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## Abstract

In order to better understand animal models of Alzheimer's disease, novel phenotyping strategies have been established for transgenic mouse models. In line with this, the current study characterised male APPxPS1 transgenic mice on mixed C57BL/6JxC3H/HeJ background for the first time for social recognition memory, sensorimotor gating, and spatial memory using the cheeseboard test as an alternative to the Morris water maze. Furthermore, locomotion, anxiety, and fear conditioning were evaluated in transgenic and wild type-like animals. APPxPS1 males displayed task-dependent hyperlocomotion and anxiety behaviours and exhibited social recognition memory impairments compared to wild type-like littermates. Spatial learning and memory, fear conditioning, and sensorimotor gating were unaffected in APPxPS1 transgenic mice. In conclusion, this study describes for the first time social recognition memory deficits in male APPxPS1 mice and suggests that spatial learning and memory deficits reported in earlier studies are dependent on the sex and genetic background of the APPxPS1 mouse line used. Furthermore, particular test conditions of anxiety and spatial memory paradigms appear to impact on the behavioural response of this transgenic mouse model for Alzheimer's disease.



## Keywords

characteristics, behavioural, disease, novel, alzheimer, model, mouse, transgenic, e9, ps1, appswe

## Disciplines

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# Novel Behavioural Characteristics of the

# *APP$_{Swe}$/PS1ΔE9* Transgenic Mouse Model of Alzheimer's Disease

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## Abstract

In order to better understand animal models of Alzheimer's disease, novel phenotyping strategies have been established for transgenic mouse models. In line with this, the current study characterised male APPxPS1 transgenic mice on mixed C57BL/6JxC3H/HeJ background for the first time for social recognition memory, sensorimotor gating, and spatial memory using the cheeseboard test as an alternative to the Morris water maze. Furthermore, locomotion, anxiety, and fear conditioning were evaluated in transgenic and wild type-like animals. APPxPS1 males displayed task-dependent hyperlocomotion and anxiety behaviours and exhibited social recognition memory impairments compared to wild type-like littermates. Spatial learning and memory, fear conditioning, and sensorimotor gating were unaffected in APPxPS1 transgenic mice. In conclusion, this study describes for the first time social recognition memory deficits in male APPxPS1 mice and suggests that spatial learning and memory deficits reported in earlier studies are dependent on the sex and genetic background of the APPxPS1 mouse line used. Furthermore, particular test conditions of anxiety and spatial memory paradigms appear to impact on the behavioural response of this transgenic mouse model for Alzheimer's disease.

Keywords: Alzheimer's disease; transgenic $APP_{Swe}$/PS1ΔE9 mice; behaviour; social recognition memory; sensorimotor gating; cheeseboard;

## 1. Introduction

Alzheimer's disease (AD) is the most common form of dementia, predicted to affect 1 in 85 people globally in 2050. Disease progression from mild to severe stages encompasses impaired learning and communication, spatial disorientation, and memory loss. Two major post-mortem histological diagnostic features describe AD: 1) cleavage of the amyloid precursor protein (APP) produces amyloid beta (Aβ) depositions, which form senile plaques, and 2) hyper-phosphorylation of tau protein causes intracellular neurofibrillary tangles [1-2]. Importantly, elevated levels of Aβ in post-mortem brain tissue correlated with AD-typical memory decline in patients diagnosed with dementia [3]. Familial AD (FAD) is the hereditary form of AD (early onset, autosomal dominant) and accounts for <10% of AD cases (the remaining are classified as sporadic forms of AD) [1]. A number of mutations in genes encoding the amyloid precursor protein (*APP*), and presenilins, a family of enzymes responsible for the processing of APP, have been identified for FAD. Presenilin 1 and 2 (*PSEN1*, *PSEN2*) are responsible for the activity of γ-secretase, one of the enzymes responsible for the cleavage of APP into Aβ isoforms [1-2].

Murine models are most commonly used to investigate the pathology of AD. The mice used in this study were generated by the co-injection of a chimeric human/murine *APP* construct bearing the Swedish double mutation ($APP_{Swe}$) and the exon-9-deleted *PSEN1* mutation (*PSEN1/*Δ*E9*) [4-5]. $APP_{Swe}/PS1\Delta E9$ (APPxPS1) double transgenic mice exhibit increased levels of Aβ at 4 months of age and develop accelerated plaque pathology, which is correlated with age [4-6]. Furthermore, impairments in cholinergic and muscarinic transmission develop alongside Aβ accumulation in the brain of APPxPS1 mice at 5-7 months of age, reminiscent of AD pathology [7-8].

Various behavioural and cognitive deficits have been documented for this transgenic AD mouse model. Most notable are spatial memory impairments in the Barnes maze and Morris

water maze (MWM), with the earliest deficits appearing at 7 and 8 months respectively [9-10]. These cognitive deficits were more pronounced with age and correlated with increasing plaque deposition [11-13], which is sex-specific [6]. Other behavioural characteristics reported for APPxPS1 mice include decreased anxiety and increased locomotor activity [14]. However, some of the reported behavioural characteristics were inconsistent across laboratories. For example, Reiserer and colleagues could not replicate the anxiolytic phenotype reported earlier [10, 14]. More importantly, spatial memory deficits in the reversal task of the MWM were detected in 9-10-month-old mice [15] whereas another study reported no deficits in the reversal task in 12-month-old mice [16]. Furthermore, some studies combined both male and female mice within one test cohort [10, 14, 17], even though other studies revealed sex-specific differences in APPxPS1 mice [6, 18].

In order to better understand animal models of AD, recent phenotyping studies in transgenic mouse models of AD have considered alternative spatial memory paradigms (i.e. cheeseboard; [19]) and also evaluated transgenic mice in novel behavioural domains such as social recognition memory [20] and sensorimotor gating [21]. In the present study we tested the APPxPS1 transgenic mouse model in these novel paradigms to determine the behavioural phenotype of this mouse model in more detail.

## 2. Materials and methods

2.1 Animals

Double transgenic mice expressing chimeric mouse/human APP (Mo/HuAPP695swe / Swedish mutations K595N/M596L) and mutant human PSEN1 (PS1/ΔE9) mice were obtained from Jackson Laboratory [Bar Harbor, USA; strain name: B6C3-Tg(APPswe,PSEN1dE9)85Dbo/Mmjax; stock no. 004462] and maintained as hemizygotes on the congenic C57BL/6JxC3H/HeJ background as described previously [4-5, 22-23]. Male double transgenic mice (APPxPS1: $n$ = 12) and their non-transgenic littermates (WT: $n$ = 17) were bred and group-housed in independently ventilated cages (Airlaw, Smithfield, Australia) at Animal BioResources (Moss Vale, Australia). Test mice were transported to Neuroscience Research Australia (NeuRA) at around 10 weeks of age, where they were group-housed in Polysulfone cages (1144B: Techniplast, Rydalmere, Australia) equipped with some tissues for nesting. Mice were kept under a 12: 12 h light: dark schedule [light phase: white light (illumination: 124 lx) – dark phase: red light (illumination: < 2 lx)]. Food and water were provided *ad libitum*, except where specified. Adult, male A/J mice from Animal Resources Centre (Canning Vale, Australia) were placed in the animal enclosures of the social preference test.

Research and animal care procedures were approved by the University of New South Wales Animal Care and Ethics Committee in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

**2.2 Behavioural Phenotyping**

Starting at 7 months of age, mice were tested in a battery of behavioural tests (for test order see Table 1; for test details see below) with an inter-test interval of at least 48h. All tests were

conducted during the first 5 h of the light phase to minimise effects of the circadian rhythm on the performance of test mice.

*2.2.1 Light-dark test (LD):* In the LD, the distance travelled and time spent in a brightly illuminated, aversive test arena compared to a dark area are indicators of anxiety in rodents [24-25]. The apparatus (for details see [26]) was an infrared photobeam-controlled open-field activity test chamber (MED Associates Inc., St Albans, USA) containing a dark box insert that covered half the chamber and was opaque to visible light. Mice were placed at the opening (faced towards the dark compartment) at the start of the experiment. The time spent as well as the distance travelled in the two chambers was recorded for 10 min.

*2.2.2 Elevated plus maze (EPM):* The EPM assesses the natural conflict between the tendency of mice to explore a novel environment and avoidance of a brightly lit, elevated and open area [27-28]. The grey plus maze was "+" shaped (for details of apparatus see [29]). Mice were placed at the centre of the + (faced towards an enclosed arm) and were allowed to explore the maze for 5 min. The time spent and distance travelled in the open and enclosed arms were recorded using AnyMaze$^{TM}$ (Stoelting, Wood Dale, USA) tracking software.

*2.2.3 Social preference test (SPT):* The SPT was used to assess sociability and social novelty preference (i.e. social recognition memory) in test mice [30-31]. The apparatus consisted of 3 chambers, a central chamber (length: 9 cm, width: 18 cm, depth: 20 cm) and two outer chambers (6 cm x 18 cm x 20 cm). The dividing walls were made of clear Plexiglas, with square passages, 4 cm high and 4 cm wide. One circular cage (i.e. mouse enclosure) was placed into each outer chamber. The mouse enclosures were 15 cm in height with a diameter of 7 cm and bars spaced 0.5 cm apart to allow nose contact between mice (i.e. test mouse and A/J mouse) but prevent fighting. The chambers and enclosures were cleaned with 30% ethanol in-between trials (inter-trial interval of 5 min) and and fresh corn cob bedding was added prior to each test trial.

