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Research report

Cognitive phenotyping of amyloid precursor protein transgenic J20 mice

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ABSTRACT

Transgenic mice that express familial Alzheimer's disease mutant forms of the human amyloid precursor protein (*hAPP*) have proved to be invaluable in determining the impact that the neurotoxic amyloid-β peptide has in vivo. In addition to the propensity to accumulate cerebral amyloid plaques, a crucial characteristic of *hAPP* mouse models is their cognitive impairments. To date the most widely used test for analyzing cognitive impairment in *hAPP* mice is the Morris water maze (MWM) which, due to the fact that mice are not "natural" swimmers, may not always be the ideal paradigm to investigate cognitive behaviours. Furthermore, not all cognitive impairments have been replicated across research laboratories. In the current study, we characterised the cognitive abilities of the J20 transgenic mouse line (expressing the Swedish $670/671_{KM->NL}$ and Indiana ($717_{V->F}$ *hAPP* mutations) and non-transgenic mice. Mice were assessed in the cheeseboard task (i.e., a 'dry version' of the MWM) and a variety of other cognitive paradigms to test fear conditioning, object recognition and short-term memory to broaden the understanding of the cognitive deficits in J20 mice. *hAPP* transgenic mice perform normally in tasks for fear conditioning, short-term object recognition and short-term memory of context familiarity. However, they were profoundly impaired in their spatial reference memory capabilities in the cheeseboard task. The cheeseboard task has potential to replace the MWM task in situations where the MWM is not suitable for particular mouse models.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative cognitive disorder affecting approximately 25–30 million people worldwide. Genetic factors play a key role in the development of AD with twin studies suggesting that 70–80% of the risk to develop the disease is inherited although epidemiological studies have shown that only around 5% of AD patients have a clear autosomal dominant inheritance (familial form of AD). Importantly, familial and sporadic (accounting for >90% of AD cases) forms of AD have an indistinguishable brain histopathology [1] and are characterised by β-amyloid (Aβ) deposits, which form senile plaques in the grey matter, and hyper-phosphorylation of tau protein, which causes intracellular neurofibrillary tangles.

The amyloid plaques are predominantly composed of Aβ peptides of 40 and 42 amino acids ($Aβ_{40}$ and $Aβ_{42}$), which are derived from proteolysis of the amyloid precursor protein (APP) [2,3]. Missense mutations in *APP* in familial AD suggest a primary pathogenic role for *APP* in the development of AD. A number of transgenic and knockout mouse models have been developed for human *APP* (*hAPP*). These models show AD-relevant pathology, as they produce amyloid plaques and exhibit varying levels of cognitive impairments (for review see [4]). Importantly, the majority of mouse models for AD have been characterised by only one or two cognitive tests (mostly testing spatial memory). Furthermore, a significant number of research groups select the Morris water maze paradigm (MWM) as the method of choice. However, *floating* behaviour, hypothermia, physical fatigue, and thigmotaxis as well as an aversion against swimming of particular inbred strains can be confounders when testing AD models with different genetic backgrounds [5–9]. More comprehensive research into the cognitive deficits of AD mouse models such as the *PD-APP* transgenic mouse [10] using a variety of tasks for spatial memory (working and reference memory), associated learning, object recognition and operant conditioning has revealed learning and memory impairments beyond spatial memory deficits (for review see [4]).

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In the current study, we characterised the cognitive abilities of an established transgenic mouse model for AD, the J20 transgenic mouse line [11], in detail. This mouse model features high levels of $A\beta_{42}$ overexpression, which result from the introduction of the Swedish ($670/671_{KM->NL}$; [12] and Indiana ($717_{V->F}$; [13]) *hAPP* mutations (i.e., *hAPPSwInd*). The J20 transgenic mice develop plaques by the age of 5–7 months and exhibit more extensive amyloid depositions in the hippocampus than other *hAPP* lines (e.g., H6, H40 and J9) [11]. J20 transgenic mice and non-transgenic control mice have been tested previously for learning and memory deficits in tasks such as the MWM, the novel object recognition task, and the Y-maze (i.e., for spontaneous alternation). Importantly, thigmotactic swimming and *floating* behaviour may confound the MWM performance of *hAPPSwInd* transgenic mice [14]. Some of the other cognitive impairments reported were inconsistent across research groups working on the J20 line: learning impairments in the cued version of the MWM were not reliable across all test sites and spatial reference memory in the hidden version of the MWM was affected by the test design used [14–18]. Thus, our study aimed to clarify the nature of the cognitive deficits of J20 mice by testing transgenic and non-transgenic control animals in contextual and cued fear conditioning, the cheeseboard task (i.e., a 'dry version' of the MWM, which avoids some of the confounding factors of MWM testing [7,19]), the Y-maze (i.e., for spatial working memory), and for short-term novel object recognition.

