2012

Cognitive phenotyping of amyloid precursor protein transgenic J20 mice

Tim Karl
Neuroscience Research Australia

Surabhi Bhatia
Neuroscience Research Australia,Prince of Wales Hopsital,Neuroscience Research Australia

David Cheng
Neuroscience Research Australia

Woojin Scott Kim
Prince of Wales Hopsital

Brett Garner
University of Wollongong, brettg@uow.edu.au

Cognitive phenotyping of amyloid precursor protein transgenic J20 mice

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.

Keywords

transgenic, j20, cognitive, mice, phenotyping, amyloid, precursor, protein

Disciplines

Medicine and Health Sciences

Publication Details

Karl, T., Bhatia, S., Cheng, D., Kim, W. & Garner, B. (2012). Cognitive phenotyping of amyloid precursor protein transgenic J20 mice. Behavioural Brain Research, 228 (2), 392-397.

Cognitive Phenotyping of Amyloid Precursor Protein Transgenic J20 Mice

Tim Karl[1,2,3¥], Surabhi Bhatia[1,3,4], David Cheng[1], Woojin Scott Kim[1,5], Brett Garner[4,6]

[1]Neuroscience Research Australia, Randwick NSW 2031, Australia

[2]Schizophrenia Research Institute, Darlinghurst NSW 2010, Australia

[3]School of Psychology, University of New South Wales, Sydney, NSW 2052, Australia

[4]Illawarra Health and Medical Research Institute, University of Wollongong, Wollongong, NSW 2522, Australia

[5]School of Medical Sciences, University of New South Wales, Sydney, NSW 2052, Australia

[6]School of Biological Sciences, University of Wollongong, Wollongong, NSW 2522, Australia

Running title: Cognition in *hAPP*-transgenic mice

Total number of pages: 19 Number of figures: 2 Number of tables: 2

Number of Words: Abstract: 276 Introduction: 524 Discussion: 1163

¥ To whom correspondence should be addressed:
Tim Karl, PhD
Neuroscience Research Australia
Barker St.
Randwick, NSW 2031
Australia
Tel: +61 2 9399 1025
Fax: +61 2 9399 1005
Email: t.karl@neura.edu.au

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-beta peptide has *in vivo*. In addition to the propensity to accumulate cerebral amyloid plaques, a crucial characteristic of *hAPP* mouse models is their demonstration of cognitive impairments that can be used as a measure of the impact that modulating numerous physiological pathways may have in the Alzheimer's disease setting. 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 as problems associated with floating behavior, hypothermia, physical fatigue and thigmotaxis have been reported in the past. In the current study, we characterized 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 using the cheeseboard task (i.e. a 'dry version' of the MWM). All mice were also assessed in a variety of other cognitive paradigms to test fear conditioning, short-term object recognition and spatial working memory to broaden the understanding of the cognitive deficits in J20 mice. The transgenic mice performed normally in these latter cognitive paradigms. However, they were profoundly impaired in their spatial reference memory capabilities in the cheeseboard task. Thus, *hAPP* transgenic mice perform normally in tasks for fear conditioning, short-term object recognition and spatial working memory but exhibit robust spatial reference memory impairment. The cheeseboard task has potential to replace the MWM task in situations where it is not suitable for particular mouse models.

Keywords: Alzheimer's disease; transgenic mouse model; J20; APP; learning and memory

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 characterized by β-amyloid (Aβ) deposits, which form senile plaques in the gray 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 ($A\beta_{40}$ and $A\beta_{42}$), 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 characterized 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* behavior, 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]).

In the current study, we characterized 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]. Importantly, 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 are inconsistent across studies [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 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: 12h 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 two 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 six days. 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 spatial working memory [21]. The apparatus consisted of three grey acrylic arms (10 cm x 30 cm x 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 x 35 x 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 x 31 x 42 mm; plastic pig: 80 x 30 x

45 mm; mini metal grater: 45 x 28 x 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) x 100].

2.2.3 Fear conditioning (FC)

Fear conditioning (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 three 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 0.4mA 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 to find the reward over days. The cheeseboard (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 and velocity as well as time spent in CB zones using Any-Maze™ software. Latency to find the target was measured using a stopwatch.

During habituation (four 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).