Test animals were isolated for an hour prior to the start of testing. During the habituation trial, WT and APPxPS1 mice were placed individually in the central chamber and allowed to freely explore the apparatus and the two empty enclosures for 5 min. For the sociability test an unfamiliar adult male A/J mouse was placed in one of the two enclosures (i.e. opponent chamber) in a quasi-randomised fashion. Then the test mouse was returned to the apparatus and allowed to explore all three chambers for 10 min. Finally, test animals were observed in a 10 min social recognition test. For this, a second, unfamiliar A/J mouse was placed in the previously empty chamber so that the test mouse had the choice to explore either the familiar A/J mouse (from the previous trial) or the novel, unfamiliar mouse. AnyMaze$^{TM}$ tracking software was used to determine the time spent in the different chambers, number of entries and distance travelled by the test mice in each trial. Time spent *sniffing* the opponent (i.e. A/J mouse) was recorded manually (i.e. snout of test mouse within the enclosure containing the opponent mouse or < 5 mm away from enclosure).

*2.2.4 Fear Conditioning:* Fear conditioning assesses associative learning whereby a previously neutral stimulus elicits a fear response after it has been paired with an aversive stimulus. On conditioning day, mice were placed into the test chamber (Model H10-11R-TC, Coulbourn Instruments, USA) for 2 min. Then an 80 dB conditioned stimulus (CS) was presented for 30 seconds with a co-terminating 0.4 mA 2 second foot shock (unconditioned stimulus; US) twice with an inter-pairing interval of 2 min). The test concluded 2 min later. The next day (context test), mice were returned to the apparatus for 7 min. On day 3 (cue test), animals were placed in an altered context for 9 min. After 2 min (pre-CS/baseline), the CS was presented continuously for 5 min. The test concluded after another 2 min with the absence of the CS. Time spent *freezing* was measured using Any-Maze$^{TM}$ software [32-33]. To avoid any influence of foot shock exposure on further testing, a inter-test interval of

several months was chosen and all following tests were carried out in other tests rooms than the fear conditioning test.

*2.2.5 Cheeseboard (CB):* The CB was used as a less stressful dry-land alternative of the MWM [19]. Mice at 10-11 months of age were trained to find a food reward on a wooden board over a number of days (for specifics of test apparatus see [34]). A total of 32 bottle caps were evenly distributed across the CB and external cues were located around the board. One cap contained a food reward (100 µl sweetened condensed milk; diluted 1:4 with water) although all caps were brushed lightly with diluted sweetened condensed milk to eliminate the chance that mice use odour cues to find the target cap. For this, all mice were food-deprived and kept at 85-90% of their pre-test body weight throughout testing (mice were fed for 1-2 h per day). A camera was mounted above the CB to measure latency to find the reward and time spent in the different CB zones (i.e. board was separated into 8 equal zones) using Any-Maze$^{TM}$ software.

During habituation, (3 days to the blank side on the inverted platform of the CB) three 2 min trials were conducted each day for three days with a 10 min intertrial interval (ITI). For spatial reference memory acquisition, mice were trained over 9 days (three trials per day with a 10 min ITI) to locate the food reward. The location of the target well was kept constant for each mouse between trials and across days but quasi-randomised and counterbalanced across genotypes. If the target well was not located within 2 min, mice were placed next to the target well and allowed to consume the food reward. To test for spatial reference memory, a probe trial was conducted on day 10, where no wells were baited and mice were given 2 min to explore the board freely. The time the mice spent in the different zones of the CB (i.e. % exploration time) was recorded (as previously described [32]).

To test reversal learning (start of training 24h post probe trial), the location of the food reward was moved to the opposite side of the CB. Mice completed 4 days of reversal training (three trials per day with a 10 min ITI) before a reversal probe trial was carried out.

*2.2.6 Sensorimotor gating (i.e prepulse inhibition: PPI):* PPI was used to test for sensorimotor gating deficits as it has been demonstrated by others that sensorimotor gating can be impaired in AD mouse models and can be directly correlated with amyloid burden [21, 35]. Test mice were placed in Plexiglas mouse enclosures of the startle chambers (SR-Lab, San Diego Instruments, San Diego, USA) and allowed to habituate to the enclosure and test apparatus for 5 min over 3 consecutive days prior to PPI testing with a consistent background noise of 70 dB. The 30 min PPI test session consisted of a 5 min acclimation period to 70 dB background noise, followed by 97 trials presented in a pseudorandom order: 5 x 70 dB trials; 5 x 100 dB trials; 15 x 120 dB trials to measure the acoustic startle response (ASR) and 15 sets of 5 trials comprising of a prepulse of either 74, 82 or 86 dB presented 32, 64, 128, or 256 ms (variable interstimulus interval; ISI) prior to a startle pulse of 120 dB to measure the PPI response. The intertrial interval (ITI) varied randomly from 10 – 20 seconds. Responses to each trial were calculated as the average mean amplitude detected by the accelerometer [36-37]. ASR was calculated as the mean amplitude to all startle trials and percentage PPI (%PPI) was calculated as [(mean startle response (120 dB) – PPI response)/mean startle response (120 dB)] x 100. %PPI was averaged across ISIs to produce a mean %PPI for each prepulse intensity. For ASR habituation, blocks of ASR to 120 dB were averaged at the beginning, middle and end of the PPI protocol (5 trials per block).

*2.2.7 Olfactory test (i.e. cookie test):* Olfactory abilities play a crucial role in social interaction between mice [38]. A simple test [24, 30] was performed to assess the gross olfactory abilities of male WT and APPxPS1 transgenic mice. Test mice were familiarised with a high carbohydrate food (Froot Loops: Kellogg Pty. Ltd., Strawberry Hills, Australia)

in their home cages, 24 h prior to the test. Consumption was observed by the experimenter to ensure the novel food was palatable for the mice. On the test day, test mice were habituated for 5 min to a large opaque cage (47 x 18 x 13 cm) containing 2 cm deep bedding. The animal was removed from the cage thereafter, and one Froot Loop was buried randomly in the cage bedding. The animal was then returned to the cage and given 10 min to locate the buried food. The latency to find the Froot Loop was recorded as a measure of olfactory abilities.

### 2.3 Statistical Analysis

Analysis of the behavioural parameters was performed using one-way analysis of variance (ANOVA) to investigate main effects of 'genotype' or repeated measures (RM) ANOVAs for effects of 'chamber' (SPT), 'time' (CB) '1 min block' (FC), 'startle block' and 'prepulse intensity' (both PPI) as published previously [34]. Performance in the CB probe trials and social preference test were also assessed using one sample t-tests to investigate whether the percentage of time spent in the target zone or novel chamber were greater than chance (12.5% and 50% respectively). Differences were regarded as significant if $p < .05$. F-values and degrees of freedom are presented for ANOVAs and significant genotype effects (ANOVA) are shown in figures and tables as '*' ($^{*}p < .05$, $^{**}p < .01$, and $^{***}p < .001$). RM ANOVA effects for chamber are presented by '#' ($^{\#}p < .05$, ($^{\#}p < .01$ and $^{\#\#\#}p < .001$). Data are shown as means ± standard error of means (SEM). Analyses were conducted using SPSS 20.0 for Windows.

## 3. Results

### 3.1 Anxiety

One-way ANOVA for total distance travelled in the LD revealed an effect of 'genotype' [$F(1,29) = 11.7$, $p < .01$; Table 2], suggesting that APPxPS1 transgenic mice exhibit a hyperlocomotor phenotype. Importantly, no effects of APPxPS1 were detected on the anxiety-related parameters time in light chamber [$F(1,29) = 0.001$, $p = .9$] and percentage distance travelled in the same zone [$F(1,29) = 0.07$, $p = .8$] (Table 2). Nevertheless, APPxPS1 mice demonstrated increased levels of anxiety in the EPM. Both, the percentage of time spent in the open arms [$F(1,28) = 4.6$, $p < .05$] as well as the percentage open arm entries [$F(1,28) = 4.5$, $p < .05$] were significantly lower in transgenic mice when compared with their WT counterparts (Table 2). Furthermore, there was a strong trend for an increase in total time spent on the open arms [$F(1,28) = 4.0$, $p = .05$] (Table 2). No other significant differences were found for any of the parameters investigated [$p > .05$ for all parameters], including the total time spent in enclosed arms and the total distance travelled in enclosed arms (data not shown).

### 3.2 Cognition

*3.2.1 Social Preference Test:* All mice demonstrated sociability in the 3-chamber social preference test. RM ANOVA detected a significant effect of test chamber for all mice for total time spent in chamber [$F(1,29) = 50.1$, $p < .001$; 'genotype' x 'chamber' interaction: $F(1,29) = 4.4$, $p < .05$] (Fig. 1A). One-way ANOVA for total time spent in opponent chamber revealed that transgenic mice spent a less time in the mouse chamber than WT control mice [$F(1,29) = 4.7$, $p < .05$]. However, one sample t-test confirmed that both WT and transgenic mice developed a preference for the opponent chamber (containing a stranger/unfamiliar mouse) [WT: $t(11) = 10.8$, $p < .001$; APPxPS1: $t(18) = 3.4$, $p < .01$].

