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Sex differences in the IntelliCage and the Morris water maze in the APP/PS1 mouse model of amyloidosis

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Abstract

Transgenic rodent models were created to decipher pathogenic mechanisms associated with Alzheimer's disease (AD), and behavioral apparatuses such as the Morris water maze (MWM) are used to assess cognition in mice. The IntelliCage was designed to circumvent issues of traditional behavioral tests, such as frequent human handling. The motivation to complete IntelliCage tasks is water consumption, which is less stressful than escaping from a pool in the MWM. Here, we examined behavioral performances of mice in the IntelliCage and MWM tasks. Twelve-month-old male and female APP/PS1 and non-transgenic mice first underwent 42 days of IntelliCage testing to assess prefrontal cortical and hippocampal function followed by MWM testing for six days. We found that females performed better in the IntelliCage while males performed superiorly in the MWM. Mechanistically, female APP/PS1 mice had a higher Amyloid-β plaque load throughout the brain, which is inconsistent with their performance in the IntelliCage. Collectively, these results inform scientists about the sex-based differences when testing animals in different behavioral paradigms that tap similar cognitive functions.

Keywords

APP/PS1 mice; IntelliCage; Morris water maze; Sex differences; Aβ; Glucose

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder leading to dementia (Alzheimer's Association, 2020). Clinically, AD is characterized by impairments in cognition, including deficits in developing new memories, loss of long-term memories as

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the disease progresses, and a severe loss in intellectual abilities coinciding with dementia (Bateman, 2015; Honjo et al., 2012). The neuropathological hallmarks of the AD brain are extra-cellular plaques composed predominately of the amyloid-β (Aβ) peptide, intraneuronal tangles of hyperphosphorylated tau, and synaptic and neuronal loss (Bakota and Brandt, 2016; Honjo et al., 2012). Currently, more than 5 million are affected in the United States, and this number is expected to reach 14 million by 2050 (Alzheimer's Association, 2020). There are no approved clinical therapies to halt the progression or treat individuals in advanced stages of AD, necessitating the need for more preclinical research (Alzheimer's Association, 2020).

Mouse models of AD were developed in an effort to decipher the underlying pathogenic mechanisms and to consequently develop and test preclinical therapies. Given that rodents do not naturally develop AD, human transgenes of familial AD mutations are incorporated into the genomes of laboratory mice to replicate the condition (Hall and Roberson, 2012; LaFerla and Green, 2012). Currently, there are 193 mouse models of AD that possess many aspects of the human condition (ALZ forums, 2020). In particular, transgenic mice overproducing mutant APP develop toxic Aβ plaques (Borchelt et al., 1997; Hall and Roberson, 2012; Jankowsky et al., 2004; LaFerla and Green, 2012; Reiserer et al., 2007). The Aβ peptide ranges from 36 to 43 amino acids in length, where Aβ_{40} and Aβ_{42} are the most abundant Aβ species (Sadigh-Eteghad et al., 2015). Aβ_{42} is more prone to aggregation and toxicity than Aβ_{40} (Sadigh-Eteghad et al., 2015). Additionally, evidence has shown that prefibrillar soluble Aβ oligomers induce AD-related synaptic dysfunction thereby contributing to cognitive deficits (Cleary et al., 2005; Ferreira et al., 2015; Sakono and Zako, 2010). Notably, most AD transgenic mouse models exhibit cognitive impairments, with these deficits appearing as a result of pathology (LaFerla and Green, 2012; Reiserer et al., 2007). One of the most widely used models is the APPswe/PSEN1dE9 (abbreviated APP/PS1) mouse, which harbors the APP human Swedish mutation and a presenilin delta 9 mutation (Borchelt et al., 1997; Jankowsky et al., 2004). APP/PS1 mice show Aβ_{42} plaques as early as 6 months of age, with abundant accumulation in the hippocampus and cortex by 9 months of age (Jankowsky et al., 2004). Cognitive deficits have been documented as early as 6 months and become more pronounced by 12 months of age (Kilgore et al., 2010; Lalonde et al., 2005; Volianskis et al., 2010).

Various behavioral tasks have been developed that assess cognition in rodents; one of the most widely used methods to assess hippocampal-dependent spatial learning and memory is the Morris water maze (MWM (Brandeis et al., 1989; Nunez, 2008; Vorhees and Williams, 2006). The MWM relies on rodents' motivation to escape a pool by locating a hidden platform using extramaze cues; however this task has been documented to elicit stress (Harrison et al., 2009). In fact, it has been shown that the levels of stress hormone corticosterone negatively correlate with spatial learning in the MWM but not with a dry land spatial cognition task, as assessed 30 minutes after the final session (Harrison et al., 2009), highlighting that performance in a water maze may be affected by test-induced stress. Notably, female mice show poorer performance in the MWM than male counterparts (Li et al., 2016). Research in recent years has raised concerns over the translatability of AD mouse models to the human condition (Cuadrado-Tejedor and García-Osta, 2014; Webster et al., 2014). Indeed, the track record of success in AD clinical trials thus far has been very poor

(Cuadrado-Tejedor and García-Osta, 2014). This high failure rate has been attributed to the premature translation of highly successful results in animal models that may not capture aspects of the human condition. A dominant criticism lies in the behavioral apparatuses being utilized to test cognition in mice and their translatability to human cognitive assessment (Puzzo et al., 2014).

In 2000, Dr. Hans-Peter Lipp developed an apparatus that relies on rodents being tested in their natural social environment with minimal human intervention (Dell'Omo et al., 2000). Lipp and colleagues' goal was to reduce variability caused by environmental factors, reduce human handling, and introduce standardized scoring methods and experimental protocols (Dell'Omo et al., 2000; Lipp, 2005; Lipp et al., 2005). The IntelliCage is a fully-automated system that allows animals to engage in behavioral tasks, using access to water as their incentive to participate (Kiryk et al., 2020; Lipp, 2005; Lipp et al., 2005; Masuda et al., 2018). Minimal handling and disruption by human experimenters is sought to minimize stress in laboratory animals being tested. Since its inception, the IntelliCage has now been used in over 150 studies (Kiryk et al., 2020). While the IntelliCage has been utilized in AD mouse models, it has yet to be determined whether animal cognitive performance in this automated system results in similar outcomes to the MWM (Lee et al., 2015; Masuda et al., 2016; Ryan et al., 2013).

The goal of the present work was to determine whether sex differences exist when APP/PS1 and NonTg animals are consecutively tested in the IntelliCage and MWM. Additionally, we aimed to determine whether peripheral glucose metabolism, a common risk factor for AD and its hallmark pathology, Aβ, are associated with behavioral results from these testing paradigms. Herein, we report that female mice performed significantly better in the IntelliCage compared to males, whereas the opposite was true for performance in the MWM. We also show that insoluble fractions of A$\beta_{40,}$ Aβ_{42} and Aβ_{42} plaque burden are significantly elevated in female APP/PS1 mice compared to male counterparts. These results highlight how sex influences cognitive performance outcomes.