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. A probe trial was conducted on days 13 and 19, where no wells were baited and mice were given 2 min to explore the board freely (only results of probe trial 19 are shown as no clear target preference was detected on day 13). On the probe trial, the board was divided into 8 zones corresponding to each line of 4 caps (the line of wells was in the centre of the zone), as well as a centre zone (40 cm diameter); the time spent in each zone (%time) was measured using Any-Maze™. Data presented for ‘zone time’ excludes the

time spent in the centre zone.

2.3 Statistical Analysis

Results were analysed using one-way analysis of variance (ANOVA: between factor: 'genotype'). Repeated measures (RM) ANOVA was used for specific tasks to control for successful learning (YM: 'arm type', NORT: 'object', FC: 'baseline freezing') and for effects across trials (CB: 'latency') or over time (FC '1 min block'). For the cheeseboard probe trials, single sample t-tests were used to assess if % time in the target zone was greater than chance (100% / 8 = 12.5%). Analyses were conducted using SPSS for Windows 19.0. Differences were regarded as significant if $p < .05$. All data are presented as means ± standard error of the mean (SEM). Significant 'genotype' effects of J20 transgenic (*hAPPSwInd*) *versus* non-transgenic control mice are indicated by '*' ($*p < .05$, $**p < .01$ and $***p < .001$), whereas repeated measures effects are indicated by '#' ($^{\#}p < .05$, $^{\#\#}p < .01$ and $^{\#\#\#}p < .001$).

3. Results

3.1 Y-maze

Test mice showed intact spatial working memory abilities. All animals distinguished between the two familiar and the novel arm of the Y maze and both genotypes exhibited a similar preference for the novel arm. This was confirmed statistically with a RM ANOVA for 'arm' [arm distance: $F(2,42) = 10.3$, $p < .001$ - arm time: $F(2,42) = 11.2$, $p < .001$; Table 1] and one-way ANOVA for 'genotype' [% novel arm time: $F(1,21) = .08$, $p = .8$ - % novel arm distance: $F(1,21) = .2$, $p = .6$] (Table1).

3.2 Novel object recognition

Object recognition was not disturbed in J20 mice. *hAPPSwInd* transgenic and non-transgenic mice had a preference to explore the novel object, as confirmed by a RM ANOVA for 'object' [% exploration time: $F(1,17) = 5.6$, $p = .03$; WT: 54.6 + 3.7 – J20: 55.7 + 2.1], and displayed equal levels of investigating the novel object [% novel exploration time: $F(1,17) = .8$, $p = .8$)].

3.3 Fear conditioning

All animals responded to the unconditioned stimulus (i.e. electric foot shock) delivered during the conditioning. We first confirmed that there were no baseline differences in *freezing* between the genotypes. The time spent *freezing* in the first 2 min across conditioning, context test and cue test was similar between genotypes [no interaction of 'baseline freezing' with 'genotype': $F(2,42) = .6$, $p = .5$ - no 'genotype' effect for conditioning: $F(1,21) = 2.2$, $p = .2$] (Table 2).

As expected, baseline freezing in the context test was significantly higher than during conditioning and in the cue test [RM ANOVA for 'baseline freezing': $F(2,42) = 24.8$, $p < 0.001$] confirming that all animals had learned to associate the context with the unconditioned stimulus (Table 2). Both genotypes displayed equal levels of total *freezing* in the context test [$F(1,21) = .2$, $p = .7$; Fig. 1A]. In the cue test, all animals associated the cue with the US and showed an increase in *freezing* after CS onset [RM ANOVA for '1 min block' comparing the last minute before and the first minute post CS onset: $F(1,21) = 45.7$, $p < 0.001$]. Similarly to the observations in the context test, there

were no differences in *freezing* between transgenic and non-transgenic mice in the cue test [F(1,21)= .6, p = .5; Fig. 1B)].

3.4 Cheeseboard

The acquisition of the cheeseboard task developed similarly across training trials in all mice. During the training period, RM ANOVA revealed a significant effect of 'day' for the latency to find the food reward [F(16,272) = 3.7, p < .001] for all mice regardless of genotype [F(1,17) = .01, p = .9], demonstrating that both genotypes learned the location of the food reward (see Fig. 2A for mean latency over days). Reference memory was tested in the probe trial. Non-transgenic control mice showed intact reference memory, as they had a clear preference for the target zone (> 12.5%) whereas J20 transgenic mice failed to display an increased exploration of the target zone. This was evidenced by a significantly greater time spent in the target zone than chance for control but not *hAPPSwInd* transgenic mice [paired t-test: WT: p = .03 - J20: p = .7] and a significant effect of 'genotype' for time spent in the target zone [F(1/16) = 4.6, p < .05] (Fig. 2B).

