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The 5XFAD mouse model of Alzheimer's disease displays age-dependent deficits in habituation to a novel environment

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ABSTRACT

Habituation is a form of learning characterized by a decrement in responsiveness to a stimulus that is repeated or prolonged. In rodents, habituation to a novel environment is characterized by a decrease in locomotion over time spent in a novel environment. Habituation to a novel environment is dependent on hippocampal function, suggesting that habituation behavior may be a relevant readout for hippocampal-dependent memory deficits that are characteristic of Alzheimer's disease (AD). Current assays that measure hippocampal-dependent memory in preclinical animal models of AD have not accurately predicted the cognitive protection of novel interventions in human trials. Here, we tested whether a behavioral habituation paradigm could detect age-associated changes in a common preclinical mouse model of AD-like amyloid pathology, the 5XFAD mouse. We exposed 5XFAD mice and age-matched wild-type (WT) littermates at 3, 6, and 9 months of age to a novel environment over two sessions separated by 24 h and measured their locomotion. WT mice habituated to the novel environment over time, while 5XFAD mice displayed age-dependent deficits in behavioral habituation. We replicated our results using publicly available open field data from 5XFAD and late-onset AD mouse models with TREM2*R47H and APOE4 mutations. Overall, we present behavioral habituation as a potentially sensitive task to assess age-associated behavioral deficits in 5XFAD mice and other mouse models of AD that could be used to test the preclinical efficacy of novel AD therapeutics.

Introduction

Alzheimer's disease (AD) is the most common form of dementia with limited treatments available. The devastating socioeconomic burden of AD is expected to increase substantially in the next 30 years as the population ages. The primary histopathological features of AD are amyloid β (Aβ) plaques and tau tangles which are associated with neurodegeneration, neuroinflammation, and cognitive deficits. As we search for novel therapeutics, the primary clinical readout for efficacy is reduced rate of cognitive decline. This highlights the need for sensitive preclinical testing in animal models that translates to protection of cognition in humans.

Many established AD mouse models use mutations in amyloid precursor protein (APP) and presenilin 1 (PS1) associated with familial AD (FAD) to drive the

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid β; APP, amyloid precursor protein; PS1, presenilin 1; FAD, familial Alzheimer's disease; WT, wild-type; MWM, Morris water maze.

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AD-associated pathognomonic feature of Aβ plaques. While novel late-onset AD models are in development, these FAD mouse models are currently relied upon for preclinical development of novel therapeutics for AD patients. However drugs that are efficacious in AD mice have not translated to efficacious human treatments for AD. A recent report performed deep phenotyping on the common 5XFAD mouse model and found that these mice, despite wide preclinical use, do not display robust, if any, age- or pathology-associated cognitive deficits despite high levels of Aβ plaque burden and neuroinflammation [24]. Other FAD mouse models lack or show inconsistent cognitive deficits using hippocampal and non-hippocampal memory tasks [12]. Many of these tasks involve a high level of stress to the mouse, laborious training of mice, and/or specialized equipment, making these assays poor choices for straightforward reproducibility and efficient screening preclinical therapeutics for cognitive efficacy. We have identified behavioral habituation to a novel environment as a potential behavioral assessment to overcome these three weaknesses. Habituation to a novel environment involves a low level of stress, relies on minimal training, and takes advantage of natural mouse behavior using readily available equipment resources.

Habituation broadly refers to a decreased response to repeated stimulation and is considered a basic form a behavioral plasticity [9,25,30]. Habituation to a novel environment is a basic form of learning behavior exhibited across species. It is characterized by a decrease in locomotor activity (e.g., velocity, distance traveled) over time spent in a novel environment from beginning to end of a session (intrasession habituation), or between two separate visits to the same environment from one session to another (intersession habituation) [16,26,31]. Intrasession or short-term habituation and intersession or long-term habituation likely have different neuroanatomical and neurophysiological correlates [1]. Intersession habituation to a novel environment requires a functional hippocampus [31], suggesting this behavioral paradigm may be a relevant readout for intact hippocampal-dependent memory. Here, we examined whether 5XFAD mice would display behavioral habituation in an open field behavioral task. We exposed 5XFAD mice and age-matched wild-type (WT) littermate control mice at 3, 5, and 9 months of age to a novel environment two days in a row and measured their locomotion. WT mice habituated to the novel environment over time, while 5XFAD mice displayed age-dependent deficits in behavioral habituation. Overall, we present behavioral habituation as a potentially sensitive task to assess age-associated behavioral deficits in 5XFAD mice.