2. Methods

2.1. Animals

The APP/PS1 mice used in this study are hemizygous for the APP Swedish mutations (KM670/671N1) and presenilin1 (PS1) deltaE9 mutation (Borchelt et al., 1997; Jankowsky et al., 2004). Mice were obtained from Jackson laboratories (Stock# 34832-JAX) and backcrossed for 12 generations into a fully congenic 129/SvJ background as we have previously reported increased litter size and improved nurturing with less cannibalism, dermatitis and aggressive behavior with this background (Branca et al., 2017; Velazquez et al., 2019a, 2019b). All protocols were approved by the Institutional Animal Care and Use Committee of Arizona State University and conform to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Mice were same-sex group housed (4–5 mice per cage) prior to being introduced into the IntelliCage. At 11.8 months of age, prior to IntelliCage testing, a radiofrequency identification transponder (RFID; Standard Microchip T-VA, DataMars, Switzerland & Troven, USA) chip was subcutaneously implanted into all mice in the dorsocervical region under isoflurane inhalation anesthesia as previously

described (Masuda et al., 2016). The RFID chip allows for identification of a mouse when it enters a corner of the IntelliCage system. Mice were allowed 1 week to recover and were then introduced into the IntelliCage. The location of the implantation did not impede animals' swim ability; the animals swim speed matched that of a different cohort of APP/PS1 and NonTg mice that did not receive RFID chip implantation from our previous publication (Velazquez et al., 2019b). Once moved into the IntelliCage setting, 14–16 same-sex mice were housed per testing apparatus.

Both male and female APP/PS1 mice (male = 8; female = 10) and NonTg (male = 8; female = 12) mice were placed in the IntelliCage to begin the battery of testing to assess performance in tasks that tap hippocampal and prefrontal cortical function (Ajonijebu et al., 2018; Kiryk et al., 2020; Voikar et al., 2018). IntelliCage testing took a total of 42 days from adaptation to the place avoidance retention phase (Fig. 1). Mice were then moved to same-sex cages of 4–5 animals per cage for a 3-day rest period followed by behavioral testing in the hippocampal-dependent MWM task for 6 days (Fig. 1). All animals underwent glucose tolerance testing (GTT) at the completion of behavioral testing. Mice were subsequently euthanized at 13.8 months of age, and one hemisphere was extracted and prepared for ELISAs and the other hemisphere was prepared for hippocampal and cortical $A\beta_{42}$ plaque load quantification.

2.2. Mouse genotyping

Genotyping was performed as previously described (Velazquez et al., 2019b, 2019a). We took 2–3 mm tail snips from all mice at postnatal day 14 and digested samples in a TrisEDTA SDS buffer containing 10/mg/mL proteinase K overnight at 55 °C. The digested tails were then vortexed and centrifuged in a microfuge at 10,000 rpms for 10 minutes. The supernatants were mixed with an equal volume of 100% isopropanol and the DNA was removed, dried and rehydrated with 100 μL Tris EDTA buffer. PCR reactions were setup using 2X Taq mix containing dNTPs and MgCl2 Tacara #R004A, 1 μL each of 10 μM primers and 1.6 μL DNA. The primers we used were PS1 Transgene 1 - AATAGAGAACGGCAGGAGCA 63.8, PS1 - Transgene 2 GCCATGAGGGCACTAACAT 63.8, PS1 Internal Control 1 - CTAGGCCACAGAATTGAAAGATCT 63.5 and PS1 Internal Control 2 - GTAGGTGGAAATTCTAGCATCATCC 64.5. The PCR ran for 35 cycles with annealing at 52 °C. PCR products were mixed with 6X loading dye and run on 1.5% agarose gels containing 1% gel red. Gels were imaged for products containing the transgene at 608 bps. All samples contain the internal positive control band of 324 bps.

2.3. Automated IntelliCage testing

The IntelliCage was used to assess water drinking behavior, spatial learning and reference memory, behavioral flexibility, attention and contextual memory (Kiryk et al., 2020; Masuda et al., 2018, 2016). The testing apparatus (39 × 58 × 21 cm) contains 4 corner chambers accessible through an open tunnel which is equipped with an antenna. Two individual doors in each corner are controlled by the computer management system and are used to regulate access to the water bottles. RFID chips are detected by a scanner and temperature sensor located at the entrance of each corner that registers when an animal enters. The animal's entire body must enter the corner to register the RFID and be counted as a visit. Nosepokes

and licks are detected by sensors on the noseport and waterspout, respectively. Mice were fed ad libitum during the entire duration in the IntelliCage with standard mouse chow. Lights in the behavior room were on between 08:00 and 20:00. A video camera was placed outside the IntelliCage and recorded the entire testing sessions (24 hours/7 days a week). The IntelliCage behavioral task sequence was as follows: (1) Adaptation phase (2) Place preference and Reversal (3) Serial Reaction Time 1 – 2 and (4) Place avoidance (Fig. 1). Any animal that failed to consume water in a 24-hour period was removed from the IntelliCage and placed in a standard cage to avoid severe dehydration. If these animals were reintroduced into the IntelliCage and failed to consume water, they were removed from the experiment. Data was extracted using the TSE analyzer software, which included visits and nosepokes per corner, licks and time of testing. For each task, the dependent variable calculated is described below.

1) Adaptation—During the first 2 days of the Adaptation phase (free adaptation) all the doors were open allowing free access to the water bottles, thereby acclimating mice to the new environment. Total visits and licks were analyzed for the first 2 days to assess exploratory behavior and water consumption. During the next 3 days, the doors to the water bottles were closed, but opened for any visit into the corner. For the last 3 days, doors were closed and could be opened with a nosepoke in a corner, thereby training animals to nosepoke to retrieve water.

2) Place preference—During the place preference phase, water was available in only one of the 4 corners for each of the mice. The correct corner for each mouse was chosen based on their previous visit habits, selecting among the least visited corners to eliminate preferential corner bias. For the first 6 days, water was available only in the selected reward corner (place preference). For the last 6 days, water was available only in the opposite corner (reversal). To prevent over-crowding of the corners and learning by imitation, the selected reward corners were balanced by number of mice and genotype, limiting the number to 4 per corner and 50–50 proportion of APP/PS1 and NonTg genotypes. The % correct visits with nosepokes was calculated by taking the number of correct corner visits with nosepokes divided by total visits per day. We also analyzed total visits and licks per day during place preference and reversal.

3) Serial Reaction Time (SRT) 1 and 2 attention task—During the 10 days of SRT 1 task, when an animal entered an assigned corner (the correct corner from the place preference reversal task), the first nosepoke for a visit initiated the illumination of a green LED for 7 seconds. The animal was required to nosepoke within the 7-second time frame to count as a correct response, resulting in the door opening allowing access to water. If the animal failed to nosepoke during the 7-second time frame, the LED turned off and the trial would reset, requiring the animal to restart the trial. During the 7 days of SRT 2, the LED cue was shortened to 3 seconds, thereby making this task more challenging. The % accuracy was calculated by taking the number of correct visits with nosepoke and lick divided by total visits per day. Additionally, we calculated the time it took to extinguish the LED on day 1 of SRT 1 and 2 to determine reaction time.

4) Place avoidance—The place avoidance tasks included both training and probe trials. For the day 1, 24-hour training trial (learned avoidance), nosepoking in the reward corner administered an aversive airpuff (~0.8 bar, 1 second airpuff). The doors in all corners remained closed and water was not available during the learned avoidance phase. We analyzed the number of corner visits with nosepokes at the airpuff corner during this phase. After the 24-hour training trial, the mice were moved to their standard home cages for a 24-hour delay with water ad libitum. After the delay, the mice were reintroduced to the IntelliCage for three days with water available at all 4 corners and the airpuff stimulus removed to assess retention and extinction. The data for retention and extinction was quantified as the % correct visits with nosepokes over total visits for each day.