4. Discussion

hAPPSwInd transgenic and non-transgenic control mice of the J20 line showed similar cognitive abilities in spatial working memory, short-term object recognition and hippocampal and amygdalar associative learning. However, transgenic mice showed a significant impairment in spatial reference memory in the cheeseboard task.

One of the first studies characterizing the behavioural phenotype of the J20 line reported that *hAPPSwInd* transgenic mice performed normally in the cued version of the MWM but exhibited impairments in task acquisition (i.e. training) and memory retention (i.e. probe trial) of the hidden platform MWM. The learning deficits correlated strongly with decreased levels of the calcium-binding protein calbindin-D_{28K} and the calcium-dependent immediate early gene product c-Fos in the dentate gyrus [16]. Since this initial study, others have shown that reducing endogenous tau levels prevents the cognitive deficits of transgenic J20 mice in the MWM without affecting Aβ levels [17]. Furthermore, hippocampal levels of arachidonic acid and its metabolites are higher in APP transgenic mice suggesting increased activity of the group IV isoform of phospholipase A_2 (GIVA-PLA_2). Indeed, MWM deficits could be rescued by removal of GIVA-PLA_2 [18].

Importantly, these and other studies on the cognitive abilities of J20 mice reported consistently deficits of *hAPPSwInd* mutant mice in the hidden version of the MWM [15-18, 28]. One study also reported reduced spontaneous alternation in the Y maze [15]. However, these studies also produced some conflicting data: learning and memory impairments in the cued version of the MWM [15-18] and in the novel object recognition [15, 28] were inconsistent across studies. In addition to cognitive deficits, *hAPPSwInd* mice have been described as hyper-locomotive in the open field, elevated plus maze and the Y maze [17, 28] and less anxious in the elevated plus maze [28].

Our finding that *hAPPSwInd* had no impact on the short-term object recognition of laboratory mice is in opposition to studies published in 2010 and 2011, in which transgenic mice exhibited a novel object recognition impairment [28-30]. Importantly, methodological variations in the NORT design can be accounted for this effect, as the test animals of the Harris study had been tested after an ITI

of 4 h (in 2-3 month old mice) or 15 min (in 5-7 month old mice, which had been exposed to familiar objects in three trials each 15 min apart) [28] and an ITI of 3 h in the studies of 2011. Cisse and co-workers also detected a deficit of *hAPPSwInd* mice in novel location recognition [29].

Test animals of the current study showed no impairments in the working memory version of the Y maze task. It is important to mention that the Y maze version used in our study tests for spatial working memory, whereas the Y maze test employed in an earlier study characterized the spontaneous alternation behaviour of the J20 line.

For the first time, fear conditioning was tested in *hAPPSwInd* mice. The experiment revealed that associative learning and emotional memory is unaffected in transgenic mice. Importantly, the hyperactive phenotype of *hAPPSwInd* animals described elsewhere [17, 28] did not affect the performance of transgenic mice, as confirmed by similar baseline *freezing* behaviour during conditioning, context test and cued test. Also, a recent study investigated passive avoidance behaviour of the J20 line and found no differences in escape latencies on training day indicating that the hyper-locomotive phenotype of *hAPPSwInd* animals had no consequences for their performance in this paradigm. However, transgenic mice exhibited an impaired ability to associate the dark chamber with the aversive stimulus received 24 h earlier [29]. Importantly, differences in cognitive performance between fear conditioning and passive avoidance have been described in the past [31]. Fear conditioning evaluates memory retrieval by *freezing* behaviour, which is considered to reflect fear memory. Furthermore, freezing is a passive coping strategy to the repeated exposure to foot shocks. In the passive avoidance paradigm, fear memory is evaluated by the attempt of mice to flee a brightly illuminated environment. Based on those differences, the latter paradigm can be more sensitive than the fear conditioning test to detect fear memory under certain conditions.