Material and methods

Mice

All procedures were approved by the Institutional Animal Care and Use Committee at University of Texas Health Science Center San Antonio. We use hemizygous 5XFAD mice (n = 9/group) and WT littermates (n = 5–8/group) of both sexes on a congenic C57BL6/J background (MMRRC #34848-JAX). 5XFAD transgenic AD mice overexpress human APP with three mutations associated with FAD (Swedish (K670N, M671L), Florida (I716V), and London (V717I)) as well as human PS1 with two mutations associated with familial AD (M146L and L286V). Expression of the transgenes is regulated by the neuron specific Thy1 promoter. These animals exhibit deposition of Aβ plaques starting at 2 months of age, gliosis and vascular changes at 3 months [8], and synapse loss [2] at 6 months of age, and loss of layer 5 cortical neurons at 12 months of age [13]. Mice were bred WT C57BL6/J (JAX stock #: 000664) female to heterozygote male. Up to five mice were housed per cage (501 cm^2 floor area) with Sani-Chips bedding (Teklad) and ad libitum access to LM-485 diet (Teklad). The light cycle was maintained on a 14:10 light:dark schedule, with lights on at 7am and off at 9 pm. At weaning, mice were genotyped according to the protocol provided by Jackson Labs and ear notched for identification. Starting at 8 weeks of age, mice were handled weekly for acclimation.

Habituation to a novel environment

All ages of mice were evaluated together as one cohort during the "lights on" phase of the light cycle with behavioral testing room lights set to approximately 500 lm. We chose the "lights on" phase of the cycle to reduce confounds due to exposure to light at night [6] but note that there are differences in open field behavior between the light and dark phase that could reduce the sensitivity of the task to detect differences [11]. On both testing days, mice were transported from their housing room to the behavioral testing room, tails labeled with non-toxic markers, and left to acclimate to the testing environment for a minimum of 60 min in their home cages. Prior to testing and between subjects, the testing arenas were sanitized with 70% ethanol. Mice were placed in open field arenas (42 cm × 20 cm × 20 cm rat cages) separated with opaque barriers and were monitored with overhead camera and tracked in real-time with Noldus EthoVision 14 software for thirty minutes on day one and then for thirty minutes on day two 24 h later; data was collected in 1-minute time bins. For data downloaded from the AD Knowledge Portal, mice were tested during the "lights on" phase of the 12:12 light cycle with testing room lights set to 500 lm. Mice were placed in open field sound-attenuated chambers (40 cm × 40 cm × 40 cm) and were monitored using infrared beam breaking for sixty minutes on one day; data was collected in 5-minute time bins. For our secondary analysis, we only used the first 30 min for consistency with our internal dataset. To assess anxiety-like behaviors, we calculated the percentage of the total time mice spent in the edge of the open field arena.

Data analysis

Total distance traveled and distance traveled each minute was collected and analyzed using GraphPad Prism 9.3.1 and was analyzed by a three-way repeated-measures

ANOVA (age × genotype × time) with significance set as $p < 0.05$ or by simple linear regression analysis. We measured intrasession habituation by comparing distance traveled in the first 5 min and within the 25 min to 30 min time bin of each session and measured intersession habituation by comparing the total distance traveled in the first and second session. For open field data downloaded from the AD Knowledge Portal, we measured intrasession habituation by comparing distance traveled in the first 5 min and between 25 min to 30 min. We calculated the "activity-change ratios" using the formula R = [last]/([last] + [first]). An activity-change ratio of 0.5 indicates no change in movement, a ratio below 0.5 indicates decreasing movement (habituation), while a ratio above 0.5 indicates an increase in movement or lack of habituation [20]. Activity change ratios and time spent in the arena edges were analyzed by one-way ANOVA (group), two-way ANOVA (age × genotype), or three-way ANOVA (age × genotype × sex) with a Tukey post-hoc for multiple comparisons.