2.4. MWM testing

To assess spatial learning, MWM testing was performed as previously described (Velazquez et al., 2019a, 2019b). Animals were tested in a circular tank of 1.5 m in diameter located in a room with extramaze cues. The platform (14 cm in diameter) was submerged 1.5 cm beneath the surface of the water, which was maintained at 23 °–25 °C throughout testing. Nontoxic white paint was added to opaque the water and hide the platform. During the learning phase, each animal was given 4 trials per day for a total of 5 days. The location of the hidden platform remained in the same quadrant for all the animals; however, the start location was pseudo-randomly selected. Each animal was given 60 seconds to locate the hidden platform. If the animal failed to reach the hidden platform in the 60 seconds, they were guided to its location by gently pushing the mouse forward from the base of their tail and maneuvering them toward the platform. Animals were allowed 10 seconds on the platform to encode extramaze cues. Mice were then returned to a resting cage with a heating pad for 25 seconds before the next trial. A probe trial was conducted 24 hours after the last training trial. During the probe trial, the platform was removed, and the mice were allowed 60 seconds to swim freely. All trials were video recorded, and the data was analyzed via the Etho-VisionXT system from Noldus Information Technology. The dependent variables used for the analysis were latency (seconds) to the platform for the learning trials and number of platform crossings, latency to first cross the platform location (seconds), and velocity (cm/second) for the probe trial.

2.5. GTT

Upon completion of behavioral testing, all mice underwent GTT as previously described (Velazquez et al., 2017). Mice were fasted overnight for 16 hours, then were weighed the following morning prior to nicking tails with a fresh razor blade to measure baseline fasting blood glucose levels. All animals received an injection of 2 mg glucose/kg of bodyweight into the intraperitoneal (i.p.) cavity. At baseline and at 15, 30, 45, 60, 90, and 120 minutes postinjection, blood glucose was sampled from the tail using a TRUEtrack glucose meter and TRUEtrack test strips (Trividia Health, Fort Lauderdale, FL, USA).

2.6. Brain tissue processing, ELISA and immunohistochemistry

At 13.8 months of age, mice were perfused with fresh 1X PBS and one hemisphere had the hippocampus and cortex dissected out and flash-frozen while the contralateral hemisphere

was fixed in a glass vial of 4% paraformaldehyde for 48 hours. Flash-frozen tissue was homogenized in a T-PER tissue protein extraction reagent, and supplemented with protease (Roche Applied Science, IN, USA) and phosphatase inhibitors (Millipore, MA, USA). The homogenized tissues were centrifuged at 4 °C for 30 minutes. The supernatant (soluble fraction) was stored at −80 °C. We then homogenized the pellet in 70% formic acid followed by centrifuging at 4 °C for 30 minutes. Hippocampal soluble and insoluble fractions of $A\beta_{40}$ and $A\beta_{42}$ were detected using the commercially available ELISA kit (CellBiolabs Inc) as previously described (Velazquez et al., 2019a, 2019b). Hippocampal soluble $A\beta$ oligomers were detected using the commercially available ELISA kit (IBL America). For the fixed hemisphere, a Leica VT1000 S vibratome was used to partition the tissue into 50-μm coronal sections and stored chronologically in a specimen plate with PBS containing 0.02% sodium azide. Three coronal sections per animal that included the hippocampus and cortex underwent immunohistochemistry for $A\beta_{42}$ staining as previously described (Velazquez et al., 2019a, 2019b). The anti-$A\beta_{42}$ antibody (Catalog #5078P, dilution 1:200), was purchased from Millipore. To quantify $A\beta_{42}$ pathology load, images from all animals were taken with a Zeiss Axio Imager A1 using a 2.5 × objective. Images were photomerged to rebuild the image, and plaque number was obtained using NIH ImageJ. The experimenter was blinded to the group allocation.

2.7. Statistical analyses

Analysis of Variance was used to analyze body weight, IntelliCage, and MWM data with repeated measures when applicable. Data was first assessed for sphericity using Mauchly's tests. Mauchly's tests confirmed that some data violated the assumption of sphericity, in which case the Greenhouse-Geisser corrections was used to correct the F statistic and assess significance. Bonferroni's corrected *post hoc* tests where performed when a significant interaction was observed. Student's unpaired *t*-tests were employed for comparison of APP/PS1 mice when appropriate. Linear correlations between $A\beta_{42}$ plaque load and the behavior variables were calculated using the Pearson r analysis. Examination of descriptive statistics revealed no other violations of any assumptions that required the use of statistical test other than the ones used. Significance was set at $p < 0.05$.

3. Results

3.1. Sex differences were observed for corner visits, total licks, and weight of mice during the adaptation phase of the IntelliCage

Animals were first tested in the IntelliCage. One NonTg female mouse did not drink during the adaptation phase of the task and was removed from the study. During the first 2 days of the adaptation phase, (Fig. 2 A), we found a significant main effect of sex ($F = 4.239$, $p < 0.05$; Fig. 2 B) and Day ($F = 34.661$, $p < 0.0001$; Fig. 2 B), where males made more visits than female mice and animals made more visits on day 1 compared to day 2, respectively. We also found a significant sex by day interaction ($F = 5.325$, $p < 0.05$). *Post hoc* analysis with Bonferroni correction revealed that males made more corner visits than females on day 1 ($p < 0.05$; Fig. 2 B). No differences were detected on day 2. Next, we analyzed total licks during the adaptation phase. We found a significant main effect of sex, where male mice licked significantly more than female mice ($F = 14.954$, $p < 0.001$; Fig. 2 C). Additionally,

we found a significant main effect of day, where the number of licks increased from day 1 to day 2 (F = 11.572, $p < 0.01$; Fig. 2 C). Lastly, we analyzed body weight and found significant main effects of genotype (F = 20.589, $p < 0.001$) and sex (F = 18.529, $p < 0.001$; Fig. 2 D), where APP/PS1 mice were significantly heavier than NonTg and males weighed significantly more than females, respectively. Weight results are consistent with previous reports (Velazquez et al., 2019a, 2019b). Collectively, these data illustrate that males weigh more than females, and initially made more corner visits and consumed more water than female mice during the adaptation phase.

3.2. Female mice performed significantly better in the place preference and reversal phases of the IntelliCage

During the learned place preference phase, animals were assigned to and only granted access to water from one corner (Fig. 3 A). Mice can use external environment cues to locate their correct corner, thereby assessing spatial learning (Kiryk et al., 2020; Lee et al., 2015; Ryan et al., 2013). We found a significant main effect of sex for % correct visits with nosepokes (F = 19.139, $p < 0.0001$; Fig. 3 B), where the female mice performed significantly better than their male counterparts, regardless of genotype. Interestingly, we found that males made more total visits (F = 22.454, $p < 0.0001$; Fig. 3 C) and licked significantly more (F = 7.789, $p < 0.01$, Fig. 4 D) across the 6 days of learned place preference than females. Next, animals were assessed in the place preference reversal phase (Fig. 3 E). We found a significant main effect of sex for % correct visits with nosepokes (F = 13.456, $p < 0.001$; Fig. 3 F), where the female mice performed significantly better than the male counterparts, regardless of genotype. Similar to the place preference phase, we found that males made more total visits (F = 13.548, $p < 0.001$; Fig. 3 G) and licked significantly more (F = 7.849, $p < 0.01$, Fig. 3 H) across the 6 days of place preference reversal than females. These results show that when animals are maintained in their social environment and tested in spatial learning tasks, female mice perform significantly better than their male counterparts.