As mentioned earlier, the value of the MWM test for mouse models has been discussed controversially in the recent past [5-9]. Nevertheless, it is one of the most commonly used tests to characterize AD mouse models. Importantly, transgenic mice of the J20 line have been reported to show a higher tendency to float (i.e. swimming at speeds lower than 0.025m/s) and to thigmotactic

swimming (i.e. swim paths restricted to 10 cm from the wall) compared to non-transgenic mice, which are both confounding factors for MWM testing [14]. Thus, we decided to test J20 mice in a 'dry version' of the MWM to circumvent the disadvantages of the MWM. Transgenic and non-transgenic mice exhibited a similar ability to learn the location of the food reward over a number of days. Earlier studies described a deficit in MWM training for transgenic mice in the hidden version of the test paradigm [14-18, 28], whereas task acquisition in the cued version of the MWM was not always impaired [16]. Studies by Galvan and Harris confirmed that test age and pre-test experience play a significant role in the MWM performance of J20 mice [14, 28]. Importantly, similar to what has been described in studies using the hidden version of the MWM paradigm, *hAPPSwInd* transgenic mice of our study displayed a significant cognitive deficit in the probe trial, during which no significant preference for the target zone was detectable. Thus, the spatial reference memory deficit of transgenic mice of the J20 line is reliable and consistent across different spatial memory tasks. Interestingly, *hAPPSwInd* have been shown to use allocentric and egocentric navigational strategies to a similar extent whereas non-transgenic C57BL/6J mice prefer allocentric strategies in spatial memory tasks. Importantly, the visual acuity is not affected in transgenic mice [32].

In line with this is an earlier study, which reported that the cheeseboard task could discriminate between non-transgenic mice and animals that express *hAPP* harboring the Swedish double mutation as well as mutant presenilin1 (i.e. *APPswe/PS1De9* mice). Importantly, there were methodological differences between this and our cheeseboard experiment (e.g. the use of both a cued and a hidden version and the length of the training period). Further, an important distinction between the two transgenic animal models used is that the addition of the PS1 mutation in *APPswe/PS1De9* can alter the processing of γ-secretase substrates (γ-secretase is one of the enzymes responsible for the endoproteolytic cleavage of APP) in addition to APP and also the substantially more severe pathology associated with the *APPswe/PS1De9* mice as compared to the J20 line [33].

In conclusion, the current study has increased our understanding of the specificity of the cognitive

impairments in *hAPP* transgenic mice of the J20 line. Transgenic mice perform normally in tasks for fear conditioning, short-term object recognition and spatial working memory but exhibit robust spatial reference memory impairment. The cheeseboard task has potential to replace the MWM task in situations where it is not suitable for particular mouse models.

5. Figures and Legends

Fig. 1A-B: Associative learning in the fear conditioning task: A) Total time spent *freezing* [s] during the context test and **B)** Time spent *freezing* [s] for 1 min before and 1 min post CS onset during the cue test. Data for non-transgenic control mice (NGT) and transgenic *hAPPSwInd* mice (hAPP) are presented as mean + SEM. Significant RM ANOVA effects of '1 min block' are indicated by '#' ($^{\#\#\#}p < .001$).

Fig. 2A-B: Spatial reference memory in the cheeseboard: A) Mean latency (averaged across two trials per day) to find the food reward [s] and **B)** Percentage time [%] spent in the target zone during the probe trial. Time spent in the zone according to chance (= 12.5%) is marked with a dotted line. Data for non-transgenic control mice (NGT) and transgenic *hAPPSwInd* mice (hAPP) are presented as mean + SEM.

6. Acknowledgements

This research was supported by the Schizophrenia Research Institute (SRI) utilizing infrastructure funding from NSW Health and the Baxter Charitable Foundation and the Alma Hazel Eddy Trust. TK is supported by an NHMRC Career Development Award (568752), by the National Alliance for Research on Schizophrenia and Depression (Young Investigator Award), and The Lindsay & Heather Payne Medical Research Charitable Foundation and Estate of the late Margaret Augusta Farrell (both managed by Perpetual). BG is supported by a Fellowship from the Australian Research Council (FT0991986) and is an honorary NHMRC Senior Research Fellow (630445). BG and TK are also supported by a NHMRC project grant (1003886). We thank Prof. Lennart Mucke (Gladstone Institute of Neurological Disease and Department of Neurology, University of California) for providing the mouse line used in this study, Jerry Tanda for critical comments on the manuscript, and the staff of the Australian BioResources.