Results

We first compared the total distance traveled on day one of open field to day two of open field to assess habituation to a novel environment in 3-, 6-, and 9-month-old 5XFADs and WT littermates of both sexes. A three-way repeated measures ANOVA (age × genotype × day) revealed a main effect of day ($p < 0.0001$) suggesting that mice habituate to a novel environment between day 1 and day 2 of testing, accounting for 44.69% of the total variation (Fig. 1A). However, there was also a significant interaction between age and genotype ($p = 0.0012$), accounting for 7.962% of the total variation. Multiple comparison testing revealed that 3-month-old and 6-month-old 5XFAD mice moved significantly less ($p = 0.0006$) on day two of the open field task, suggesting they habituated to the novel environment. In contrast, 9-month-old 5XFAD mice did not habituate to the novel environment between testing days ($p = 0.1239$) and were significantly more active on the second day of testing compared to 3-month-old 5XFAD mice ($p = 0.0182$). WT littermates showed habituation between day one and day two of open field at 3, 6, and 9 months of age ($p = 0.0023$, $p = 0.0526$, and $p = 0.0448$, respectively). We next used simple linear regression to test if mice decreased their distance moved over each subsequent minute elapsed, inclusive of day one and day two of testing (Fig. 1B). Each of the groups (age × genotype) had significantly different slopes ($F(5,2748) = 9.678$, $p < 0.0001$). 9-month-old 5XFAD mice demonstrated the weakest relationship between decreasing distance moved over time elapsed (slope = −1.055, $R^2 = 0.02$, $F(1,538) = 14.84$, $p = 0.0001$), while younger 5XFAD mice decreased their distance traveled over time elapsed (for 3-month-old 5XFAD mice, slope = −2.903, $R^2 = 0.29$, $F(1,538) = 224.3$, $p < 0.0001$ and 134.5; for 6-month-old 5XFAD mice slope = −2.563, $R^2 = 0.20$, $F(1,538) = 134.5$, $p < 0.0001$). Younger 5XFAD mice were more comparable to WT mice ($p < 0.0001$, slope = −2.493, −1.842, −2.813; $R^2 = 0.27$, 0.13, 0.23; $F(1, 298) = 112.6$, $F(1, 478) = 69.30$, $F(1, 358) = 108.2$ for 3-, 6-, and 9-month-old WT mice, respectively). We then evaluated intrasession and intersession habituation by measuring the activity change ratio within or between sessions. A two-way ANOVA revealed that 5XFAD and WT mice had significantly different intrasession habituation behavior on day 1 (($F(1,40) = 8.591$, $p = 0.0056$) and there was a significant interaction between genotype and age that affected intersession habituation behavior ($F(2,40) = 5.14$, $p = 0.103$). Post-hoc analyses revealed that 9-month-old 5XFAD mice had a significantly higher intrasession activity change ratio on day 1 compared to 9-month-old WT mice (Fig. 1C), while 9-month-old 5XFAD mice had a significantly higher intersession activity change ratio compared to 3-month-old 5XFAD mice (Fig. 1E). There was no change in intrasession habituation on day 2 (Fig. 1D). Overall, our results indicate that 5XFAD mice display significant decreases in effective behavioral habituation to a novel environment.

To validate our findings in a larger cohort of 6- and 12-month-old 5XFAD mice, we downloaded open field from the AD Knowledge Portal; this larger sample size would allow us to evaluate sex differences (n = 12 mice per genotype of each sex). Hyperactivity had been observed in 5XFAD mice in this study, but habituation had not been measured [24]. We were only able to evaluate intrasession habituation because only one day of open field data was collected at each age. A two-way repeated measures ANOVA (genotype × age; mice were tested twice longitudinally) revealed that 5XFAD mice had significantly increased activity change ratios compared to WT mice (main effect of genotype, $F(1,46) = 25.59$, $p < 0.0001$) with multiple comparisons testing showing significant differences between 5XFAD and WT mice at both ages ($p < 0.001$) (Fig. 2A). We then evaluated the contribution of sex with a three-way repeated measures ANOVA (genotype × age × sex) which confirmed that 5XFAD mice had significantly increased activity change ratios compared to WT mice (main effect of genotype, $F(1,44) = 25.63$, $p < 0.0001$) but there was no effect of sex on intrasession habituation (Fig. 2B). Overall, these data suggest that intrasession defects in habituation to a novel environment in 5XFAD mice is replicable across multiple testing sites despite small procedural differences.

We then tested whether habituation behavior to a novel environment was sensitive to pharmacological manipulation of Aβ levels by evaluating data downloaded from the AD Knowledge Portal wherein 5XFAD mice were treated with verubecestat in chow (0, 10, 30, or 100 mg/kg day) from 3 to 6 months of age. Verubecestat is a beta-secretase (BACE) inhibitor that dose-dependently inhibits the production of Aβ in 5XFAD mice. In the original analyses of these open field data, verubecestat did not alter total distance moved in the open field [23]. A one-way ANOVA ($F(4,160) = 4.943$) on intrasession habituation data revealed that 5XFAD mice treated with vehicle or the low dose (10 mg/kg/day) of verubecestat had significantly increased activity change ratios ($p = 0.0014$ and 0.0235, respectively), while 5XFAD mice treated with the high dose (100 mg/kg/day) of verubecestat had reduced activity