3.3. No differences were detected in the SRT attention tasks

To determine whether animals show impairments in attention and reaction time, we next tested animals in the SRT attention task (Fig. 4 A). Notably, 1 NonTg and 2 APP/PS1 males died due to unknown causes at the beginning of the SRT tasks. For % accuracy (number of correct visits with nospoke and lick/total visits), we found that all mice performed equally during the 10 days of testing (Fig. 4 B). We also analyzed time to extinguish the LED on day 1 of SRT 1 and found no significant differences among the 4 groups (Fig. 4 C). Next, mice were tested for 7 days by the SRT 2 attention task, which was identical to SRT 1, however, the LED cue duration was shortened to 3 seconds (Fig. 4 D). We found no significant differences in % accuracy between the 4 groups (Fig. 4 E). Additionally, we found no significant differences on time to extinguish the LED on day 1 of SRT 2 among the 4 groups. These results are consistent with a recent report showing no deficits in attention in APP/PS1 mice (Shepherd et al., 2019).

3.4. Sex and genotype differences were detected in the place avoidance task

Lastly in the IntelliCage, we tested all mice in a place avoidance learning task to assess both working and contextual memory. During the 24-hour period of airpuff exposure, we found a

significant main effect of sex, where males entered the airpuff corner more than females ($F = 85.33$, $p < 0.0001$; Fig. 5 B). We also found a significant genotype by sex interaction ($F = 10.230$, $p < 0.01$; Fig. 5 C) for nosepokes within the airpuff corners. *Post hoc* analysis revealed that the APP/PS1 male mice entered the airpuff corner and nosepoked significantly more than the NonTg male and all female mice regardless of genotype ($p < 0.05$), illustrating deficits in working memory. After the 24-hour airpuff exposure, mice were removed from the IntelliCage and placed in a standard cage for 24 hours. The mice were then returned to the IntelliCage to assess memory and extinction by measuring corner visits with nosepokes to the previously assigned airpuff corner (Fig. 5 D). We found no significant differences in the 3-day retention phase between male APP/PS1 and NonTg mice (Fig. 5 E). For females, we found a significant main effect of genotype, where NonTg mice entered the airpuff corner and nosepoked significantly more than APP/PS1 mice ($F = 4.669$, $p < 0.05$; Fig. 5 F). This effect appears specific to day 2 and 3, suggesting extinction in the NonTg female mice. In conclusion, the male APP/PS1 mice enter the airpuff corner and nosepoke significantly more, illustrating working memory errors, and female NonTg mice extinguish the previous memory after the first day of the retention phase.

3.5. Sex and genotype differences were observed in the MWM task

After a 3-day rest period, mice were tested in the MWM. After correcting for a violation of sphericity using the Greenhouse-Geisser correction, during the first 5 days of the MWM, we found a significant main effect of day for latency to the platform, indicating learning throughout the five days of the training trials ($F = 9.473$, $p < 0.0001$; Fig. 6 A). We also found a significant main effect of genotype ($F = 5.158$, $p < 0.05$), where APP/PS1 mice took significantly longer to find the platform than NonTg mice. Additionally, we found a significant main effect of sex ($F = 33.990$, $p < 0.0001$), where males found the platform faster than females. On the day 6 probe trial, the hidden platform was removed, and the mice had 60 seconds to swim freely throughout the pool to assess spatial reference memory. We found no significant differences in platform crosses or latency to first cross the platform location during the 60 seconds among the 4 groups (Fig. 6 B, C). Next, we examined swim speed and found that all 4 groups performed equally (Fig. 6 D). Additionally, we did not observe any floating or thigmotaxis of mice during any phases of the MWM. Collectively, these results show that female mice perform worse in the learning phase of the MWM than male mice, and that APP/PS1 mice are significantly impaired compared to NonTg mice. No differences were found in the memory phase of the MWM.

3.6. Female mice show increased glucose levels and APP/PS1 females have a higher insoluble fraction of $A\beta_{40-42}$ and plaque burden than male counterparts

Impairments in peripheral glucose metabolism has been shown to be associated with cognitive deficits and is a risk factor for AD (Haan, 2006; Petersen and Shulman, 2018; Zilliox et al., 2016). Various AD mouse models have been shown to exhibit peripheral insulin resistance (Macklin et al., 2017; Rodriguez-Rivera et al., 2011; Velazquez et al., 2017). Notably, reports of impaired insulin resistance in APP/PS1 mice have been inconsistent (Denver et al., 2018; Jiménez-Palomares et al., 2012; Macklin et al., 2017). To assess changes in peripheral glucose metabolism, we performed a GTT in all mice. We found a significant sex by GTT time interaction ($F = 4.998$, $p < 0.0001$). *Post hoc* analysis

revealed that female mice had a higher level of glucose ($p < 0.001$) when compared to the male mice (310.07 ± 32.87 mg/dL for female and 183.13 ± 23.70 mg/dL for male mice) at the 15-minute timepoint after the bolus glucose injection (Fig. 7 A). Next, we performed an area under the curve analysis as this has been reported to be a better assessment of glucose tolerance (Rodriguez-Rivera et al., 2011; Velazquez et al., 2017). We found no significant sex nor genotype differences for GTT area under the curve (Fig. 7 B). These results are consistent with previous reports illustrating gender difference in GTT, where females show a higher plasma glucose than men during GTT (Mauvais-Jarvis, 2018).

Next, mice were euthanized, and their brains were prepared for assessment of soluble A$\beta_{40–42}$ fractions, soluble Aβ oligomers, and insoluble fractions of A$\beta_{40–42}$ via ELISA. We found no significant sex differences in APP/PS1 mice for both soluble Aβ_{40} and Aβ_{42} levels (Fig. 7 C). Additionally, we did not find any significant differences in the levels of Aβ oligomers (Fig. 7 D). We did find that the insoluble levels of both Aβ_{40} ($t = 9.177$, $p < 0.0001$; Fig. 7 E) and Aβ_{42} ($t = 9.276$, $p < 0.0001$) were significantly higher in female APP/PS1 mice compared to male counterparts. Next, we assessed Aβ_{42} plaque load via immunohistochemical staining. Quantitative analysis of the Aβ_{42} plaque load reveals that female APP/PS1 mice have a significantly higher number of Aβ_{42} plaques within the hippocampus ($t = 9.823$, $p < 0.0001$; Fig. 7 F, H) and cortex ($t = 6.902$, $p < 0.0001$; Fig. 7 G, I) compared to male APP/PS1 mice, consistent with previous reports (Li et al., 2016; Wang et al., 2003). Lastly, we performed Pearson's r analysis to determine if Aβ_{42} hippocampal and cortical plaque load correlated with the various behaviors tested in the Intellicage and MWM (Supplementary Table 1). For male APP/PS1 mice, the only significant correlation was between visits during airpuff exposure and Aβ_{42} hippocampal plaque load ($r = -0.8118$ $p < 0.05$), illustrating that as plaque load goes up, the entrance into the airpuff corner went down. For female APP/PS1 mice, we found a significant positive correlation in cortical Aβ_{42} plaque load and day 1 retention after airpuff exposure, where entrance into the aversive corner went up as plaque load did ($r = 0.6801$, $p < 0.05$). Collectively, these results suggest fractions of A$\beta_{40–42}$ and Aβ_{42} plaque load may not be the best predictor of cognition deficits given the sex-based differences observed in the cognitive tasks and a lack of significant correlations.

4. Discussion

Our results highlight sex differences when mice were tested in the fully automated IntelliCage and the MWM. In the IntelliCage, during day 1 of the adaptation phase, males made more corner visits than females, suggesting increase exploration of male mice. On day 2, all mice made the same number of corner visits. Interestingly, male mice licked significantly more during the adaptation and the place preference tasks, thereby consuming more water than female mice. These findings are consistent with previous reports showing that male mice consume more water due to increased weight (Bachmanov et al., 2002). While increased thirst/water consumption is a common symptom of impaired glucose tolerance in humans (Verbalis, 2003), reports of impaired insulin resistance in APP/PS1 have been inconsistent (Denver et al., 2018; Jiménez-Palomares et al., 2012; Macklin et al., 2017). Our results show that female mice, regardless of genotype, show increased glucose levels during the GTT, which is consistent with reports showing that women have a higher 2-

hour plasma glucose than men (Mauvais-Jarvis, 2018). No differences were observed between APP/PS1 and NonTg mice in GTT, which is consistent with previous work (Denver et al., 2018).