7. References

[1] Rosenberg RN. The molecular and genetic basis of AD: the end of the beginning: the 2000 Wartenberg lecture. Neurology. 2000;54:2045-54.
[2] Gotz J, Ittner LM. Animal models of Alzheimer's disease and frontotemporal dementia. Nat Rev Neurosci. 2008;9:532-44.
[3] Gotz J, Streffer JR, David D, Schild A, Hoerndli F, Pennanen L, et al. Transgenic animal models of Alzheimer's disease and related disorders: histopathology, behavior and therapy. Mol Psychiatry. 2004;9:664-83.
[4] Kobayashi DT, Chen KS. Behavioral phenotypes of amyloid-based genetically modified mouse models of Alzheimer's disease. Genes Brain Behav. 2005;4:173-96.
[5] Gerlai R. Behavioral tests of hippocampal function: simple paradigms complex problems. Behav Brain Res. 2001;125:269-77.
[6] Iivonen H, Nurminen L, Harri M, Tanila H, Puolivali J. Hypothermia in mice tested in Morris water maze. Behav Brain Res. 2003;141:207-13.
[7] Llano Lopez L, Hauser J, Feldon J, Gargiulo PA, Yee BK. Evaluating spatial memory function in mice: a within-subjects comparison between the water maze test and its adaptation to dry land. Behav Brain Res. 2010;209:85-92.
[8] Mizunoya W, Oyaizu S, Hirayama A, Fushiki T. Effects of physical fatigue in mice on learning performance in a water maze. Biosci Biotechnol Biochem. 2004;68:827-34.
[9] Wolfer DP, Stagljar-Bozicevic M, Errington ML, Lipp HP. Spatial Memory and Learning in Transgenic Mice: Fact or Artifact? News Physiol Sci. 1998;13:118-23.
[10] Games D, Adams D, Alessandrini R, Barbour R, Berthelette P, Blackwell C, et al. Alzheimer-type neuropathology in transgenic mice overexpressing V717F beta-amyloid precursor protein. Nature. 1995;373:523-7.
[11] Mucke L, Masliah E, Yu GQ, Mallory M, Rockenstein EM, Tatsuno G, et al. High-level neuronal expression of abeta 1-42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. J Neurosci. 2000;20:4050-8.
[12] Mullan M, Crawford F, Axelman K, Houlden H, Lilius L, Winblad B, et al. A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of beta-amyloid. Nat Genet. 1992;1:345-7.
[13] Murrell J, Farlow M, Ghetti B, Benson MD. A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease. Science. 1991;254:97-9.
[14] Galvan V, Zhang J, Gorostiza OF, Banwait S, Huang W, Ataie M, et al. Long-term prevention of Alzheimer's disease-like behavioral deficits in PDAPP mice carrying a mutation in Asp664. Behav Brain Res. 2008;191:246-55.
[15] Galvan V, Gorostiza OF, Banwait S, Ataie M, Logvinova AV, Sitaraman S, et al. Reversal of Alzheimer's-like pathology and behavior in human APP transgenic mice by mutation of Asp664. Proc Natl Acad Sci U S A. 2006;103:7130-5.
[16] Palop JJ, Jones B, Kekonius L, Chin J, Yu GQ, Raber J, et al. Neuronal depletion of calcium-dependent proteins in the dentate gyrus is tightly linked to Alzheimer's disease-related cognitive deficits. Proc Natl Acad Sci U S A. 2003;100:9572-7.
[17] Roberson ED, Scearce-Levie K, Palop JJ, Yan F, Cheng IH, Wu T, et al. Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. Science. 2007;316:750-4.
[18] Sanchez-Mejia RO, Newman JW, Toh S, Yu GQ, Zhou Y, Halabisky B, et al. Phospholipase A2 reduction ameliorates cognitive deficits in a mouse model of Alzheimer's disease. Nat Neurosci. 2008;11:1311-8.
[19] Chesworth R, Downey L, Logge W, Killcross S, Karl T. Cognition in female transmembrane domain neuregulin 1 mutant mice. Behav Brain Res. 2011;226:218-23.
[20] Duffy L, Cappas E, Lai D, Boucher AA, Karl T. Cognition in transmembrane domain neuregulin 1 mutant mice. Neuroscience. 2010;170:800-7.