In the social recognition test, RM ANOVA revealed a significant effect of 'chamber' for all mice for total time spent in test chambers [$F(1,29) = 4.4$, $p < .05$] (Fig. 1B) and time spent *sniffing* [$F(1,29) = 7.8$, $p < .01$] (Fig. 1C). Importantly, only WT mice demonstrated a preference for the chamber containing the novel mouse [time spent in chamber: $F(1,11) = 5.9$, $p < .05$ – time spent *sniffing*: $F(1,11) = 8.9$, $p = .01$] while transgenic mice spent an equal amount of time with the familiar and the novel mouse [time spent in chamber: $F(1,18) = 0.1$, $p = .7$ – time spent *sniffing*: $F(1,18) = 0.9$, $p = .4$]. T-tests for percentage time spent with novel mouse and percentage time *sniffing* the novel mouse confirmed that WT [$t(11) = 2.2$, $p < .05$ – $t(11) = 3.6$, $p < .01$] but not APPxPS1 transgenic mice [$t(18) = .3$, $p = .7$ – $t(18) = 1.0$, $p = .3$] developed a clear preference for the chamber containing the novel mouse (data not shown). One-way ANOVA revealed no significant genotype differences for percentage of time spent in the novel chamber [$F(1,29) = 2.3$, $p = .1$] and percentage of time *sniffing* the novel opponent [$F(1,29) = 2.2$, $p = .2$]. Locomotion of WT and APPxPS1 mice was identical in both the familiar [$F(1,29) = 1.3$, $p = 0.3$] and the novel chamber [$F(1,29) = 0$, $p = 1.0$] (Table 2).

*3.2.3 Fear Conditioning:* All mice responded to the electric foot shocks delivered during the conditioning phase (i.e. vocalisation). Furthermore, the baseline *freezing* prior to conditioning was similar across genotypes [$F(1,29) = .3$, $p = .5$; Table 3]. Contextual fear conditioning (i.e. total time spent *freezing* during context test) of APPxPS1 mice was WT-like [$F(1,29) = 2.9$, $p = .1$]. In the cue test, all mice demonstrated the ability to associate the CS with the US as evidenced by a significant increase in *freezing* behaviour in response to the presentation of the cue [RM ANOVA for '1 min block': $F(1,29) = 9.3$, $p < .01$ - no '1 min block' by 'genotype' interactions; Table 3].

*3.2.4 Cheeseboard:* Mice of both genotypes showed normal task acquisition as indicated by RM ANOVA for 'time' [$F(8,208) = 38.8$, $p < .001$ – no interaction with 'genotype'; Fig. 2A],

demonstrating a significant decrease in latency to find and consume the reward across days. In the probe trial, all mice demonstrated a preference for the target zone [WT: $t(11) = 3.1$, $p < .01$; APPxPS1: $t(15) = 3.7$, $p < .01$], as they spent significantly more time than chance (i.e. 12.5%) in the target zone, indicating successful recall of the reward location (Fig. 2B). Furthermore, one-way ANOVA for percentage time in target zone revealed the performance of transgenic mice did not differ significantly from that of WT mice [$F(1,26) = .3$, $p = .6$].

During reversal learning, all test animals adapted to the change in reward location and exhibited decreased latencies to find the food reward over days [RM ANOVA for 'time': $F(3,78) = 4.7$, $p < .001$ – no interaction with 'genotype'; Fig. 2C]. Finally, all mice developed a preference for the new target zone in the reversal probe trial as they spent significantly more time than chance in the designated zone [WT: $t(11) = 3.0$, $p < .05$; APPxPS1: $t(15) = 2.9$, $p < .05$; Fig. 2D]. Transgenic mice did not differ significantly in their preference to explore the target zone [$F(1,26) = .2$, $p = .6$].

### 3.3 Sensorimotor gating

*3.3.1 Acoustic startle response (ASR) and ASR habituation:* RM ANOVA revealed a significant effect of 'pulse intensity' [$F(2,50) = 25.3$, $p < .001$] on the ASR of all mice with 120 dB pulses generating the highest startle responses (Fig 3A). A trend was found for the effect of 'genotype' [$F(1,25) = 3.9$, $p = .06$], suggesting that ASR was generally higher in transgenic APPxPS1 mice compared to WT mice. However, one-way ANOVAs for the different startle pulses revealed no significant differences between WT and APPxPS1 mice [$p > .05$ for all startle pulses].

Statistical analysis suggested that mice did not habituate significantly to the 120 dB pulse [RM ANOVA for 'startle block': $F(2,50) = 2.6$, $p = .08$], although this appeared to be due to a failure of APPxPS1 rather than WT mice to habituate to a 120 dB startle stimulus [trend for

'startle block' x 'genotype' interaction: $F(2,50) = 2.7$, $p = .08$] (Fig. 3B). Indeed, when data were split by 'genotype', it was found that WT mice demonstrated significant habituation towards the 120 dB pulse [WT: $F(2,20) = 6.8$, $p < .01$], while transgenic mice exhibited no reduction in ASR across trials [APPxPS1: $F(2,30) = .002$, $p = 1.0$] (Fig. 3B].

*3.3.2 Prepulse inhibition:* Prepulse intensities had a significant effect on %PPI as increasing prepulse intensities resulted in more pronounced prepulse inhibition [RM ANOVA: $F(2,50) = 28.4$, $p < .001$] (Fig. 3C). Importantly, sensorimotor gating was not altered in transgenic mice as no effects of 'genotype' were found at any prepulse intensity [$p > .05$ for all parameters investigated; Fig. 3C].

### 3.4 Olfaction (Cookie test)

All mice found and consumed the buried food reward within the allotted time as measured in seconds (WT: 300.6 ± 61.6 - APPxPS1: 227.8 ± 46.4). The performance of transgenic mice in the olfactory test was comparable to WT mice [latency to find buried food: $F(1,27) = .9$, $p = .3$], suggesting WT-like olfactory abilities of transgenic AD mice.

## 4. Discussion

This is the first report that APPxPS1 males develop social recognition memory impairments. Furthermore, transgenic males displayed task-dependent hyperlocomotion and anxiety behaviours. Spatial learning and memory in the CB paradigm as well as sensorimotor gating and fear conditioning were all unaffected in 10-month-old APPxPS1 mice.

Agitation and increased motor activity (restlessness) is one characteristic of AD patients [39]. Measuring the locomotor activity of APPxPS1 mice revealed that transgenic animals developed a hyperlocomotive phenotype in the LD test at the age of 7 months. This finding is in line with a study testing 8-months old APPxPS1 male in the open field [11] although other studies reported wild type-like locomotion of APPxPS1 [10, 14, 40]. Importantly, a detailed comparison of all these studies suggests that the characteristics of the particular APPxPS1 mouse model tested (i.e. number of backcrosses onto C57BL/6J background), the sex of test animals and the methodology used to analyse locomotion (e.g. test duration and the level of stress caused by test apparatus) may account for inconsistent behavioural responses across studies. Methodological differences might also explain why hyperlocomotion of APPxPS1 males of the current study was detected in the LD test but not the EPM.

Male APPxPS1 mice displayed wild type-like anxiety levels in the LD test, which is consistent with earlier reports [10]. However, transgenic males were more anxious in the EPM compared to control animals. This task-specific anxiety phenotype may be related to the human clinical setting as there are AD patients who experience symptoms of anxiety [41]. In contrast, Lalonde and co-workers detected decreased anxiety levels in APPxPS1 mice (males and females were tested together) and interpreted this phenotype as a loss of behavioural inhibition, akin to dis-inhibitory tendencies observed in AD patients [14].

While control mice exhibited a clear preference for the novel opponent as expected [30], APPxPS1 males did not differentiate between the novel and familiar opponent mouse

suggesting deficits in social recognition memory (as measured by time spent in chamber and time spent *sniffing* opponent). This effect was not confounded by the hyperactive phenotype of APPxPS1 mice observed in the light-dark test as locomotion was identical in both chambers across genotypes. All test mice were also characterised in the cookie test, as the test performance is dependent on olfactory abilities and as AD patients and some mouse models of AD exhibit impaired olfaction [42-43]. All test animals showed normal olfactory abilities in the cookie test. In addition, Rey and colleagues showed that the olfactory discrimination under baseline conditions (using a 5 min delay between first and second exposure to novel/familiar odours) was identical for control and APPxPS1 mice [44]. Interestingly, transgenic mice of that study exhibited impaired odour retention with a 15 min delay. However, as the inter-trial interval of the social preference test in our study was 5 min, it is unlikely that the social recognition memory deficit of APPxPS1 mice was influenced by a reduced ability of transgenic mice to recall odours they had encountered earlier. Furthermore, control and APPxPS1 males displayed normal sociability (i.e. preference to investigate a mouse over an empty chamber) although this preference was more pronounced in WT mice. Importantly, the task-dependent anxiety phenotype of transgenic mice in the elevated plus maze (but not the light-dark test) did not impact on the natural drive of mice to explore another mouse. Both WT and APPxPS1 mice exhibited a clear preference to investigate the social stimulus presented during the sociability test. However, the intact but compared to control mice reduced levels of social interaction/investigation of another mouse observed in APPxPS1 mice may be influenced by the anxiety phenotype detected in the elevated plus maze.

In support of an impaired social recognition memory in AD mice is a recent study reporting impaired social recognition in the Thy1-hAPP(Lond/Swe+) transgenic mouse model [20]. In this context, it is interesting to note that AD patients have difficulties to recognise familiar

faces [45]. Brain regions responsible for recognition memory are the perirhinal cortex and hippocampus [46], both regions are compromised in AD patients [47]. Furthermore, the amygdala, which is associated with social behaviours, undergoes atrophy in AD patients [48]. Thus, impairments in social recognition memory may be caused by pathological changes in these brain regions in APPxPS1 mice. Further research will have to address potential histological differences in these regions between WT and APPxPS1 mice.