Notably, females performed significantly better on the place preference and reversal phases of the IntelliCage, which assess spatial learning (Lee et al., 2015; Ryan et al., 2013). Male mice made more incorrect corner visits during place preferences phases, and while there might be a myriad of explanations of why male mice did so, as there is no real disincentive to visit an incorrect corner, the fact that they drank more throughout these phases suggest that they likely entered these corners in search for water. This highlights a deficit in males' ability to learn that one corner has unlimited water access. Moreover, female mice were also capable of extinction in the place avoidance tasks, illustrating enhanced performance in behavioral flexibility, which is mediated by the prefrontal cortex (Ajonijebu et al., 2018; Voikar et al., 2018). Notably, no significant differences were detected in the SRT 1 or 2 attention tasks among the 4 groups, which is consistent with a very recent report (Shepherd et al., 2019). Lastly, we found that female mice performed significantly worse in the MWM than male mice, and that APP/PS1 mice were significantly impaired when compared to their NonTg counterparts. This is consistent with previous work showing poorer performance of female mice in the MWM than male counterparts (Li et al., 2016). Collectively, these results highlight how sex influences cognitive performance outcomes.

Our results demonstrate that sex is a stronger predictor of cognitive performance in the NonTg and the APP/PS1 model. Notably, since the IntelliCage does involve social interactions and development of hierarchical systems, it may also induce some forms of stress. To this end, while aggression between male mice may be one explanation for poor performance in the IntelliCage, we found no video evidence of physical aggression (i.e., fighting) beyond the first day of the adaptation phase. This suggests that other mechanisms may be at play. Heightened stress has been shown to impair learning and memory, in particular those associated with hippocampal function (Kim et al., 2015). Female mice may be more sensitive to the stressful stimuli of the MWM which includes daily handling and fear of remaining in water. Indeed, previous reports have shown that the levels of the stress hormone corticosterone negatively correlate with spatial learning in the MWM but not with a dry land spatial cognition task, as assessed 30 minutes after the final session (Harrison et al., 2009). Furthermore, higher stress hormone corticosterone levels in female mice have been found both at baseline and during stressful conditions (Aoki et al., 2010). A more recent report found that female APP/PS1 mice exposed to chronic unpredictable mild stress show significantly higher corticosterone expression than males (Dominguez et al., 2020). Interestingly, previous studies have found that Aβ plaque load is significantly elevated with increased stress (Dong et al., 2008, 2004; Kang et al., 2007). Thus, it is tempting to speculate that heightened susceptibility to stress in females may be a contributing factor to Aβ plaque accumulation during their lifespan, and that when tested in the MWM, elevations in stress may have impaired performance. Moving to a task such as the IntelliCage, where human intervention is low may truly capture cognitive deficits.

The deposition of Aβ plaques has long been seen as a link to cognitive decline in AD (Reiss et al., 2018; Sadigh-Eteghad et al., 2015). We found that the insoluble levels of Aβ_{40} and

Aβ_{42} were significantly elevated in the hippocampus of female APP/PS1 mice. Additionally, we found that Aβ plaque load was significantly elevated in both the hippocampus and cortex of female APP/PS1 mice compared to their male counterparts, which is consistent with previous reports (Jiao et al., 2016; Li et al., 2016; Wang et al., 2003). While female APP/PS1 mice have a higher Aβ plaque load than the males, it is interesting to note that the female mice performed significantly better in the complex cognitive testing of the IntelliCage. Notably however, in the place avoidance task, we did find that female APP/PS1 cortical Aβ plaque load positively correlated with contextual memory deficits. In male APP/PS1 mice however, no such correlation was significant. No correlations between Aβ plaque load and MWM performance was found in male APP/PS1 mice. Collectively, these results suggest Aβ may not be an accurate predictor of cognitive deficits and that other sex-specific molecular mechanisms, independent of Aβ, may be affecting cognitive function. To this end, recent reports in humans have shown consistent findings (Arboleda-Velasquez et al., 2019; Khosravi et al., 2019). For example, a recent report found that a woman with a familial presenilin 1 (PSEN1) mutation did not show cognitive decline associated with AD due to the homozygotic presence of the APOE3 Christchurch mutation (Arboleda-Velasquez et al., 2019). When they examined the brain of this patient with PET scan, it was observed that Aβ levels were very high in her brain, but she did not develop the cognitive deficits like others in her family. Additionally, various reports have shown that Aβ may be present in various individuals that do not show clinical signs of cognitive deficits (Aizenstein et al., 2008). This aligns with our results illustrating that Aβ levels in APP/PS1 mice may not be associated with cognitive function.

5. Conclusions

In conclusion, our results show that sex is a strong determinant of cognitive performance in the APP/PS1 mouse model of amyloidosis as well as in NonTg controls. Our results highlight how behavioral tasks that require human intervention, and which rely on motivation to escape water, such as the MWM, may influence results particularly in female mice. Our results will inform scientists to consider sex-based differences when testing NonTg and APP/PS1 mice in the automated IntelliCage and the MWM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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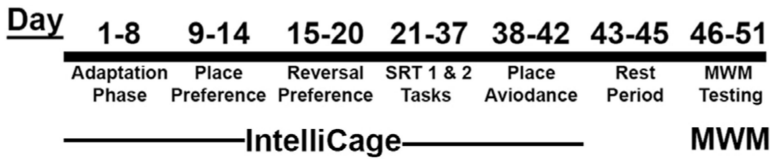

Fig. 1.

Behavioral battery timeline for the study. Starting at 12 months of age, NonTg and APP/PS1 male and female mice were introduced into the IntelliCage to begin the battery of testing assessing prefrontal cortical and hippocampal brain function. After a 3-day rest period, mice were behaviorally tested in the hippocampal-dependent Morris water maze (MWM) task. Mice then underwent a glucose tolerance test (GTT) and were sacrificed the following day to harvest brain tissue. Abbreviations: SRT, Serial reaction time task.

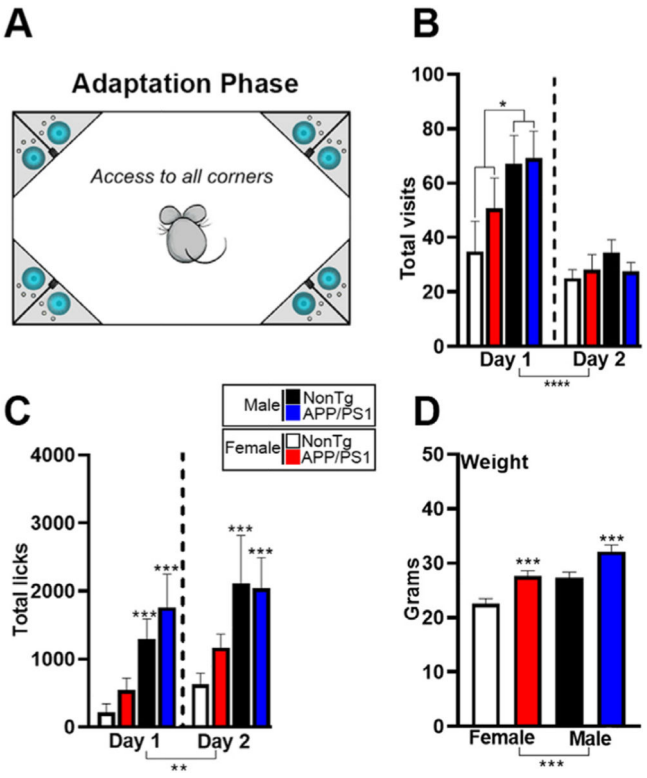

Fig. 2.