[21] Hughes RN. The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory. Neurosci Biobehav Rev. 2004;28:497-505.
[22] Pang TY, Stam NC, Nithianantharajah J, Howard ML, Hannan AJ. Differential effects of voluntary physical exercise on behavioral and brain-derived neurotrophic factor expression deficits in Huntington's disease transgenic mice. Neuroscience. 2006;141:569-84.
[23] Dere E, Huston JP, De Souza Silva MA. The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. Neurosci Biobehav Rev. 2007;31:673-704.
[24] Holmes A, Wrenn CC, Harris AP, Thayer KE, Crawley JN. Behavioral profiles of inbred strains on novel olfactory, spatial and emotional tests for reference memory in mice. Genes Brain Behav. 2002;1:55-69.
[25] McNally GP, Westbrook RF. Predicting danger: the nature, consequences, and neural mechanisms of predictive fear learning. Learn Mem. 2006;13:245-53.
[26] Radulovic J, Tronson NC. Molecular specificity of multiple hippocampal processes governing fear extinction. Rev Neurosci. 2010;21:1-17.
[27] Stiedl O, Spiess J. Effect of tone-dependent fear conditioning on heart rate and behavior of C57BL/6N mice. Behav Neurosci. 1997;111:703-11.
[28] Harris JA, Devidze N, Halabisky B, Lo I, Thwin MT, Yu GQ, et al. Many neuronal and behavioral impairments in transgenic mouse models of Alzheimer's disease are independent of caspase cleavage of the amyloid precursor protein. J Neurosci. 2010;30:372-81.
[29] Cisse M, Halabisky B, Harris J, Devidze N, Dubal DB, Sun B, et al. Reversing EphB2 depletion rescues cognitive functions in Alzheimer model. Nature. 2011;469:47-52.
[30] Cisse M, Sanchez PE, Kim DH, Ho K, Yu GQ, Mucke L. Ablation of cellular prion protein does not ameliorate abnormal neural network activity or cognitive dysfunction in the J20 line of human amyloid precursor protein transgenic mice. J Neurosci. 2011;31:10427-31.
[31] Takao K, Tanda K, Nakamura K, Kasahara J, Nakao K, Katsuki M, et al. Comprehensive behavioral analysis of calcium/calmodulin-dependent protein kinase IV knockout mice. PLoS One. 2010;5:e9460.
[32] Deipolyi AR, Fang S, Palop JJ, Yu GQ, Wang X, Mucke L. Altered navigational strategy use and visuospatial deficits in hAPP transgenic mice. Neurobiol Aging. 2008;29:253-66.
[33] Pillay NS, Kellaway LA, Kotwal GJ. Early detection of memory deficits and memory improvement with vaccinia virus complement control protein in an Alzheimer's disease model. Behav Brain Res. 2008;192:173-7.