The deficit in recognition memory was specific as fear conditioning (i.e. associative learning) was intact in 7-month-old transgenic mice, which is similar to what had been reported in 4-month-old APPxPS1 females [49]. Furthermore, task acquisition and retention of spatial memory of APPxPS1 males were not impaired in the hidden version of the CB paradigm. APPxPS1 mice have been described to develop spatial learning and memory deficits, which are most often evaluated in the MWM. In females, deficits in spatial learning were evident in 9-10-month-old APPxPS1 mice [15, 50] and retention deficits were detected in 12-month-old transgenic animals [51]. However, only one study has investigated male APPxPS1 mice on C57BL/6JxC3H/HeJ background to date. Cao and co-workers reported intact task acquisition but impaired spatial memory retention for 8-month-old transgenic males [9], whereas APPxPS1 males backcrossed to C57BL/6J developed spatial learning and memory deficits at the age of 9-15 months. This suggests an influence of the genetic background of APPxPS1 males on the development of cognitive deficits [52-53]. Importantly, the CB paradigm used in current study is classified as the dry version of the MWM [54] and has been validated as an alternative spatial memory test to detect cognitive impairments in AD transgenic mice [19]. Nonetheless, comparing results between MWM and CB testing requires caution as the MWM can impact severely on the stress response of mice (for this and other issues relevant to MWM testing of mice see [54-58]). Thus, the anxiety phenotype of male APPxPS1 mice may explain the differences between the cognitive performance of transgenic animals in the

MWM [9] and the CB of the current study. Two previous studies found deficits in spatial learning and memory of APPxPS1 males using the CB paradigm. However, one study used WT and transgenic mice at the age of 24 months [59] and both studies detected cognitive impairments in the cued (but not the hidden) version of the CB only [21, 59].

A meta-analysis has found social withdrawal is among the first symptoms displayed by AD patients, occurring up to 33 months on average prior to the diagnosis of AD [39]. In line with this, 10-month-old APPxPS1 males appear to demonstrate social recognition memory impairments in the absence of any other cognitive deficits. Thus, testing APPxPS1 males on a mixed background, which are significantly older than the cohort tested in the current study, might result in spatial memory deficits in the hidden versions of both CB and MWM.

Studies have identified suppression of the P50 event-related potential of sensorimotor gating in AD patients [60]. The present study found that sensorimotor gating as measured by prepulse inhibition was unaltered in 10-month-old APPxPS1 males. This is supported by a previous study that found no PPI deficits in a mixed cohort of 12-month-old male and female mice of another APPxPS1 line [61]. However, a more recent study showed that female APPxPS1 of the same line developed sensorimotor gating deficits at the age of 7 months [35]. However, sex and PPI protocol-specific effects are likely [6, 37, 62-63].

In conclusion, this investigation describes for the first time social recognition memory deficits in male APPxPS1 mice. Furthermore, this deficit manifests at least 3 months prior to any evidence of other cognitive deficits such as spatial learning and memory impairments. The deficits in social recognition could be linked to possible impairments of the prefrontal cortex and hippocampus caused either by the deposition of Aβ or other underlying pathological symptoms. The observed anxiety phenotype and the absence of any spatial deficits in 10-month-old male APPxPS1 mice on a mixed background emphasize the

necessity to consider sex and genetic background effects in AD mouse models and to pay attention to details of the cognitive paradigms undertaken.

## 5. Figure Legends

**Fig. 1A-B: Sociability (A) and social recognition memory (B and C) in the social preference test: A)** Total time spent in test chambers containing either an unfamiliar mouse (i.e. opponent) or an empty mouse enclosure (i.e. empty) [s]; **B)** Time spent in a test chamber containing either a familiar or an unfamiliar (i.e. novel) mouse [s]. **C)** Time spent *sniffing* a familiar or an unfamiliar (i.e. novel) opponent (i.e. A/J mouse) [s]. Data for non-transgenic control (WT) and double transgenic $APP_{Swe}$/PS1ΔE9 (APPxPS1) males are shown as means + SEM. Significant genotype effects of ANOVA are indicated with '*' ($^{*}p < .05$) whereas RM ANOVA for chamber effects are presented by '#' ($^{\#}p < .05$, ($^{\#}p < .01$ and $^{\#\#\#}p < .001$).

**Fig. 2A-D: Spatial learning and memory in the cheeseboard (CB): A)** Latency [s] to find the food reward (averaged across 3 daily trails) during training; **B)** Percentage time [%] spent in the target zone of the CB (i.e. in close proximity to the reward well) during the 2 min probe trial; **C)** Latency [s] to find the food reward (averaged across 3 daily trials) during reversal training; **D)** Percentage time [%] spent in the target zone of the CB during the 2 min reversal probe trial. Data for non-transgenic control (WT) and double transgenic $APP_{Swe}$/PS1ΔE9 (APPxPS1) males are shown as means + SEM.

**Fig. 3A-C: Sensorimotor gating: A)** Acoustic startle response (ASR: startle amplitude in arbitrary units) to different startle pulses (i.e. 70 dB = background noise, 100 dB, 120 dB); **B)** Habituation of the ASR to a 120 dB startle pulse over blocks of trials; **C)** Percentage prepulse inhibition (%PPI) averaged over trials for different prepulse intensities (72/74/78 dB). Data for non-transgenic control (WT) and double transgenic $APP_{Swe}$/PS1ΔE9 (APPxPS1) males are shown as means + SEM. Significant genotype effects (ANOVA) are indicated by '*' ($^{*}p < .05$).

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| Test age [d] | Behavioural paradigm |
|---|---|
| 194 ± 12 | Light-dark test (LD) |
| 196 ± 12 | Elevated plus maze (EPM) |
| 207 ± 12 | Social preference test (SPT) |
| 214 ± 12 | Contextual and cued fear conditioning (FC) |
| 308 ± 6 | Cheeseboard (CB) |
| 321 ± 6 | Reversal cheeseboard (rCB) |
| 340 ± 12 | Sensorimotor gating (Prepulse inhibition: PPI) |
| 379 ± 12 | Olfaction (Cookie test) |

**Table 1:** Test age [d] and test biography of non-transgenic control (WT) and double transgenic $APP_{Swe}$/PS1ΔE9 (APPxPS1) male mice are shown.

| | WT | APPxPS1 |
|---|---|---|
| **LD** | | |
| Total distance travelled [cm] | 1315.3 ± 114.5 | 2256.0 ± 204.8 ** |
| Distance travelled in the light chamber [%] | 35.0 ± 4.4 | 33.8 ± 1.9 |
| Time spent in the light chamber [%] | 33.6 ± 4.3 | 33.7 ± 2.1 |
| Time spent in the light chamber [s] | 191.9 ± 24.3 | 194.6 ± 12.3 |
| **EPM** | | |
| Time spent on open arms [%] | 21.3 ± 5.6 | 16.2 ± 3.4 * |
| Time spent on open arms [s] | 29.9 ± 8.7 | 13.0 ± 3.8 $^{+}$ |
| Entries into open arms [%] | 28.0 ± 4.6 | 16.2 ± 3.4 * |
| **SPT** | | |
| Familiar mouse chamber Total distance travelled [cm] | 321.4 ± 29.5 | 397.9 ± 49.1 |
| Novel mouse chamber Total distance travelled [cm] | 416.7 ± 54.5 | 416.2 ± 43.1 |

**Table 2:** Locomotion (total distance travelled) and anxiety behaviours (percentage locomotion and time spent in aversive zones) in the light-dark test (LD), the elevated plus maze (EPM) and the social preference test (SPT: total distance travelled in social recognition test only) of non-transgenic control (WT) and double transgenic $APP_{Swe}$/PS1ΔE9 (APPxPS1) male mice are shown as mean ± SEM. Significant effects of 'genotype' are indicated with '*' ($^{*}p < .05$ and $^{**}p < .01$) whereas trends of 'genotype' are shown with '+' ($^{+}p = .05$).

| Fear conditioning | WT | APPxPS1 |
|---|---|---|
| **Conditioning** | | |
| Baseline freezing [s] | 9.4 ± 4.5 | 6.9 ± 2.3 |
| **Context** | | |
| Total time spent freezing [s] | 61.2 ± 13.3 | 100.4 ± 16.4 |
| Freezing - first 2 min [s] | 17.2 ± 5.5 | 21.7 ± 5.7 |
| **Cue** | | |
| Time spent freezing 1 min prior to cue onset [s] | 6.7 ± 2.8 | 9.9 ± 2.2 |
| Time spent freezing 1 min post cue onset [s] | 10.2 ± 2.0 | 16.3 ± 3.4 |
| Freezing – first 2 min [s] | 10.2 ± 4.4 | 12.4 ± 3.0 |

**Table 3:** Fear-associated memory: Time spent freezing [s] at baseline (first 2 min of conditioning trial), during the context test, and 1 min prior to and post tone presentation in the cue version of the fear conditioning paradigm is shown for non-transgenic control (WT) and double transgenic $APP_{Swe}$/PS1ΔE9 (APPxPS1) male mice. Data are presented as mean ± SEM.