Sex differences identified on corner visits, total licks, and weight of mice. (A) During the 2-day adaptation phase, animals freely entered any of the 4 corners and could access water; cage not drawn to scale. (B) We found that males made more corner visits than females on day 1 ($p < 0.05$). We also found that all mice made more visits on day 1 compared to day 2 ($p < 0.0001$). By day 2, no differences were detected. (C) We also found that male mice licked significantly more often than female mice, thereby consuming more water ($p < 0.001$). The number of licks increased from day 1 to day 2 ($p < 0.01$). (D) Body weight analysis revealed that APP/PS1 mice were significantly heavier than NonTg ($p < 0.001$) and that males weighed significantly more than females ($p < 0.001$). Data are presented as means ± SEM. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

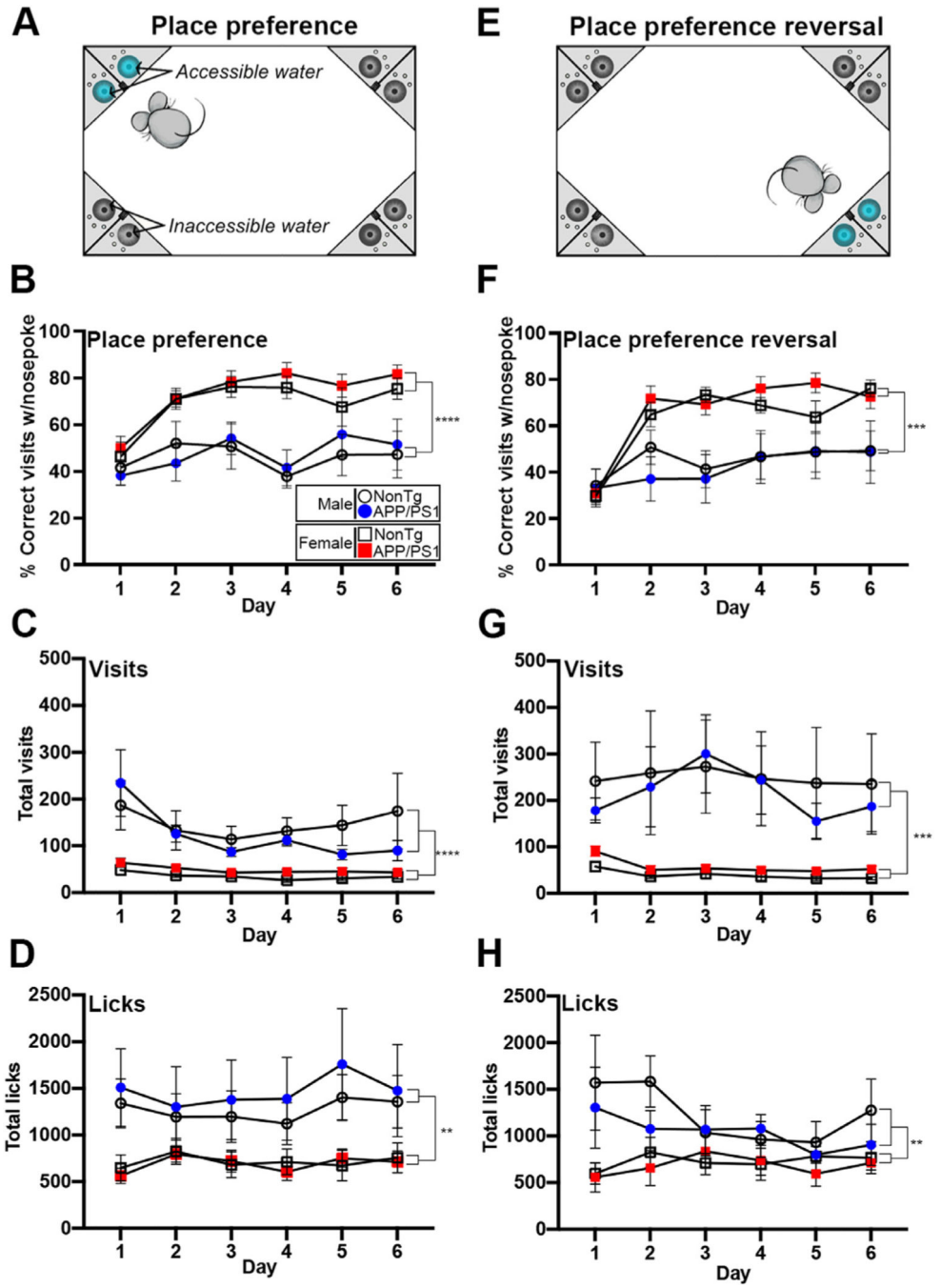

Fig. 3.

Female mice perform significantly better in the place preference and place preference reversal phases of the IntelliCage. (A) During the 6 days of the learned place preference phase, animals were assigned to one corner where they could access water. All other corners were counted as incorrect and no access to water was granted; cage not drawn to scale. (B) We found a significant main effect of sex for % correct visits with nosepokes ($p < 0.0001$), where the female mice performed significantly better than the male counterparts, regardless of genotype. (C-D) Interestingly, we found that males made more total visits ($p < 0.0001$)

and licked significantly more ($p < 0.01$) across the 6 days of learned place preference than females. (E) During the 6 days of the place preference reversal phase, animals could access water by entering and nosepoking the opposite corner from the corner assigned during the learned place preference phase; cage not drawn to scale. (F) We found a significant main effect of sex for % correct visits with nosepokes ($p < 0.001$), where the female mice performed significantly better than the male counterparts, regardless of genotype. (G-H) Males made more total visits ($p < 0.001$) and licked significantly more ($p < 0.01$) across the 6 days of the place preference reversal than females. Data are presented as means ± SEM. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