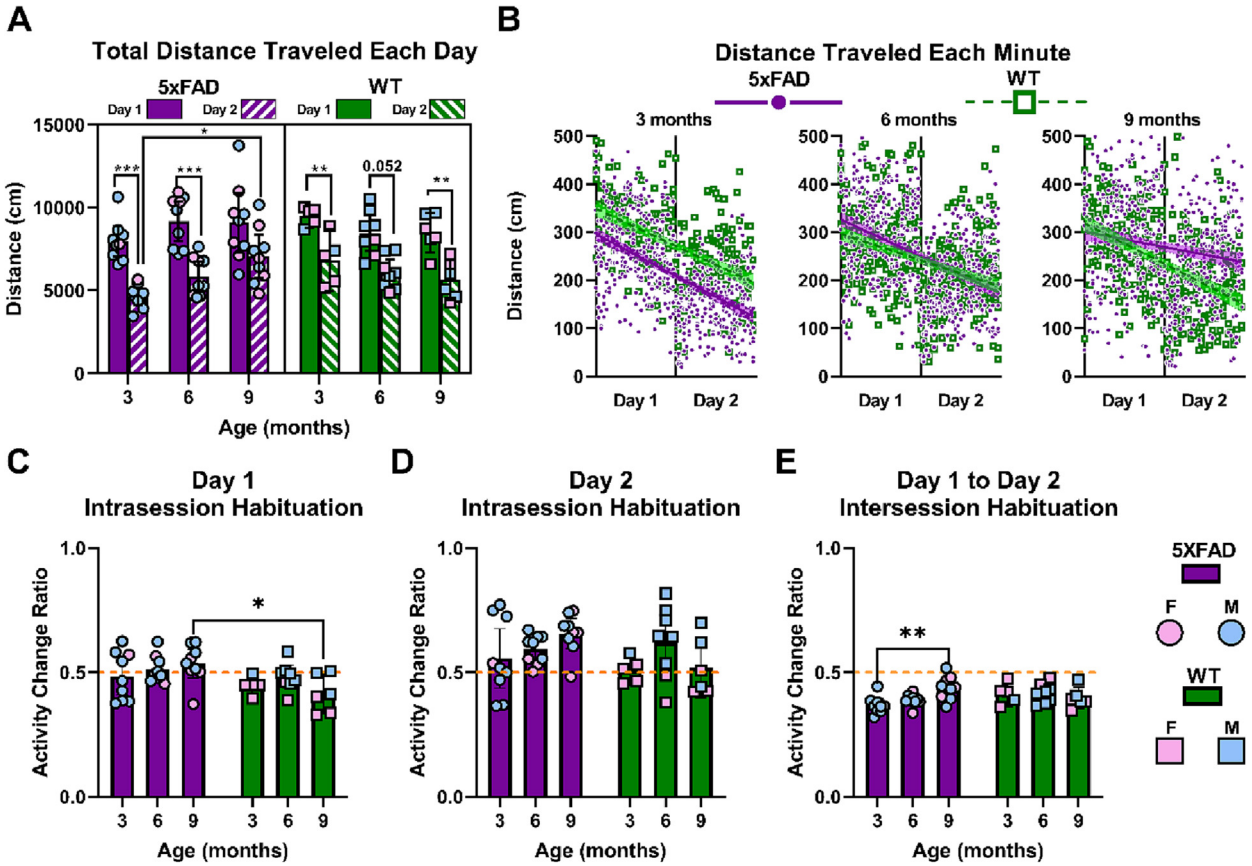

Fig. 1. 5XFAD mice display significant age-dependent decreases in behavioral habituation to a novel environment. Orange dotted line marks an activity-change ratio of 0.5 indicates no change in movement; a ratio below 0.5 indicates decreasing movement (habituation), while a ratio above 0.5 indicates an increase in movement or lack of habituation. Each dot represents an individual mouse (females = pink, males = blue). ***p < 0.001, **p < 0.01, *p < 0.05 A. 9-month-old 5XFAD mice do not reduce activity in a novel environment over two days of testing as measured by 3-way ANOVA. There was a main effect of day (p < 0.0001) and a significant interaction between age and genotype on distance traveled (p = 0.0012). 3- and 6-month-old 5XFAD and 3-, 6, and 9-month-old WT mice moved significantly less on day two while 9-month-old 5XFAD mice did not move significantly less on day two and were significantly more active than 3-month-old mice on day two. B. 9-month-old 5XFAD mice display reduced rates of habituation as measured by simple linear regression. Data is shown as the line-of-best-fit with 95% confidence intervals shown the shaded area around the line. Each dot represents an individual mouse at an individual timepoint. In the first panel, 3-month-old WT and 5XFAD mice show similar rates of habituation (slope = −2.493 and −2.903, respectively). In the second panel, 6-month-old WT and 5XFAD mice also show similar rates of habituation (slope = −1.842 and-2.563, respectively). In the third panel, 9-month-old WT and 5XFAD mice (slope = −2.813 and −1.055, respectively). C. Intrasession habituation between the first five minutes and last five minutes of open field on Day 1 of testing was significantly impaired in 9-month-old 5XFAD mice compared to age-matched WT mice as measured by 2-way ANOVA with Tukey post-hoc (main effect of genotype). D. There was no intrasession habituation between the first five minutes and last five minutes of open field on Day 2 of testing. E. Intersession habituation between the Day 1 and Day 2 of open field testing was significantly impaired in 9-month-old 5XFAD mice compared to age-matched WT mice as measured by 2-way ANOVA with Tukey post-hoc (interaction between age and genotype). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