Figure 1A

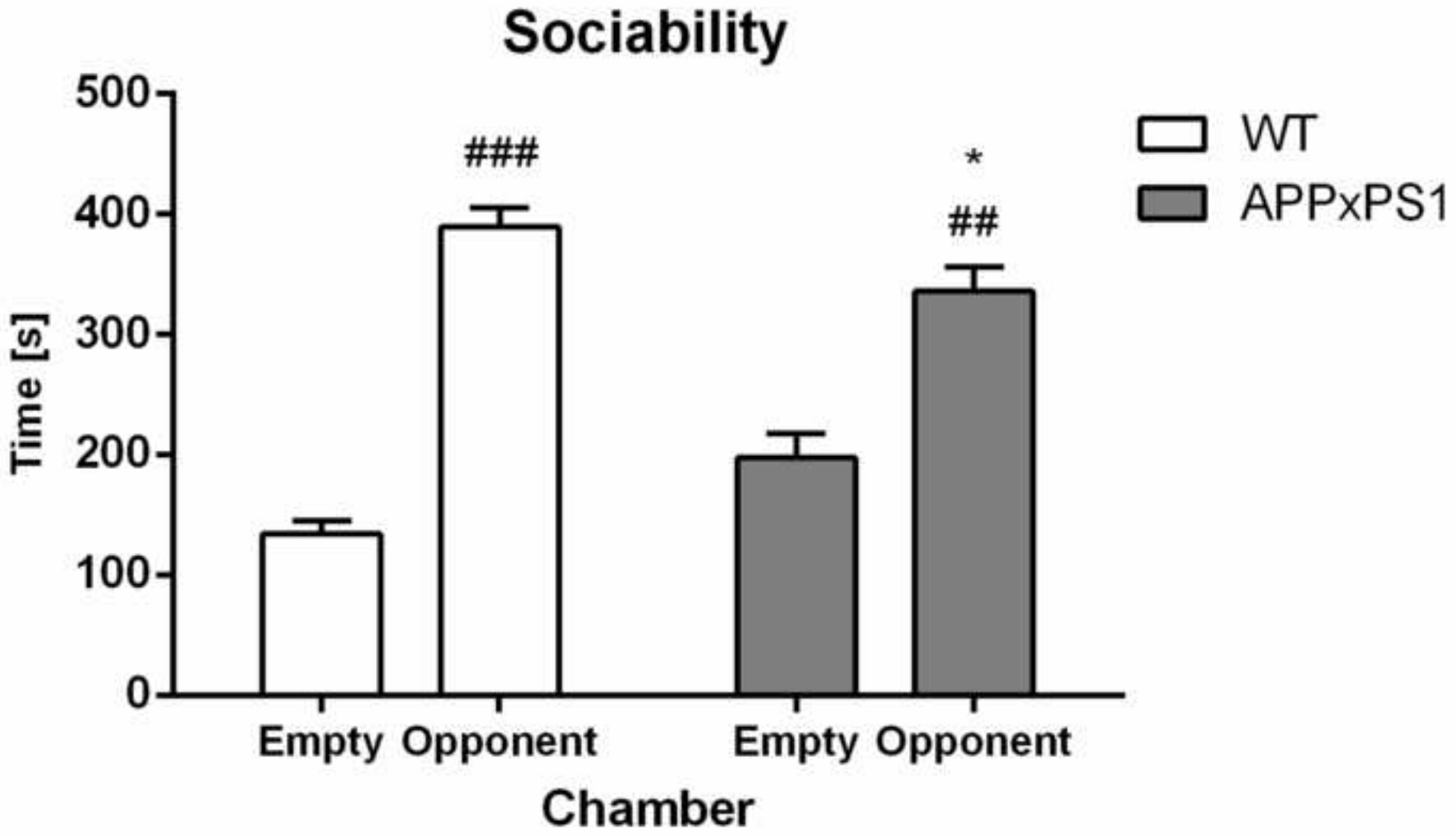

Sociability
500
400
300
200
100
0
Time [s]
###
*
##
WT
APPxPS1
Empty
Opponent
Empty
Opponent
Chamber

Figure 1B

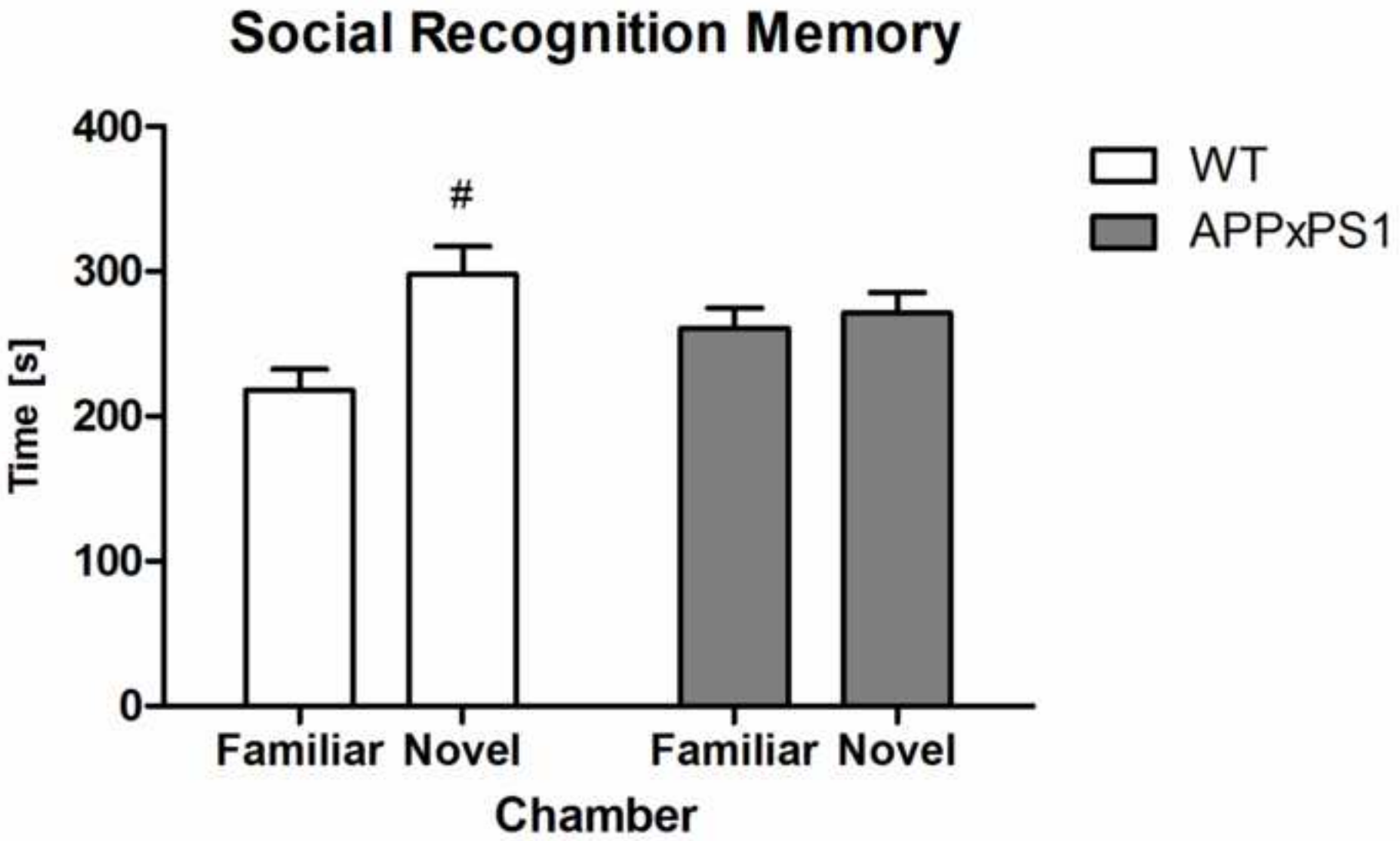

Social Recognition Memory
400
300
200
100
0
Time [s]
#
WT
APPxPS1
Familiar
Novel
Familiar
Novel
Chamber

Figure 1C

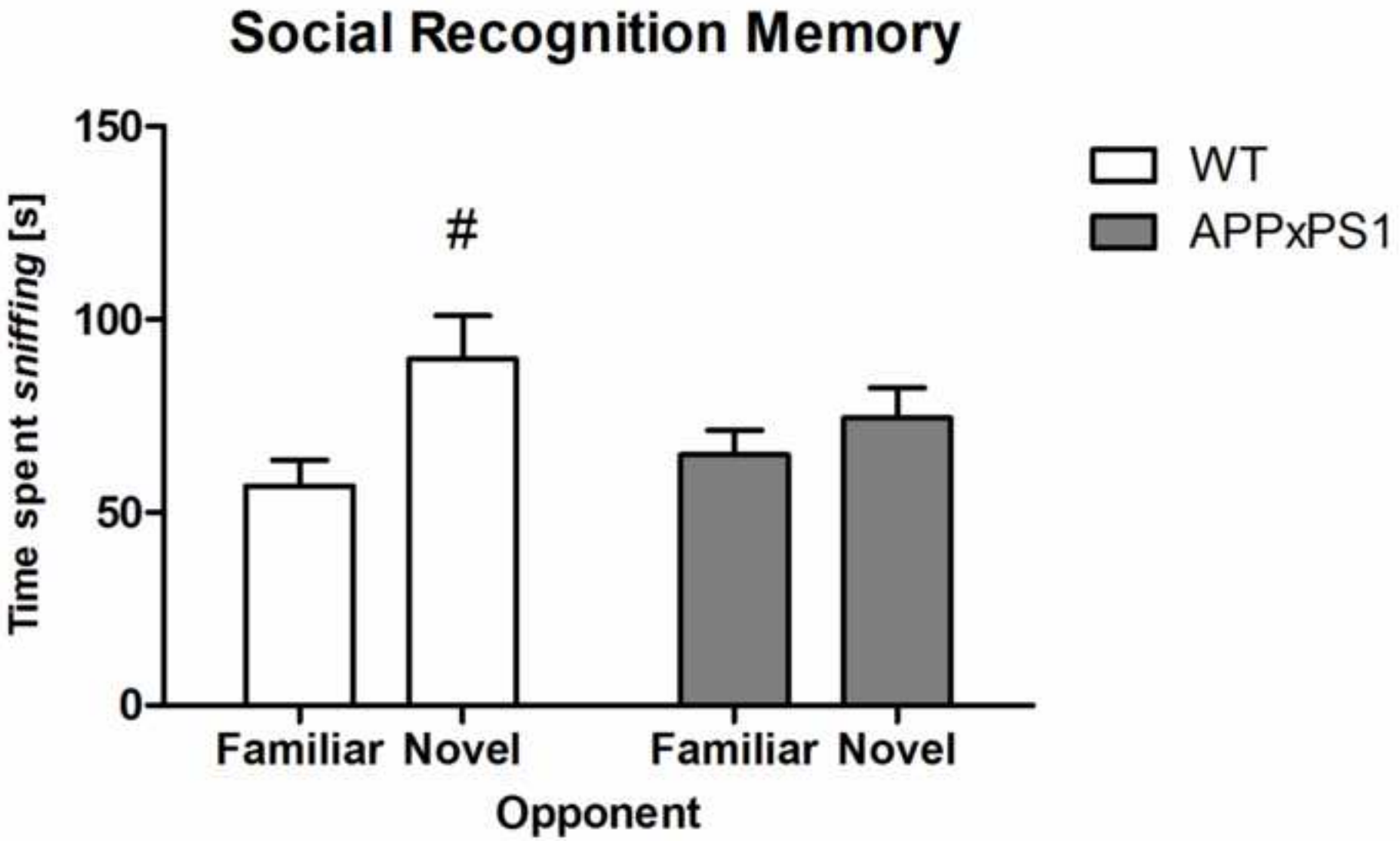

Social Recognition Memory
150
100
50
0
Time spent sniffing [s]
#
WT
APPxPS1
Familiar
Novel
Familiar
Novel
Opponent