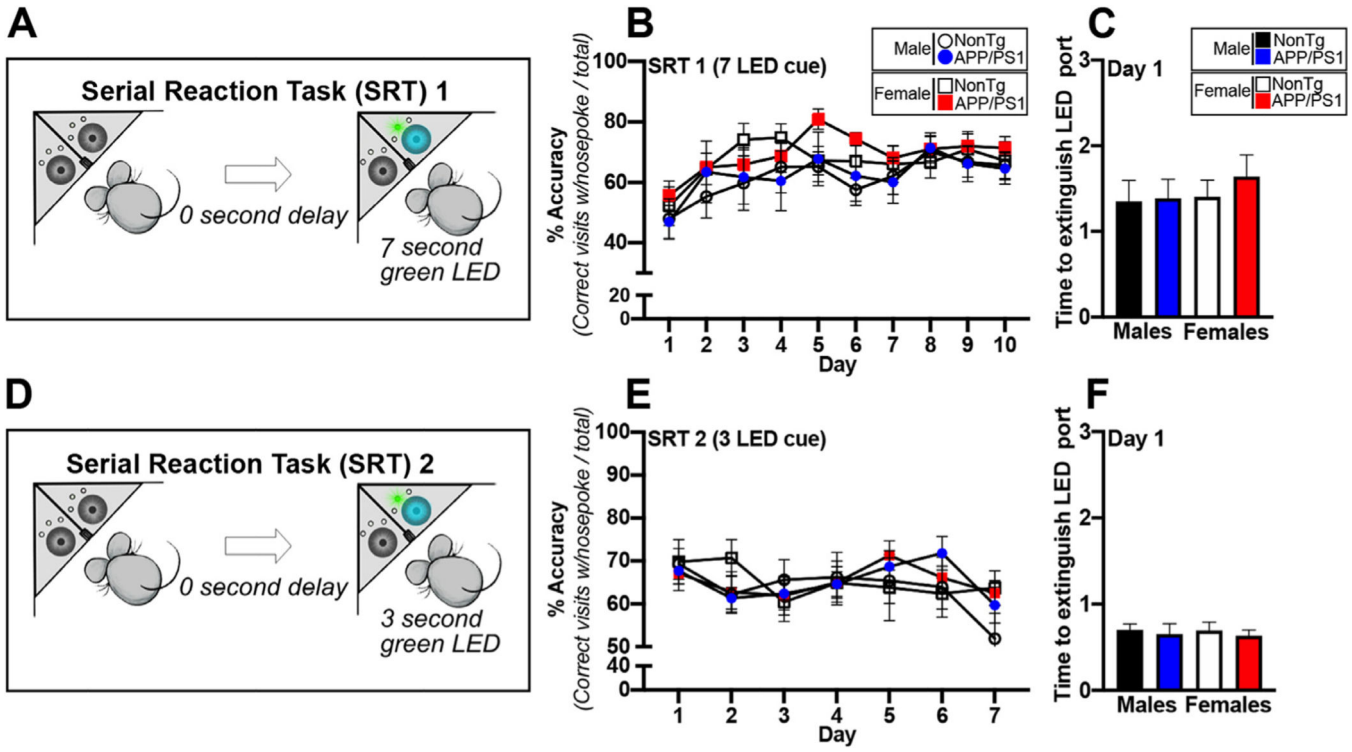

Fig. 4.

No differences detected on the Serial Reaction Time (SRT) attention task. (A) During the first 10 days of the Serial Reaction Time (SRT 1) attention task, animals were required to enter an assigned corner and nosepoke to initiate a trial. Then, a green LED illuminated in one of the 2 noseports and the animal had 7 seconds to extinguish the LED with a nosepoke. Correct response resulted in access to water, while an incorrect response reset the trial and the animal was required to leave the corner before initiating a new trial; corner not drawn to scale. (B) No significant differences in % accuracy were detected in the SRT 1 attention tasks among the 4 groups. (C) We also analyzed reaction time to extinguish the LED during day 1 and found no significant differences among the 4 groups. (D) During the 7 days of the SRT 2 attention task, the green LED illuminated in one of the 2 noseports and the animals had three seconds to extinguish the LED with a nosepoke; corner not drawn to scale. (E) No significant differences in % accuracy were detected in the SRT 2 attention tasks among the 4 groups. (F) No significant differences were detected among the 4 groups on the time to extinguish the LED during day 1 of SRT 2.

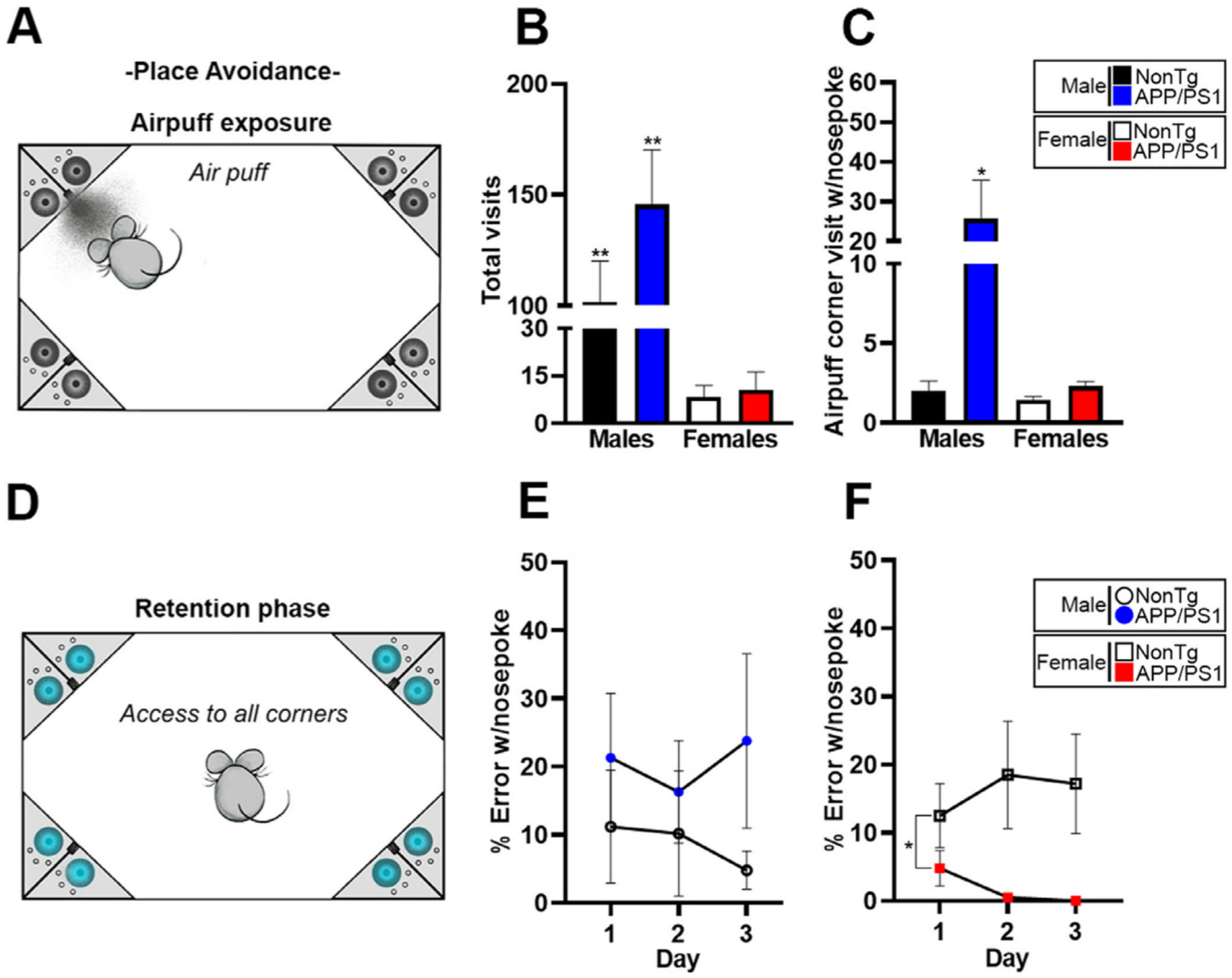

Fig. 5.

Sex and genotype differences detected in the place avoidance task. (A) For a 24-hour period, entry into the assigned corner with a nosepoke resulted in an airpuff (~0.8 bar, 1 second airpuff); cage not drawn to scale. (B-C) We found a significant main effect of sex, where males entered the airpuff corner more than females ($p < 0.0001$). We also found a significant genotype by sex interaction ($p < 0.01$) for airpuff corners with nosepokes. *Post hoc* analysis revealed that the APP/PS1 male mice entered the airpuff corner and nosepoked significantly more than the NonTg male and all female mice ($p < 0.05$). (D) Mice were removed from the IntelliCage after the airpuff exposure and placed in a standard cage for 24 hours, then returned to the IntelliCage to assess memory and extinction by measuring corner visits to the previously assigned airpuff corner; cage not drawn to scale. (E-F) We found no significant differences in the 3-day retention phase between male APP/PS1 and NonTg mice. For females, we found a significant main effect of genotype, where NonTg mice entered the airpuff corner and nosepoked significantly more than APP/PS1 mice ($p < 0.05$). This effect appears specific to day 2 and 3, suggesting extinction in the NonTg female mice. Data are presented as means ± SEM. * $p < 0.05$, ** $p < 0.01$.