change ratios compared to vehicle-treated 5XFAD mice (p = 0.0467) (Fig. 2C). These data suggest that habituation to a novel environment is sensitive to treatment with verubecestat in 5XFAD mice.

We next tested whether deficits in habituation to a novel environment were present in a mouse model of late-onset AD (LOAD) by downloading open field data from the AD Knowledge Portal from 4-, 8-, 12-, or 24-month-old LOAD mice with either a knock-in of the AD risk mutation TREM2*R47H, humanized apolipoprotein E (ApoE) with the AD risk allele ApoE4, or both alleles [14]. A two-way ANOVA (genotype × age) revealed a significant main effect of genotype and an interaction between age and genotype (F(3,379) = 13.10 and F(9,379) = 2.822, respectively). Post-hoc analyses revealed that 12- and 24-month-old mice with the TREM2*R47H mutation and significant deficits in habituation to a novel environment compared to other genotypes at the same age and across ages (Fig. 2D). Take together with our data above, these data suggest that intrasession deficits in habituation to a novel environment may be sensitive for evaluation of behavioral changes in both FAD and LOAD mouse models of AD.

Finally, we assessed anxiety-like behavior by measuring the proportion of time that mice spent in the open field arena edge; more time in the edge of the arena is indicative of increased anxiety-like behaviors while more time spent in the center of the arena is indicative of decreased anxiety-like behaviors. A two-way ANOVA (genotype × age)