Figure 2A

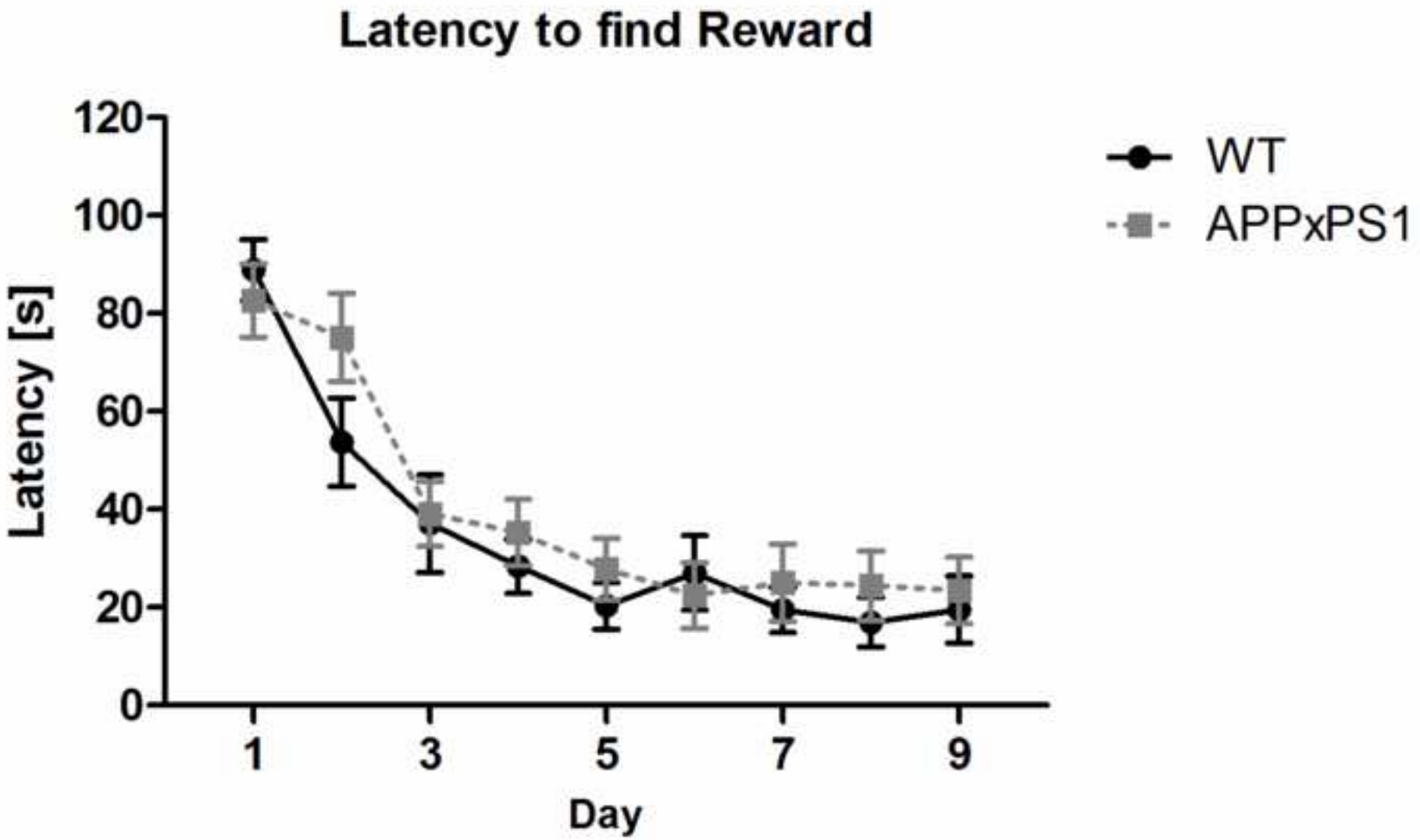

Latency to find Reward
Latency [s]
120
100
80
60
40
20
0
1
3
5
7
9
Day
WT
APPxPS1