2. Materials and methods

2.1. Animals

The generation of the J20 line [JAX Stock No. 006293: B6.Cg-Tg(PDGFB-APPSwInd)20Lms/2Mmjax] has been described elsewhere [11]. *Prof. Mucke* (Gladstone Institute of Neurological Disease and Department of Neurology, University of California) provided the transgenic J20 breeders for this study. Genotypes were determined after weaning by tail biopsy and polymerase chain reaction as described previously [11]. All transgenic mice (*hAPPSwInd*) were heterozygous with respect to the transgene (the original study developing *hAPPSwInd* transgene mice as well as all following studies investigated heterozygous transgenics only [10]) and backcrossed to C57BL/6J for >10 generations). C57BL/6JArc mice served as non-transgenic controls (NTG). Test animals were adult (J20: 61 ± 8 weeks, $n = 11$; control: 52 ± 1 weeks, $n = 12$) male mice. Mice were bred and housed in independently ventilated cages (Airlaw, Smithfield, Australia) at Animal BioResources (Moss Vale, Australia). Following transport to the holding facility of Neuroscience Research Australia (NeuRA), mice were pair-housed in Polysulfone cages (1144B: Tecniplast, Rydalmere, Australia) with minimal environmental enrichment in the form of a red, transparent, polycarbonate igloo (certified polycarbonate mouse igloo: Bioserv, Frenchtown, USA), tissues for nesting material (Kimwipes®, Kimberley-Clark, Australia) and a metal ring (3 cm diameter) in the cage lid. 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 available ad libitum. Behavioural phenotyping commenced not earlier than 2 weeks after the arrival of the test animals at NeuRA. 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

Animals were tested in a battery of cognitive tasks, which are well-established at NeuRA [19,20] using an inter-test interval of at least 6 days (the least aversive/disruptive cognitive tasks were carried out first): Y-maze, novel object recognition task, fear conditioning, and cheeseboard test. All devices (and objects) were cleaned thoroughly with 70% ethanol in between trials and sessions.

2.2.1. Y-maze (YM)

The version of Y-maze used for this study assesses short-term memory of the familiarity to a specific context [21]. The apparatus consisted of three grey acrylic arms (10 cm × 30 cm × 17 cm) placed at 120° with respect to each other. Arms were equipped with different internal visual cues (horizontal stripes, spotted, and cross-shaped patterns), which covered both sides and the end panel of each arm. Corn-cob bedding covered the apparatus floor and was changed in between sessions. The Y-maze test consisted of two trials (training and test), with a 1 h inter-trial interval (ITI). The trial duration for training and test was 10 and 5 min respectively [22]. During training, one arm was blocked off (novel arm); mice were placed facing the end of one of the other two accessible arms (start arm). In the test trial, all arms were accessible, and mice were placed facing the end of the start arm then allowed to explore the apparatus freely. The apparatus was cleaned thoroughly with 70% ethanol in between each trial. Time, entries and distance travelled in arms was recorded using Any-Maze™ video tracking software (Stoelting Co., Wood Dale, USA). An arm entry was scored whenever an animal entered an arm with more than half of its body length. The percentage of novel arm time was calculated using [(novel arm time/total arm time) × 100]. The corresponding calculations were performed for novel arm distance travelled and novel arm entries.