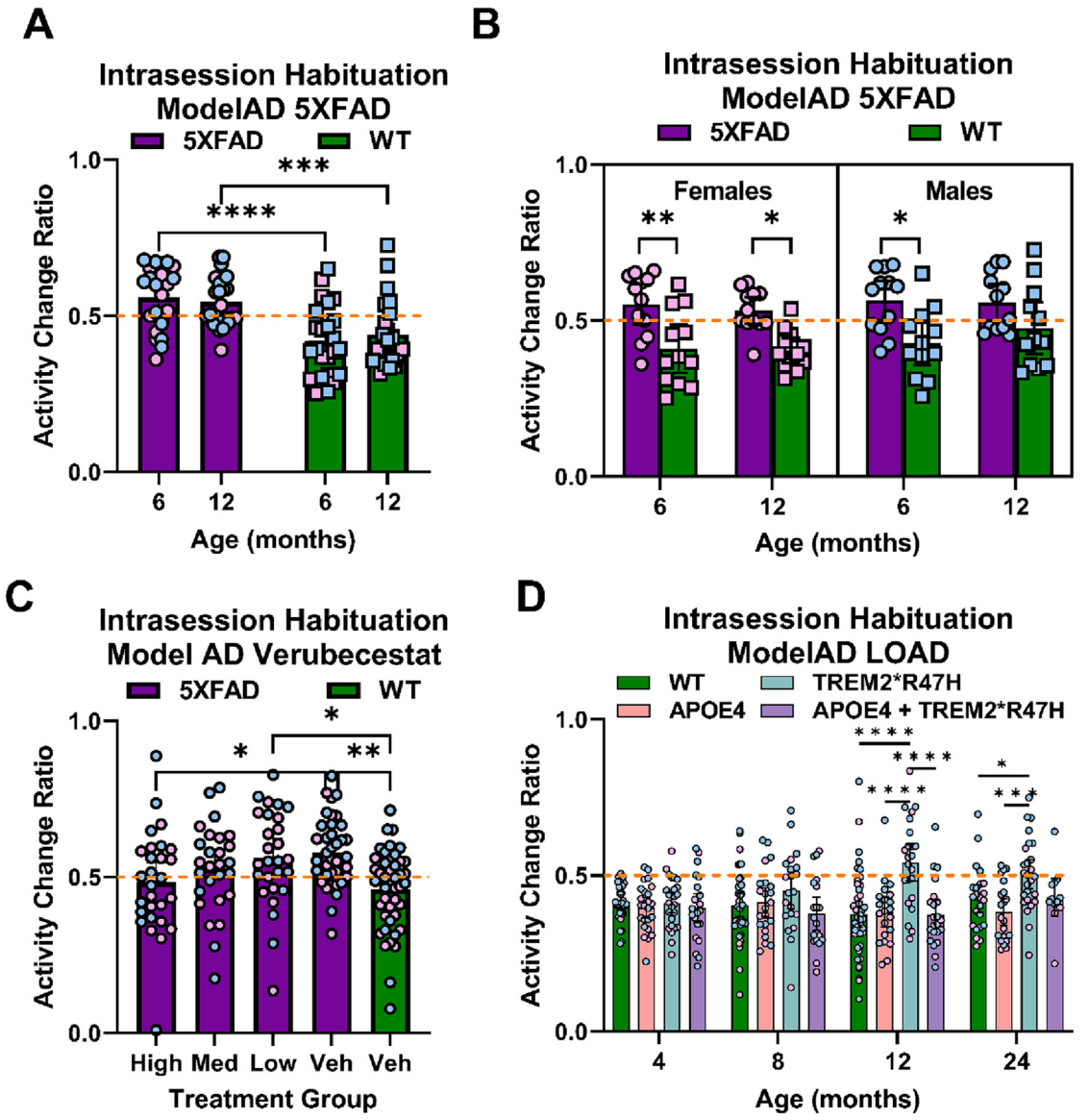

Fig. 2. Deficits in habituation to a novel environment are replicable in 5XFAD mice, change with BACE inhibition, and are also present in LOAD mice. Orange dotted line marks an activity-change ratio of 0.5 indicates no change in movement; a ratio below 0.5 indicates decreasing movement (habituation), while a ratio above 0.5 indicates an increase in movement or lack of habituation. Each dot represents an individual mouse (females = pink, males = blue). ***p < 0.001, **p < 0.01, *p < 0.05 A. Intrasession habituation between the first five minutes and last five minutes of open field on Day 1 of testing was significantly impaired in 6- and 12-month-old 5XFAD mice compared to age-matched WT mice as measured by 2-way ANOVA with Tukey post-hoc (main effect of genotype). B. There was no main effect of sex on habituation to a novel environment as measured by 3-way ANOVA (main effect of genotype). C. 6-month-old 5XFAD mice treated with vehicle or the low dose of verubecestat (10 mg/kg/day) showed significant impairments in intrasession habituation compared to age-matched WT mice. Treatment with a high dose of verubecestat (100 mg/kg/day) significantly reduced the activity change ratio in 5XFAD mice as measured by 1-way ANOVA. D. There was a significant effect of genotype and an interaction between age and genotype on intrasession habituation in LOAD mice as measured by 2-way ANOVA. Post-hoc analyses revealed that TREM2*R47H mice had significant impairments in habituation compared to age-matched WT, ApoE4, and ApoE4 + TREM2*R47H mice at 12 months of age and WT and ApoE4 mice at 24 months of age. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

revealed a main effect of genotype and age on anxiety-like behavior in 5XFAD ($F(1,91) = 4.57$ and $F(1,91) = 7.602$, respectively) and LOAD ($F(9,379) = 6.025$ and $F(9,379) = 7.824$, respectively) mice (Fig. 3A–B). Post-hoc analyses revealed that 6-month-old 5XFAD mice, 8-month-old ApoE4, TREM2*R47H, and ApoE4 + TREM2*R47H mice, and 12-month-old TREM2*R47H mice spend less time in the edges of the open field arena compared to age-matched WT mice. Verubecestat dose did not modulate the significantly reduced anxiety-like behavior in 5XFAD mice (Fig. 3C). Taken together with our above data, these data suggest that defects in anxiety-like behavior does not parallel defects in habituation behavior in FAD or LOAD mice.