Figure 2B

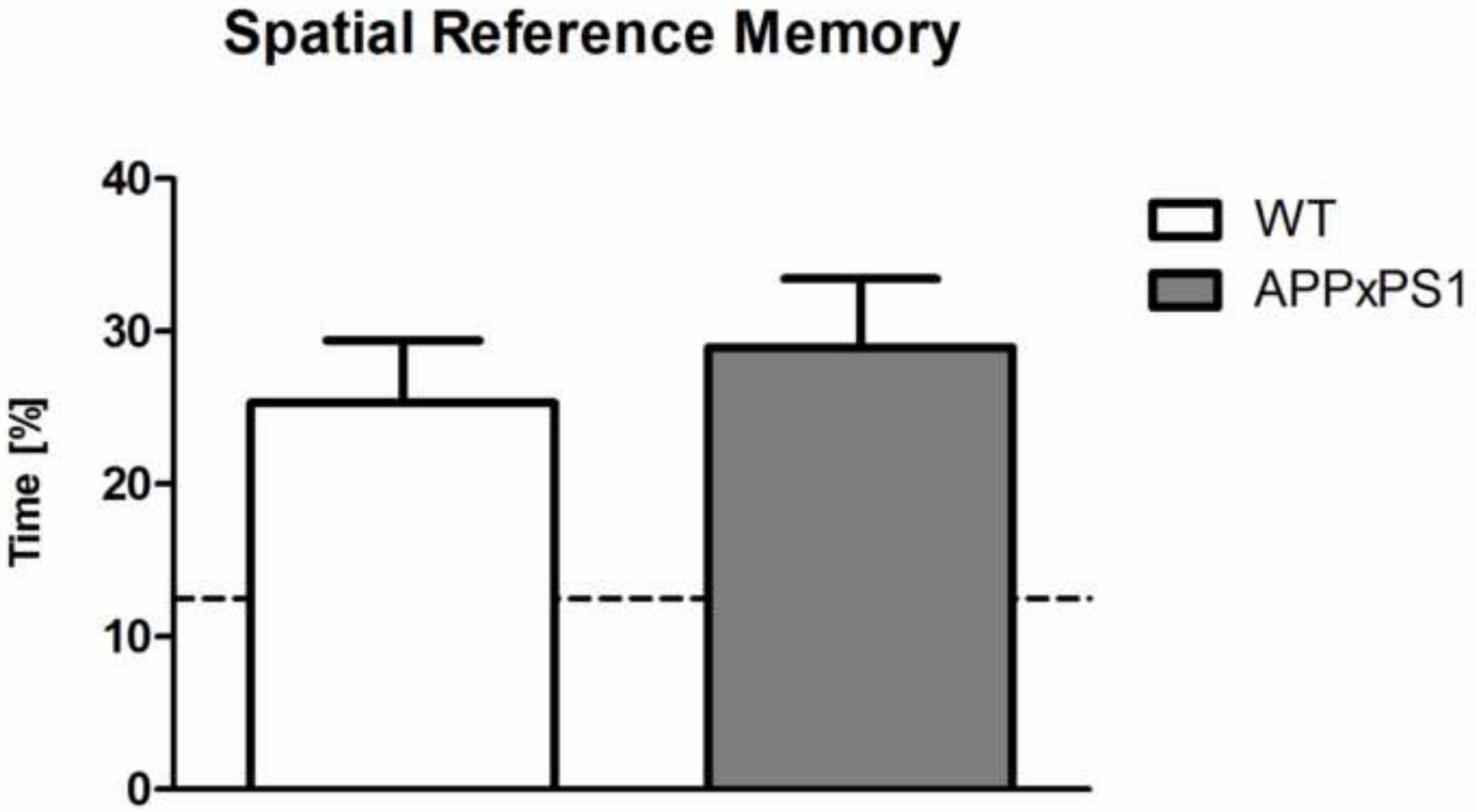


Figure 2C

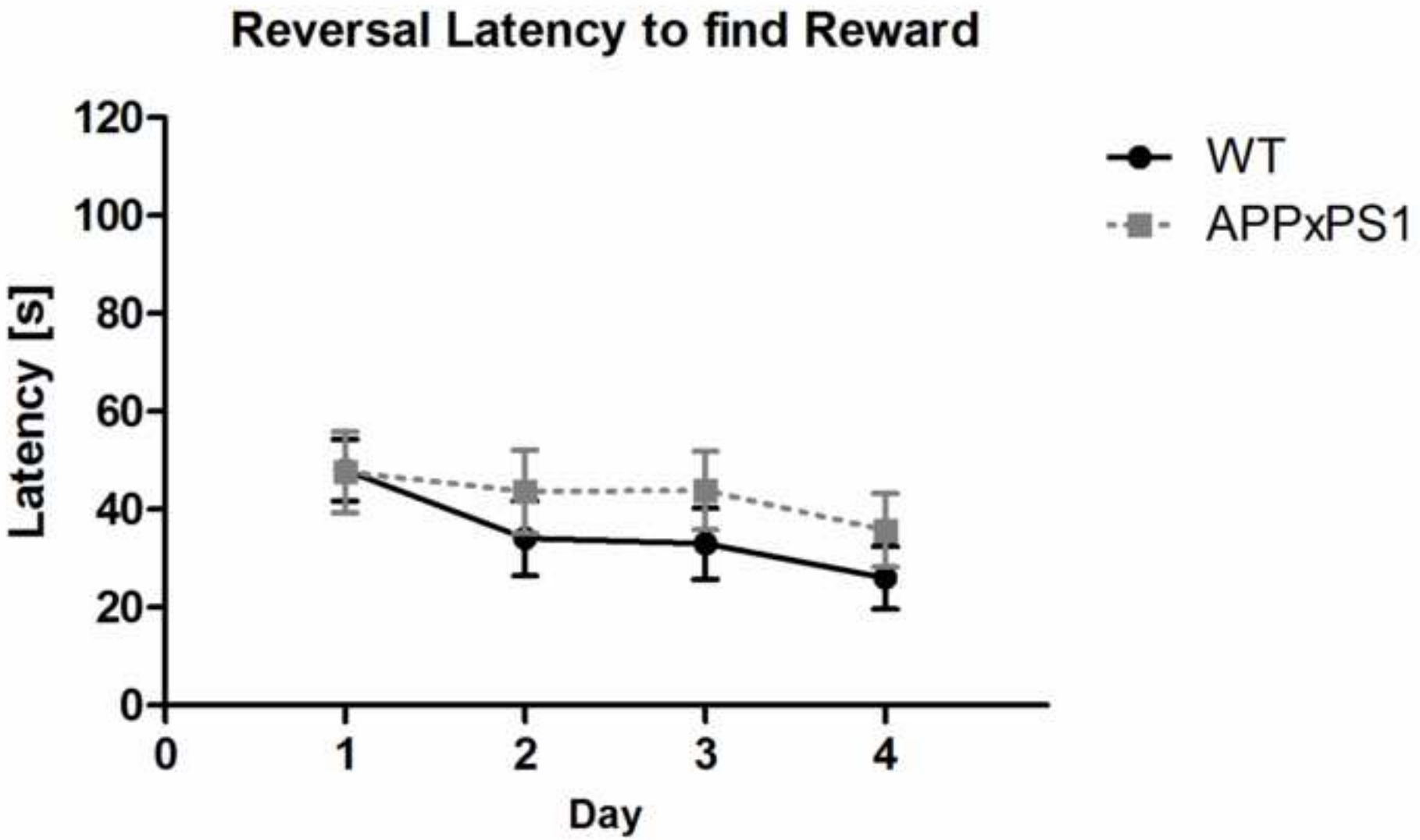

Reversal Latency to find Reward
Latency [s]
120
100
80
60
40
20
0
0
1
2
3
4
Day
WT
APPxPS1