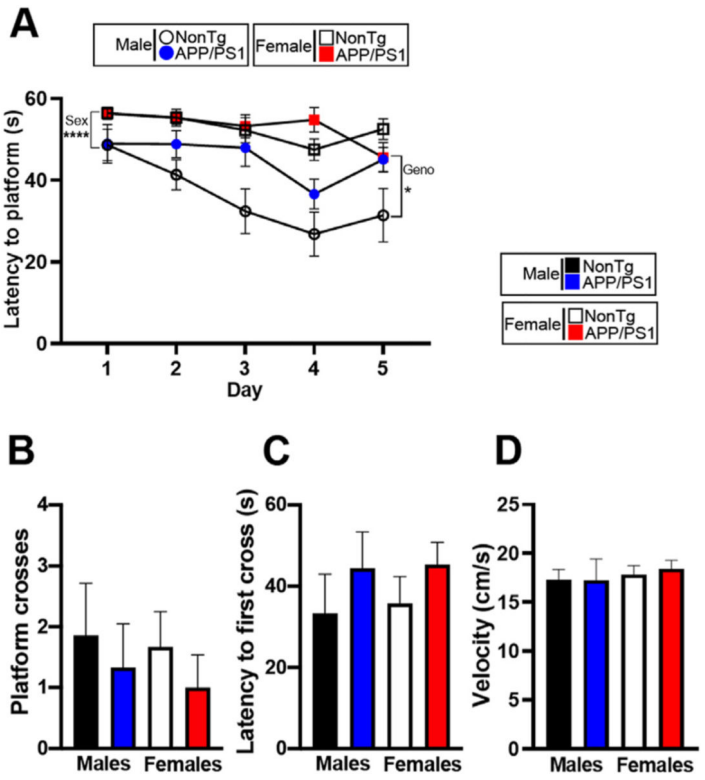

Fig. 6.

Genotype and sex differences identified in the Morris water maze task (MWM). (A) During the five day learning phase of the MWM, we detected a significant main effect of genotype ($p < 0.05$) and sex ($p < 0.0001$) for escape latency to the platform, where APP/PS1 mice took significantly longer to find the platform than NonTg mice, and males found the platform faster than females. (B, C) During the day 6 probe trial, we found no significant differences for number of platform crosses and latency to first cross the platform location. (D) No differences in swim speed were detected among the four groups. Data are presented as means ± SEM. * $p < 0.05$, **** $p < 0.0001$.

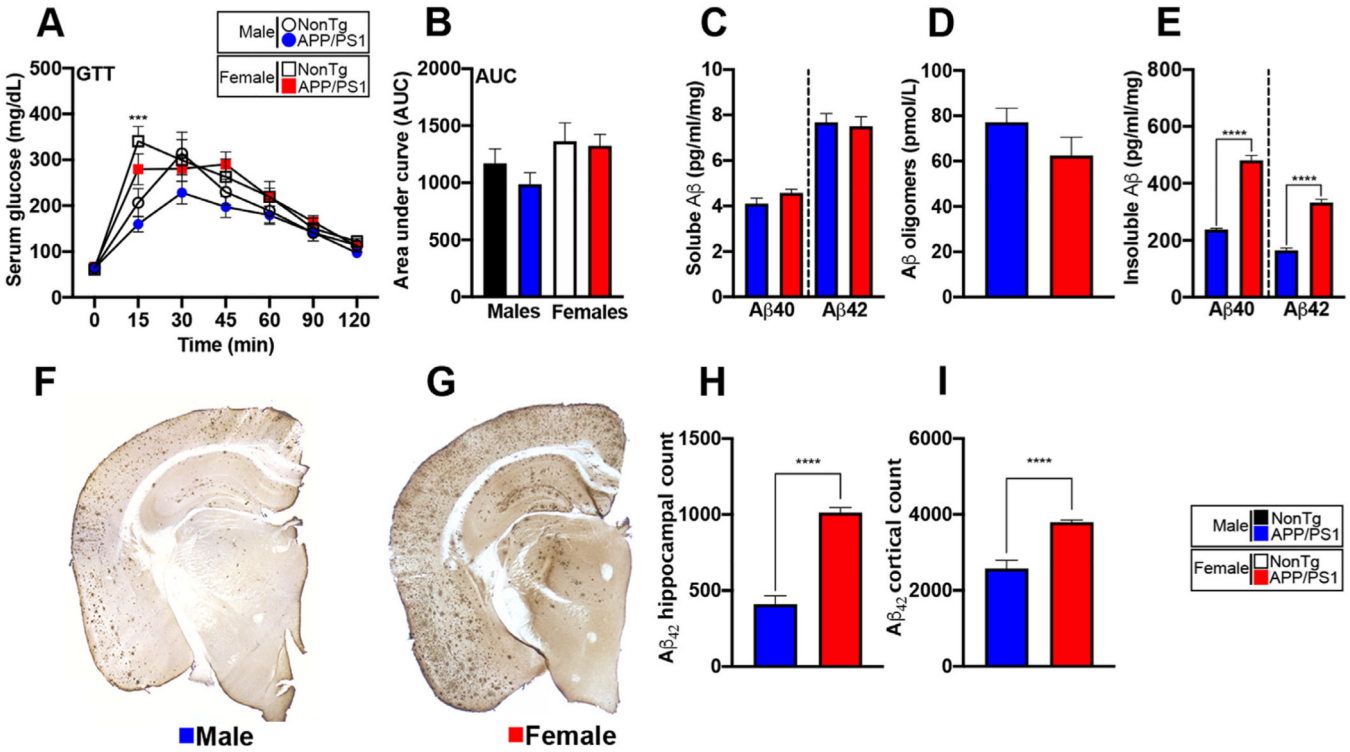

Fig. 7.

Female mice show higher glucose levels during the GTT and APP/PS1 females have a higher Aβ_{42} plaque burden in the hippocampus and cortex than male counterparts. (A) We performed a glucose tolerance test (GTT) in all mice. We found a significant sex by GTT time interaction ($p < 0.0001$). *Post hoc* analysis revealed that female mice had a higher level of glucose when compared to the male mice (310.07 ± 32.87 mg/dL for female and 183.13 ±23.70 mg/dL for male mice) at the 15-minute timepoint after the bolus glucose injection ($p < 0.001$). (B) There were no significant differences in area under the curve (AUC) for GTT between the groups. (C) We found no significant sex differences in APP/PS1 mice for both soluble Aβ_{40} and Aβ_{42} levels. (D) We found no significant sex differences in APP/PS1 mice for Aβ oligomers. (E) The insoluble levels of both Aβ_{40} ($p < 0.0001$) and Aβ$_{42}$ ($p < 0.0001$) were significantly higher in female APP/PS1 mice compared to male counterparts. (F, G) Photomicrographs of a coronal section illustrating Aβ_{42} plaques within the hippocampus and cortex of male and female APP/PS1 mice. (H, I) Quantitative analysis reveals that female APP/PS1 mice have a significantly higher number of Aβ$_{42}$ plaques within the hippocampus ($p < 0.0001$) and cortex ($p < 0.0001$) compared to male APP/PS1 mice. Data are presented as means ± SEM. *** $p < 0.001$, **** $p < 0.0001$.