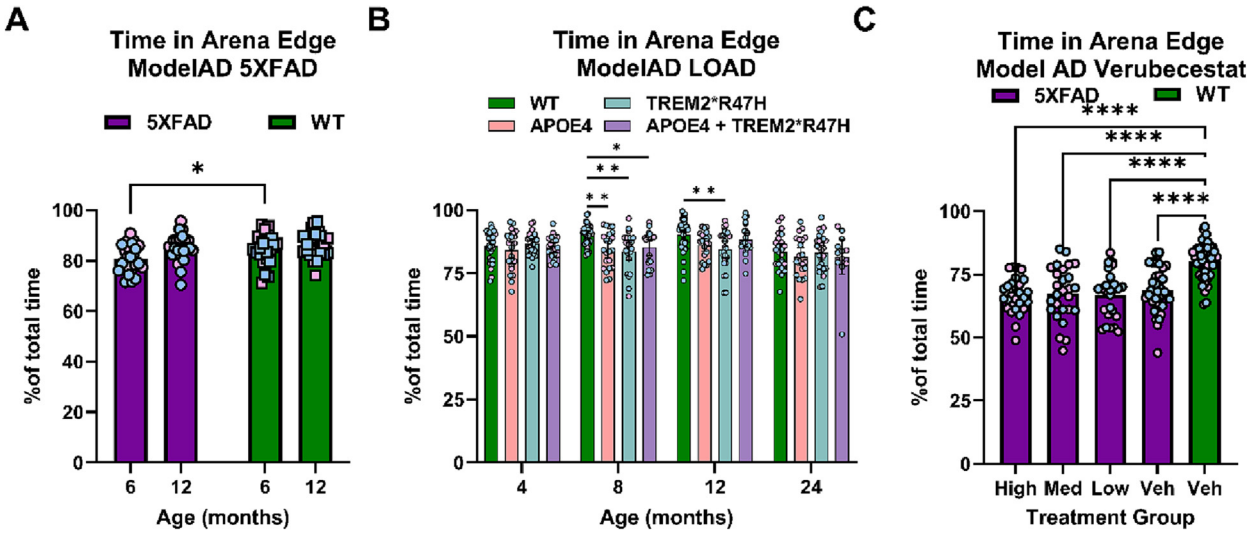

Fig. 3. 5XFAD and LOAD mice show reduced anxiety-like behavior in the open field that does not parallel habituation deficits. Each dot represents an individual mouse (females = pink, males = blue). ****p < 0.0001, **p < 0.01, *p < 0.05 A. 6-month-old 5XFAD mice spent a significantly smaller percentage of the total session time in the edges of the open field arena compared to age-matched WT mice. As measured by 2-way ANOVA with Tukey post-hoc (main effect of age and genotype). B. There was a significant effect of age and genotype on the percentage of the total session time spent in the arena edges in LOAD mice as measured by 2-way ANOVA. Post-hoc analyses revealed that 8-month-old ApoE4, TREM2*R47H, and ApoE4 + TREM2*R47H mice and 12-month-old TREM2*R47H mice spent significantly less time in the arena edges compared to age-matched WT mice. C. 6-month-old 5XFAD mice spent significantly less time in edges of the arena compared to age-matched WT mice regardless of verubecestat dose. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Discussion

Here, we show that 5XFAD mice show age-associated deficits in habituation to a novel environment. Our data suggests that habituation to a novel environment is a sensitive task to assess age-associated behavioral deficits in 5XFAD mice. Behavioral habituation overcomes drawbacks to current cognitive behavioral tasks such as Morris water maze (MWM) by reducing stress to the mouse, reducing laborious training, and by not necessitating the need for specialized equipment or large amounts of space. We demonstrate that habituation deficits are replicable in two additional datasets of 5XFAD mice (Fig. 2A-C), utility across different mouse strains (Fig. 2D), and sensitivity to therapeutic intervention (Fig. 2C), but association with specific neuropathological features would provide stronger evidence for the utility of this task for preclinical translational relevance to human AD.

The reversal of habituation deficits with verubecestat in 5XFAD mice (Fig. 2C) suggests that behavioral habituation may be related to plaque load or soluble Aβ which are both reduced by verubecestat treatment in 5XFAD mice. However, these changes may be due to noted changes in motor coordination measured by rotarod following verubecestat treatment [23]. Human clinical trials with verubecestat have failed due to worsened cognition and increased risk of falls [4,5]. Further, the habituation deficits we found in the TREM2*R47H mice, which do not develop Aβ pathology, suggest that other mechanisms underlie deficits in habituation to a novel environment we found in both FAD and LOAD mice. TREM2*R47H mice show age-related downregulation in transcriptomic pathways related to lysosomes, phagosomes, antigen processing, cytokine signaling, and the complement cascade [14], suggesting that immune pathways may in some way regulate habituation behavior. However, verubecestat treatment did not modulate immune signaling pathways in the 5XFAD mice [23]. Together, these data suggest divergent pathways may regulate habituation to a novel environment in FAD and LOAD mice.