2.2.2. Novel object recognition task (NORT)

The distinction between familiar and unfamiliar objects is an index of recognition memory, and its measurement is aided by the innate preference of rodents for novel over familiar objects [23]. The NORT was conducted over 3 days; two trials were conducted per day with a 1 h ITI. On day 1, mice were placed in an empty grey Perspex square arena (35 cm × 35 cm × 30 cm) and allowed to explore the arena freely for 10 min in both trials. On day 2, mice were placed in the empty arena for 10 min in trial 1. In the second trial, two identical objects were placed 5 cm from each wall in the centre of the apparatus and mice were allowed to explore freely for 10 min. On day 3, mice were exposed to two identical objects for 10 min in trial 1 (sample trial), and then one familiar and one novel object for 5 min in trial 2 (test trial). Objects (plastic hose nozzles: 31 mm × 31 mm × 42 mm; plastic pig: 80 mm × 30 mm × 45 mm; mini metal grater: 45 mm × 28 mm × 81 mm) and their location were counterbalanced across genotypes. Using an ITI of 1 h to test the mice for short-term memory. The frequency and duration of *nosing* and *rearing* the objects were recorded offline using Any-Maze™ tracking software. The percentage of time spent *nosing* and *rearing* on the object (i.e., exploration) was calculated using [(novel object time/time for both objects) × 100].

2.2.3. Fear conditioning (FC)

FC is a form of associative learning that occurs when a previously neutral stimulus (e.g., context or tone) elicits a fear response after it has been paired with an aversive stimulus (e.g., foot shock). Contextual and cued fear conditioning is mediated by hippocampal and amygdalar brain processes and involves emotional memory [24–26]. The present fear conditioning task was conducted over 3 days (24 h ITI). On day 1 (conditioning), animals were placed in the test chamber (Model H10-11R-TC: Coulbourn Instruments, Whitehall, USA) for 120 s. A 80 dB conditioned stimulus (CS) was then 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 120 s. The test concluded 120 s later. On day 2 (context test), the animals were returned to the apparatus for 7 min. On day 3 (cue test), animals were placed in an altered context (i.e., grid floor replaced by a flat plastic floor, clear Perspex walls replaced with pink acrylic panels) for 9 min. After 120 s (pre-CS / baseline), the CS was presented continuously for 5 min. The test concluded after another 120 s without the CS. There was a 68 dB white noise background for all tests. Baseline *freezing* behaviour is recorded to rule out that motor activity differences between transgenic and non-transgenic mice are a confounding factor in this paradigm. Time spent *freezing* and distance travelled were measured using Any-Maze™ software (Any-Maze™ *freezing* parameters: freezing on: 3, freezing off: 13), where *freezing* was defined as complete behavioural immobility except for natural respiratory motions [27].

2.2.4. Cheeseboard (CB)

The cheeseboard paradigm was employed as a less stressful dry-land equivalent of the MWM [7]. Mice were trained to find a food reward over a number of days; spatial reference memory was indexed by a decreased latency (and distance) to find the reward over days. The CB was a grey painted circular wooden board 1.1 m in diameter, elevated 60 cm from the floor. The illumination on the board was 60 lx during habituation, but was dropped to 20 lx during reference acquisition. There were 32 bottle caps (3.1 cm diameter, 1.3 cm deep) evenly distributed across the CB (spaced in a radial pattern with 8 lines of 4 wells each radiating from the centre area; each well was 5 cm from the next well and the last well was 10 cm from the edge of the board). One of the caps contained the food reward (100 μl sweetened condensed milk; diluted 1:4 with water). All caps were brushed lightly with diluted sweetened condensed milk at the beginning of each test day to exclude the use of odour cues to find the target. External cues were located around the CB. A camera was mounted above the CB to measure distance travelled, latency to find the reward as well as time spent in CB zones using Any-Maze™ software. Latency to find the target was measured using a stopwatch.

During habituation (4 days to the blank side of the CB) two 2 min trials were conducted each day with a 20 min ITI. Mice were food-restricted for 4 days prior to habituation and kept at 85–90% of their pre-test body weight throughout testing (mice were fed for 1–2 h per day).

2.2.4.1. Spatial reference memory acquisition. Mice were trained over 18 days (two trials per day with a 20 min ITI) to locate the food reward. The location of the target well was kept constant for each mouse between trials and across days. The target well location was different for each mouse and was 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.