Figure 2D

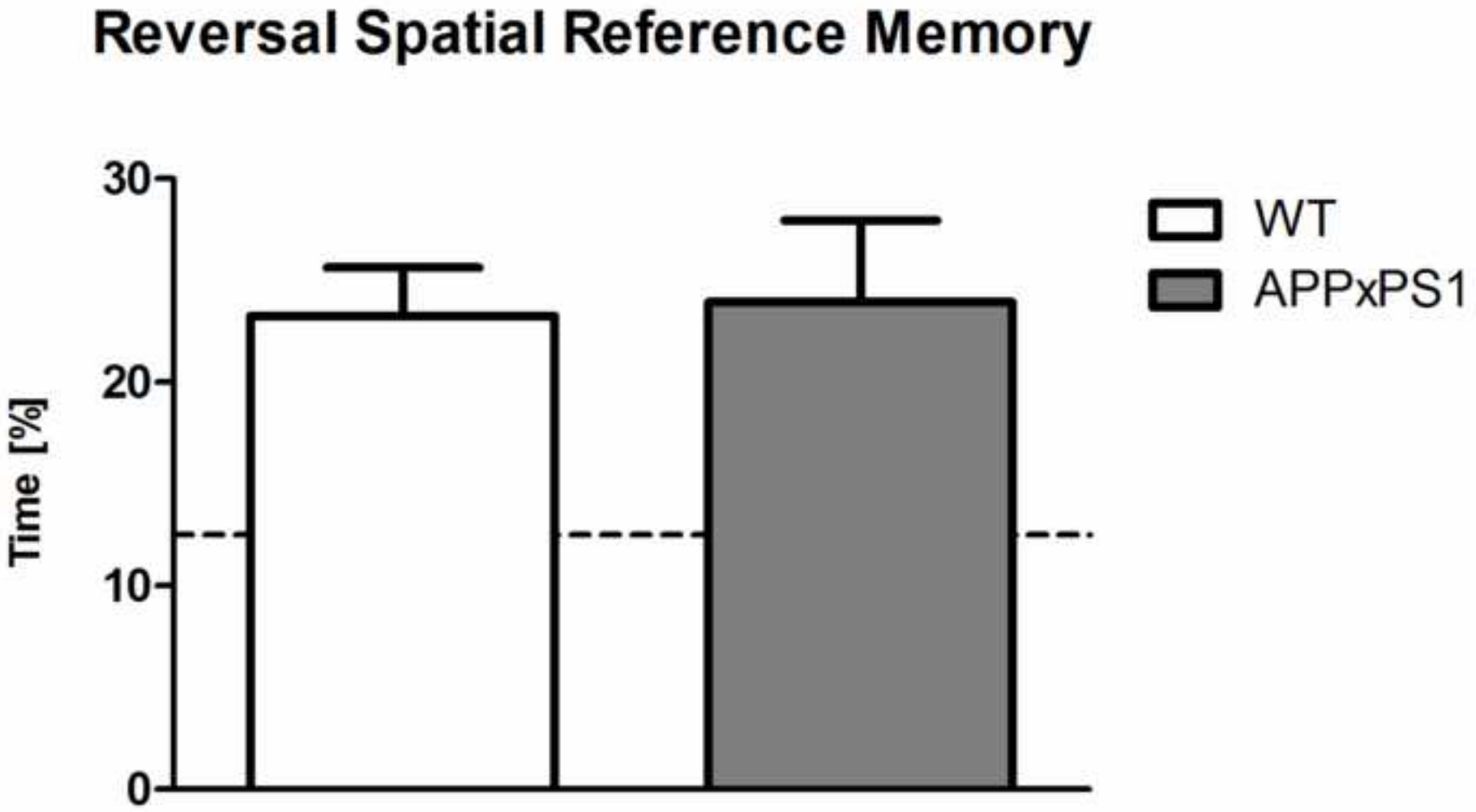

Reversal Spatial Reference Memory
Time [%]
30
20
10
0
WT
APPxPS1

Figure 3A

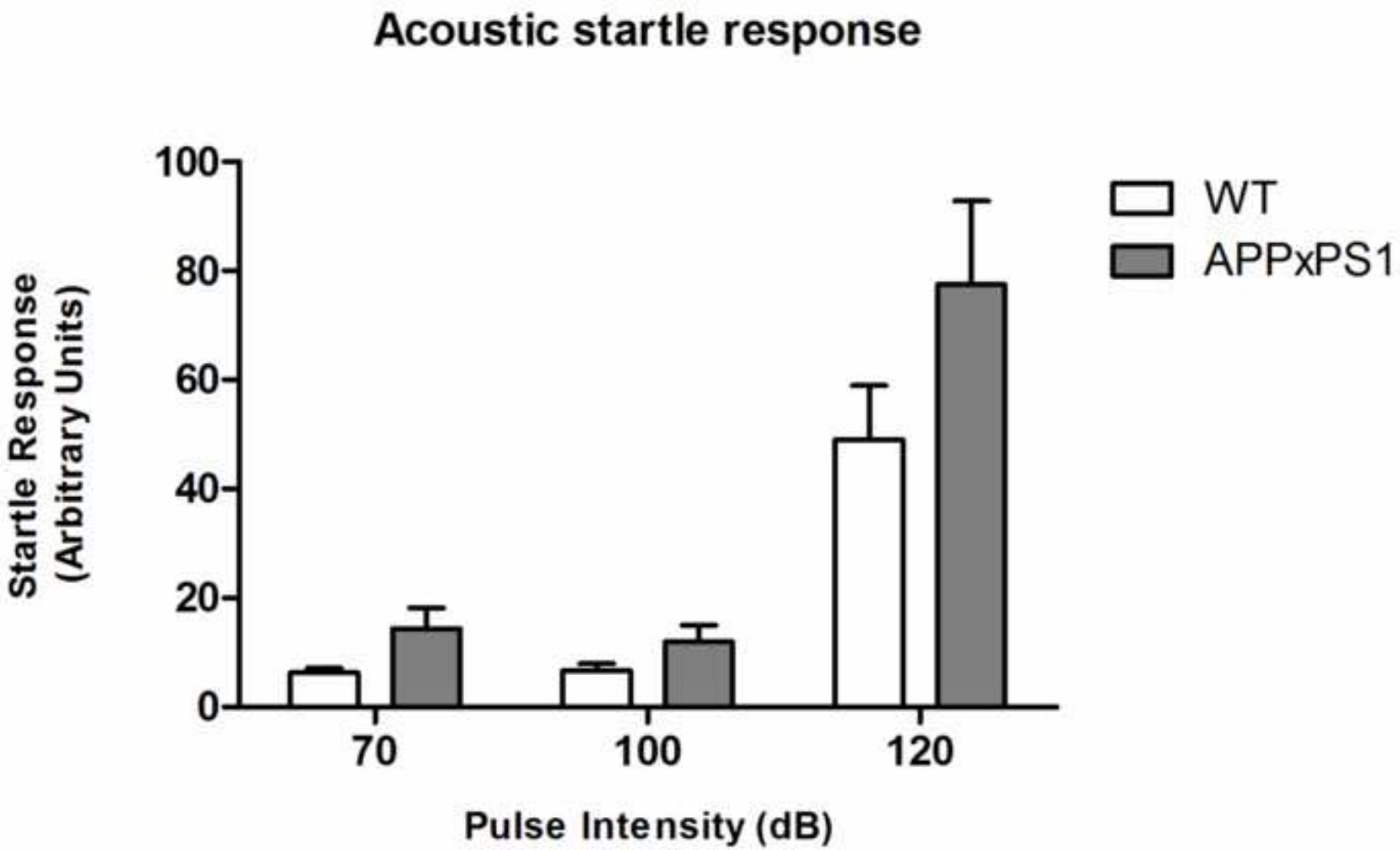

Acoustic startle response
Startle Response
(Arbitrary Units)
100
80
60
40
20
0
70
100
120
Pulse Intensity (dB)
WT
APPxPS1

Figure 3B

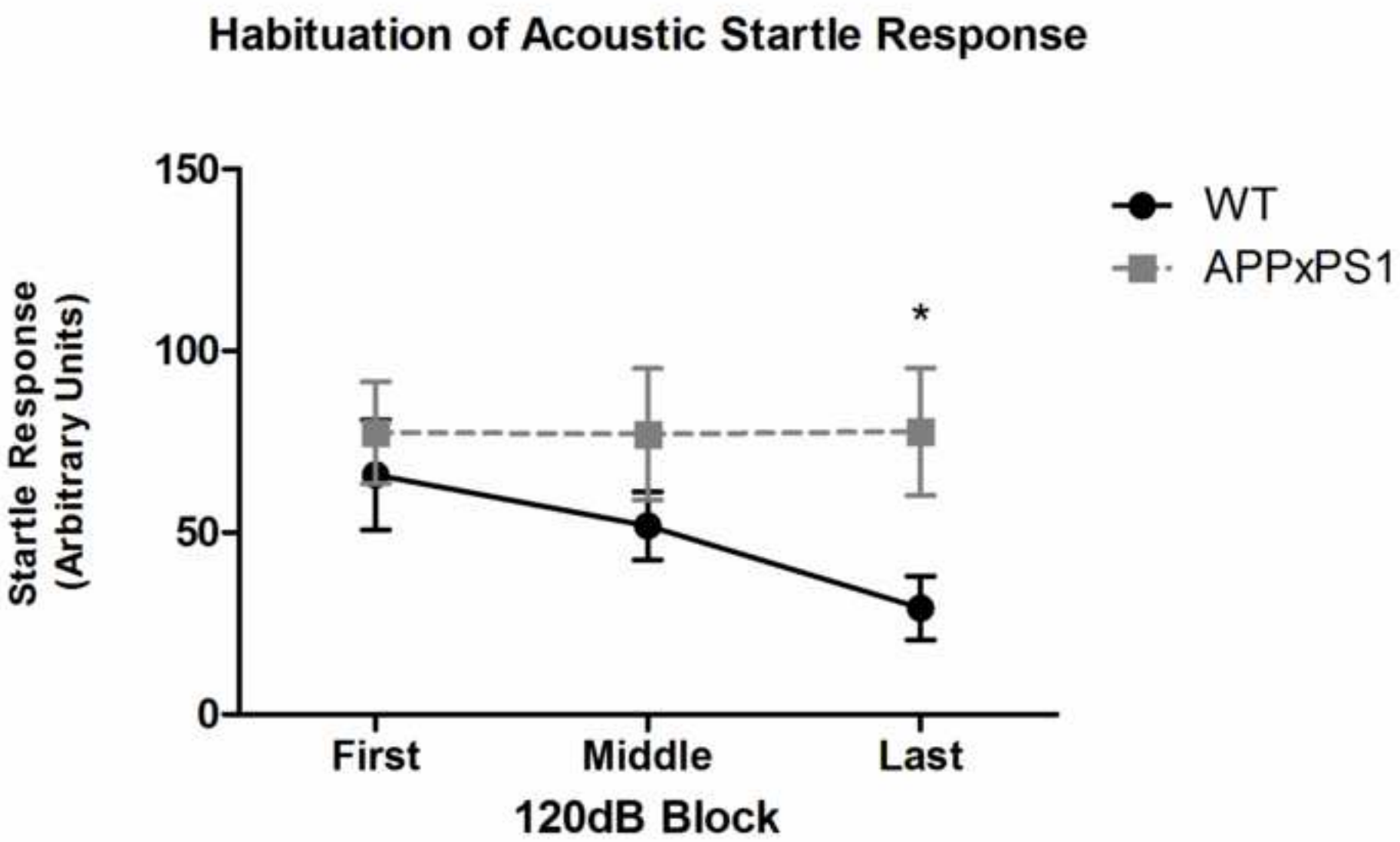
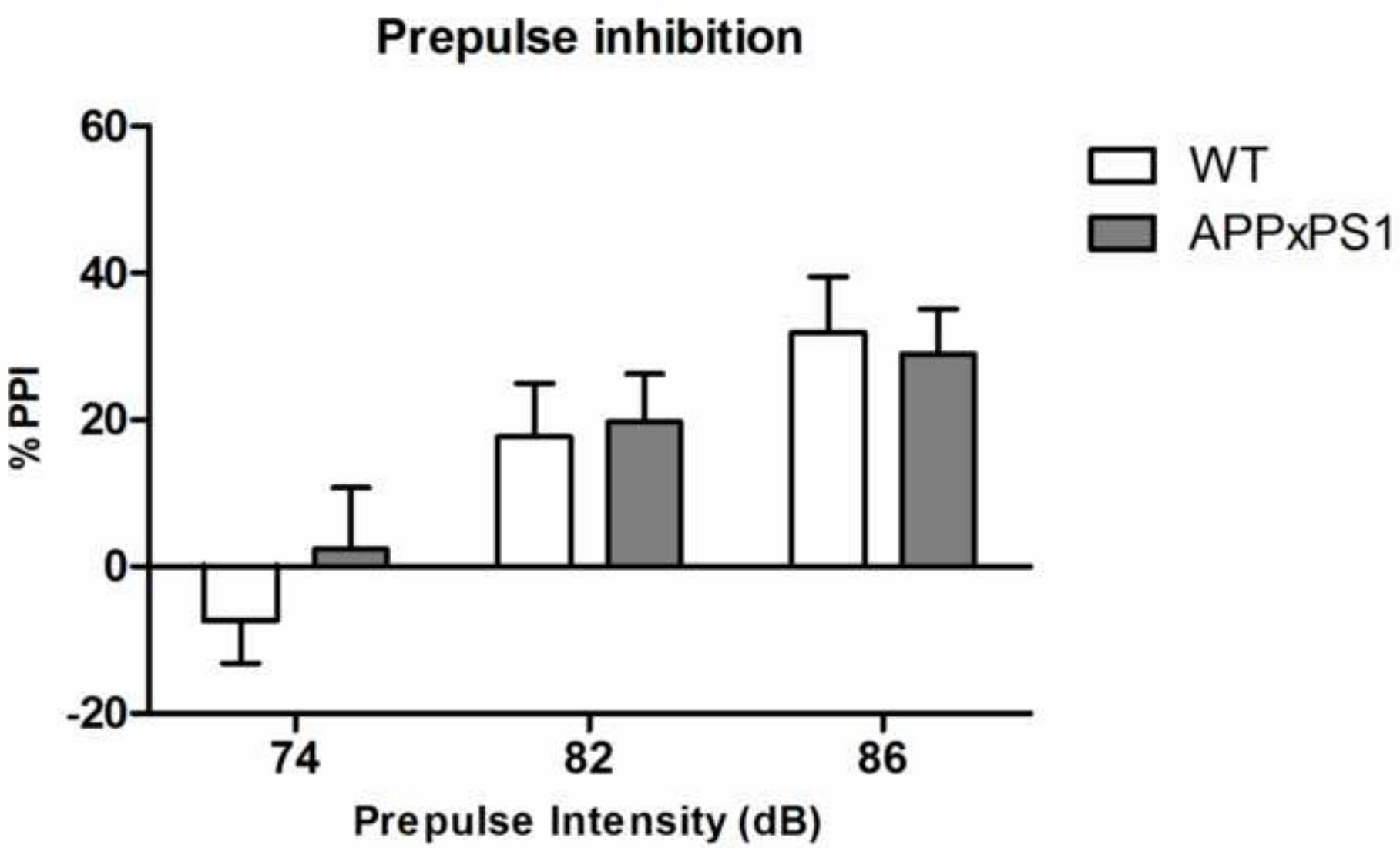

Habituation of Acoustic Startle Response
Startle Response
(Arbitrary Units)
150
100
50
0
*
WT
APPxPS1
First
Middle
Last
120dB Block

Figure 3C


Prepulse inhibition
60
40
20
0
-20
% PPI
74
82
86
Prepulse Intensity (dB)
WT
APPxPS1