The reduced habituation behavior in 5XFAD and TREM2*R47H mice may indicate alterations in novelty reactivity, stress coping, or anxiety-like behaviors. Exposure to a novel environment is a mild psychological stress. Corticosterone enhances motor activity in rodents [27]and corticosterone levels increase in adult mice within 15 min of exposure to a novel environment, remain elevated above baseline levels for at least 90 min. In normal mice, motor activity gradually decreases (intrasession habituation) as corticosterone levels wane, while adrenalectomized mice show blunted intrasession habituation [15]. Previous studies show that 5XFAD mice and mice deficient in TREM2 have enhanced serum corticosterone [17,34]which may underlie the changes in habituation that we observe herein. Stress may also influence AD pathology, suggestive of a vicious cycle between dysregulated stress response and disease progression [18]. Increased anxiety can also contribute to habituation deficits by suppressing exploration, resulting in altered mapping of the novel environment [1]. However, we found that 6-month-old 5XFAD, 8-month-old LOAD, and 12-month-old TREM2*R47H mice showed significantly less anxiety-like behavior in the open field (Fig. 3), suggesting their habituation deficits are not related to increased anxiety.

One major confounding factor in studies of AD model mice is background strain. Intrasession and intersession habituation phenotypes vary dramatically across different inbred mouse strains; C57BL6/J mice show intermediate levels of both types of habituation [1]. Here, we used the 5XFAD mouse on the congenic C57BL6/J background and performed replicative analysis on 5XFAD and LOAD mice also on the congenic C57BL6/J background. Pathology progresses less rapidly in the congenic C57BL6/J mice compared to the hybrid B6SJL 5XFADs [7,19,22], demonstrating the importance of consistent background strain on consistent pathology and behavior. However, many studies using 5XFAD mice do not report the background strain that they use, hampering cross-lab replicability and comparability. Some groups generate congenic C57BL6/J transgenic mice by backcrossing for 5 generations [13,33], while others, including ourselves herein, use mice generated using a speed congenic approach to ensure genetic uniformity [7,24]. The 5XFAD-BXD mice intentionally incorporate genetic diversity into congenic 5XFAD mice, demonstrating how genetic background influences both behavioral and pathological phenotypes [21]. While incorporation genetic diversity into mouse models of FAD may better recapitulate human AD, unintentional addition of mutations through different breeding practices could alter pathology and ultimately affect reproducibility.

Replicability of robust cognitive deficits is an important challenge in animal models of AD. Analyses of cognitive deficits in 5XFAD mice on a congenic C57BL6/J background strain highlight issues with replicability. For example, a common task used to assess spatial working memory is measuring the rate of alternating arm entries in a three-arm T or Y-maze or four-arm cross maze. A recent deep phenotyping study found no deficits in 5XFADs compared to WT controls at 6 or 12 months of age in the Y-maze [24], while other studies found deficits at 6, 9, and 12 months of age (but not 3 months) in the cross maze [13] or at 7, 11, and 18 months of age (but not 3 months) in the Y-maze [32]. Others have also noted deficits in spontaneous alternation as late as 10 months of age [3]. In common spatial reference memory test, the MWM, a wide variety of different protocols are used across studies in congenic 5XFAD mice, generating divergent data [3,10,29,32,33]. Numerous factors can add variance to animal behavior data including genetics but also experimenter, time of day, and differences in animal husbandry, among others [28]. Here, we show that deficits in habituation to a novel environment is replicable in 5XFAD mice across different institutions even with small changes to behavioral procedures and equipment. Simplified behavioral procedures, such as the habituation protocol described herein, could have utility in reducing variability by reducing the number of potential confounding factors.

Conclusions

In the study herein, we describe age-dependent changes in behavioral habituation in 5XFAD mice that are not present in WT mice that are replicable in others' datasets. Current commonly used behavioral paradigms do not reveal consistent cognitive deficits in the 5XFAD mice. Behavioral habituation deficits are also present in the TREM2*R47H model of LOAD. Current preclinical mouse behavioral assays have not yielded therapeutics that protect cognition in human clinical trials. We posit that the behavioral habituation assay may overcome weaknesses of current cognitive and memory tasks used for preclinical assessment of mouse models of AD.

Significance statement

Accessible and reproducible models that can translate protection of cognition from mouse to humans are important for the development of therapies to treat age-associated dementia causing diseases such as Alzheimer's disease. Here, we demonstrate that a common preclinical mouse model of Alzheimer's disease displays age-associated deficits in behavioral habituation, which is an easy-to-perform behavioral task that overcomes many of the issues with current cognitive behavioral paradigms.

CRediT authorship contribution statement

Sabrina Smith: Investigation, Resources, Writing – review & editing. **Sarah C. Hopp:** Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Supervision, Project administration, Funding acquisition, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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