Figure 1

Figure 1A

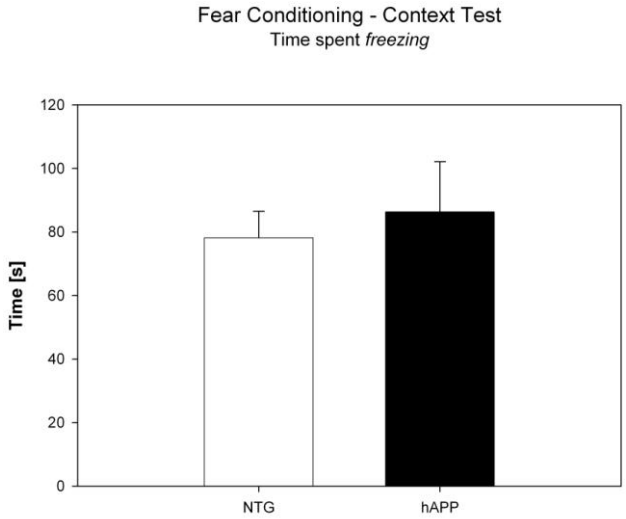

Figure 1B

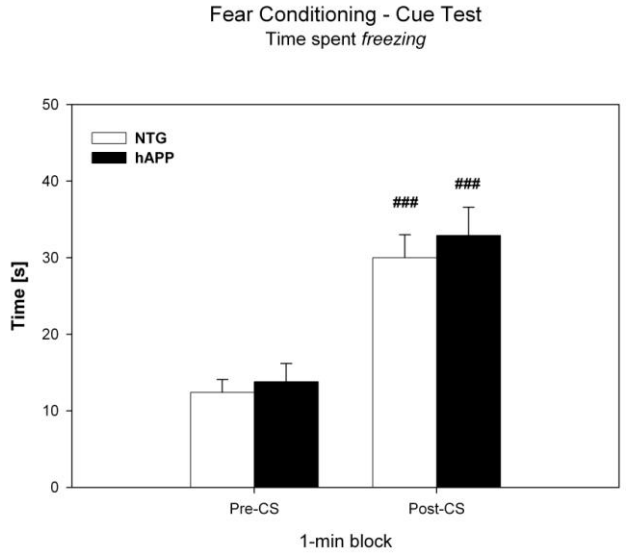

Figure 2

Figure 2A

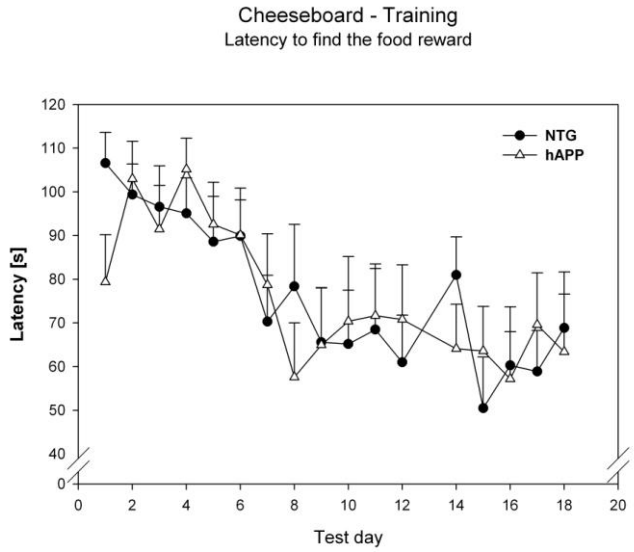

Figure 2B

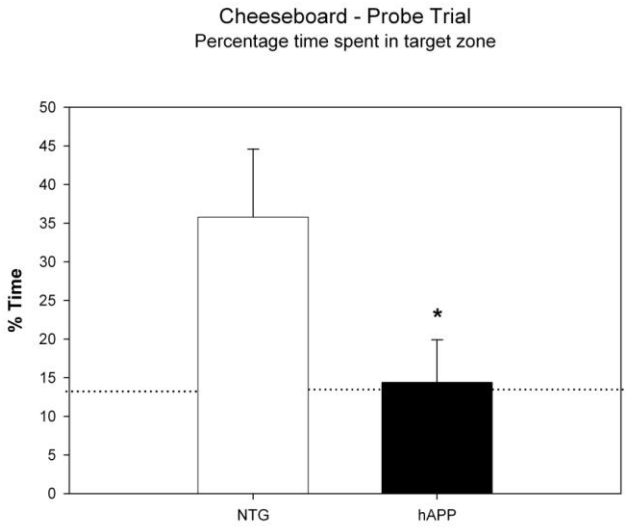

Table 1

	NTG	hAPP
%Novel Arm Time	43.1 ± 2.5	44.3 ± 3.7
%Novel Arm Distance	47.6 ± 5.7	44.1 ± 3.9

Table 1: Spatial memory in the Y maze task. Time spent [%] and travelled distance [%] in the novel arm of the Y maze for non-transgenic control mice (NGT) and transgenic *hAPPSwInd* mice (hAPP). Data are presented as mean ± SEM.

Table 2

	NTG	hAPP
Conditioning [s]	0.3 ± 0.3	1.6 ± 0.9
Context test [s]	16.7 ± 2.8	21.4 ± 6.5
Cue test [s]	14.8 ± 2.1	15.5 ± 4.7

Table 2: Baseline *freezing* activity in the fear conditioning paradigm. Duration of baseline *freezing* (in the first 120s of the test session) for non-transgenic control mice (NGT) and transgenic *hAPPSwInd* mice (hAPP). Data are presented as mean ± SEM.