors in mice as a means of better identifying the initial onset of AD-like symptoms.

Although an imperfect analogy to human behavioral studies, with time it is hoped that this paradigm and other behavioral and biochemical analyses of Alzheimer's pathology, more sensitive than those existing today, can be developed and deployed in a clinical setting. Through development and refinement of analyses that yield greater sensitivity at early timepoints, the efficacy of existing therapeutics could be improved enormously, potentially allowing greater preservation of cognitive function, memory, and independence in patients with AD. As the prevalence of Alzheimer's increases with the aging of the US and global population, the importance of identifying early warning signs of AD progression will only increase.

References

1.   Association As. 2019 ALZHEIMER'S DISEASE FACTS AND FIGURES. Alzheimer's Association, 2019 2019. Report No.

2.   Reiss AB, Arain HA, Stecker MM, Siegart NM, Kasselman LJ. Amyloid toxicity in Alzheimer's disease. Rev Neurosci. 2018;29(6):613-27. doi: 10.1515/revneuro-2017-0063. PubMed PMID: 29447116.

3.   Naseri NN, Wang H, Guo J, Sharma M, Luo W. The complexity of tau in Alzheimer's disease. Neurosci Lett. 2019;705:183-94. Epub 2019/04/25. doi: 10.1016/j.neulet.2019.04.022. PubMed PMID: 31028844.

4.   Lane CA, Hardy J, Schott JM. Alzheimer's disease. Eur J Neurol. 2018;25(1):59-70. Epub 2017/10/19. doi: 10.1111/ene.13439. PubMed PMID: 28872215.

5.   Hansen DV, Hanson JE, Sheng M. Microglia in Alzheimer's disease. J Cell Biol. 2018;217(2):459-72. Epub 2017/12/01. doi: 10.1083/jcb.201709069. PubMed PMID: 29196460.

6.   Yeh FL, Wang Y, Tom I, Gonzalez LC, Sheng M. TREM2 Binds to Apolipoproteins, Including APOE and CLU/APOJ, and Thereby Facilitates Uptake of Amyloid-Beta by Microglia. Neuron. 2016;91(2):328-40. doi: 10.1016/j.neuron.2016.06.015. PubMed PMID: 27477018.

7.   Heneka MT, Golenbock DT, Latz E. Innate immunity in Alzheimer's disease. Nat Immunol. 2015;16(3):229-36. doi: 10.1038/ni.3102. PubMed PMID: 25689443.

8.   Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, Bennett ML, Münch AE, Chung W-S, Peterson TC, Wilton DK, Frouin A, Napier BA, Panicker N, Kumar M, Buckwalter MS, Rowitch DH, Dawson VL, Dawson TM, Stevens B, Barres BA.

Neurotoxic reactive astrocytes are induced by activated microglia. Nature. 2017;541(7638):481-7. Epub 2017/01/18. doi: 10.1038/nature21029. PubMed PMID: 28099414.

9.   Agostinho P, Cunha RA, Oliveira C. Neuroinflammation, oxidative stress and the pathogenesis of Alzheimer's disease. Curr Pharm Des. 2010;16(25):2766-78. doi: 10.2174/138161210793176572. PubMed PMID: 20698820.

10.   Oakley H, Cole SL, Logan S, Maus E, Shao P, Craft J, Guillozet-Bongaarts A, Ohno M, Disterhoft J, Van Eldik L, Berry R, Vassar R. Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. J Neurosci. 2006;26(40):10129-40. doi: 10.1523/JNEUROSCI.1202-06.2006. PubMed PMID: 17021169.

11.   Kahn MS, Kranjac D, Alonzo CA, Haase JH, Cedillos RO, McLinden KA, Boehm GW, Chumley MJ. Prolonged elevation in hippocampal Aβ and cognitive deficits following repeated endotoxin exposure in the mouse. Behav Brain Res. 2012;229(1):176-84. Epub 2012/01/12. doi: 10.1016/j.bbr.2012.01.010. PubMed PMID: 22249135.

12.   Lee JW, Lee YK, Yuk DY, Choi DY, Ban SB, Oh KW, Hong JT. Neuro-inflammation induced by lipopolysaccharide causes cognitive impairment through enhancement of beta-amyloid generation. J Neuroinflammation. 2008;5:37-. doi: 10.1186/1742-2094-5-37. PubMed PMID: 18759972.

13.   Jack CR, Jr., Albert MS, Knopman DS, McKhann GM, Sperling RA, Carrillo MC, Thies B, Phelps CH. Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):257-62. Epub 2011/04/21. doi: 10.1016/j.jalz.2011.03.004. PubMed PMID: 21514247.

14.   Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, Gamst A, Holtzman DM, Jagust WJ, Petersen RC, Snyder PJ, Carrillo MC, Thies B, Phelps CH. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):270-9. Epub 2011/04/21. doi: 10.1016/j.jalz.2011.03.008. PubMed PMID: 21514249.

15.   McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Jr., Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):263-9. Epub 2011/04/21. doi: 10.1016/j.jalz.2011.03.005. PubMed PMID: 21514250.

16.   Bryan KJ LH, Perry G, et al. Boca Raton (FL): CRC Press/Taylor & Francis; . Chapter 1. Available from: https://www.ncbi.nlm.nih.gov/books/NBK5231/. Transgenic Mouse Models of Alzheimer's Disease: Behavioral Testing and Considerations. In: JJ B, editor. Methods of Behavior Analysis in Neuroscience. 2nd edition ed. Boca Raton, FL: CRC Press/Taylor & Francis; 2009.

17.   Quirk GJ, Mueller D. Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology. 2008;33(1):56-72. Epub 2007/09/19. doi: 10.1038/sj.npp.1301555. PubMed PMID: 17882236.

18.   Bonardi C, de Pulford F, Jennings D, Pardon MC. A detailed analysis of the early context extinction deficits seen in APPswe/PS1dE9 female mice and their relevance to preclinical Alzheimer's disease. Behav Brain Res. 2011;222(1):89–97. doi:10.1016/j.bbr.2011.03.041

19.	Kilgore M, Miller CA, Fass DM, et al. Inhibitors of class 1 histone deacetylases reverse contextual memory deficits in a mouse model of Alzheimer's disease. Neuropsychopharmacology. 2010;35(4):870–880. doi:10.1038/npp.2009.197

20.	Kimura R, Ohno M. Impairments in remote memory stabilization precede hippocampal synaptic and cognitive failures in 5XFAD Alzheimer mouse model. Neurobiol Dis. 2009;33(2):229–235. doi:10.1016/j.nbd.2008.10.006