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Research report

Novel behavioural characteristics of the $APP_{Swe}/PS1\Delta E9$ transgenic mouse model of Alzheimer's disease

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HIGHLIGHTS

- There are inconsistencies in the behavioural phenotype of APPxPS1 transgenic mice.
- Male APPxPS1 mice showed task-dependent hyperlocomotion and increased anxiety.
- Transgenic mice exhibited normal spatial cognition and fear conditioning.
- APPxPS1 mice displayed impaired social recognition memory.
- Sensorimotor gating of APPxPS1 males was unaffected.

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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.

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

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PSEN1 mutation (*PSEN1/ΔE9*) [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 et al. 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(APP_{Swe},*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 48 h. 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.

Table 1
Test age [d] and test biography of non-transgenic control (WT) and double transgenic $APP_{Swe}/PS1\,\Delta E9$ (APPxPS1) male mice are shown.

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)

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™ (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 × 18 cm × 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™ 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 s with a co-terminating 0.4 mA 2 s 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™ software [32,33]. To avoid any influence of foot shock exposure on further testing, an inter-test interval of several months was chosen and all following tests were carried out in tests rooms other 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