A rapidly acquired foraging-based working memory task, sensitive to hippocampal lesions, reveals age-dependent and age-independent behavioural changes in a mouse model of amyloid pathology

AUTHORS

Charles Evans[a,b], Martha Hvoslefeide[a,d], Rhian Thomas[b,c], Emma Kidd[b], Mark A. Good[a]

[a] School of Psychology, Cardiff University, Park Place, Cardiff CF10 3AT, UK [b] School of Pharmacy & Pharmaceutical Sciences, Cardiff University, King Edward VII Avenue, Cardiff CF10 3NB, UK [c] Department of Applied Sciences, University of the West of England, Coldharbour Lane, Bristol BS16 1QY, UK [d] Department of Biosciences, University of Oslo, Postboks 1066, Blindern 0316, Oslo, Norway

Corresponding author.   E-mail address: Good@cardiff.ac.uk (M.A. Good).

ABSTRACT

Three experiments examined the ability of mice to forage efficiently for liquid rewards in pots located in an open field arena. Search behaviour was unconstrained other than by the walls of the arena. All mice acquired the task within 4 days of training, with one trial per day. Experiment 1 tested the hypothesis that hippocampal lesions would disrupt foraging behaviour using extramaze cues. Mice with hippocampal lesions showed normal latency to initiate foraging and to complete the task relative to sham-operated mice. However, lesioned mice showed increased perseverative responding (sensitization) to recently rewarded locations, increased total working memory errors and an increased propensity to search near previously rewarded locations. In Experiment 2, the extramaze cues were obscured and each pot was identified by a unique pattern. Under these conditions, mice with hippocampal lesions showed comparable working memory errors to control mice. However, lesioned mice continued to display increased perseverative responding and altered search strategies. Experiment 3 tested the hypothesis that age-related accumulation of amyloid would disrupt foraging behaviour in transgenic PDAPP mice expressing the V717F amyloid precursor protein (APP) mutation. Consistent with previous findings, PDAPP mice showed both age-dependent and age-independent behavioural changes. More specifically, 14–16 month-old PDAPP mice showed a deficit in perseverative responding and working memory errors. In contrast, changes in search behaviour, such as systematic circling, were present throughout development. The latter indicates that APP overexpression contributed to some features of the PDAPP behavioural phenotype, whereas working memory and flexible responding was sensitive to ageing and β-amyloid burden. In conclusion, the present study provided novel insight into the role of the hippocampus and the effects of APP overexpression on memory and search behaviour in an open-field foraging task.

1. Introduction

Spatial working memory tasks, such as the radial arm maze and Barnes maze, often take advantage of rodent's natural propensity to forage for food. Such studies have informed our understanding of neural networks involved in spatial navigation and helped characterise the functional properties of hippocampal place cell and entorhinal grid cells in encoding location and movement information (Shapiro et al., 1997; Brunel & Trullier, 1998; Derdikman et al., 2009). There is a growing body of evidence that hippocampal and entorhinal networks are

sensitive to the early stages of Alzheimer's disease. For example, hippocampal place cells in amyloid precursor protein (APP) transgenic mice show reduced spatial resolution (Cacucci, Yi, Wills, Chapman, & O'Keefe, 2008; Zhao, Fowler, Chiang, Ji, & Jankowsky, 2014) and mice expressing human tau mutations show disrupted grid cell activity (Fu et al., 2017); similar to individuals possessing an APOE4 genotype (Kunz et al., 2015). Changes in spatial behaviour are well-documented in patients with Alzheimer's disease (Graham, 2015). For example, formal assessment of navigation strategies in patients indicates an early decline in path integration and allocentric memory processes in tasks analogues to the watermaze and the radial maze (Laczó et al., 2010; Lee, Kho, Yoo, Park, & Choi, 2014; Mokrisova et al., 2016). In addition, foraging for rewards in an open field arena has revealed deficits in allocentric memory in patients with Down syndrome, who are at increased risk of developing dementia (Lavenex et al., 2015).

Perhaps the most well-known foraging task is the radial arm maze designed by David Olton (Walker & Olton, 1979; Olton, Walker, & Wolf, 1982). In the simplest version of a radial arm maze task, all arms of the maze are baited and the animal has one opportunity to retrieve a food reward from an arm during the trial. As noted by Olton (1987), rodents may adopt a number of different strategies to solve the radial arm maze task. In order to restrict the development of certain spontaneous strategies, such as circling behaviour, rats can be confined to the central hub of the maze between arm selections. While the radial arm maze task can elicit accurate spatial working memory performance in rodents, it can also take several days to achieve such high levels of accuracy (e.g., Clark et al., 2015) and may limit assessment of alternative strategies that may also guide performance.

In the present study, an unconstrained open-field task was used to assess the nature of spontaneous foraging strategies that developed in mice following hippocampal cell loss and in mice developing amyloid pathology with age. The use of an unconstrained procedure can provide insights into the structure of mouse behaviour (c.f., Fonio et al., 2009; Benjamini et al., 2011), the underlying brain circuitry (Gordon et al., 2014) and thus the impact of disease on brain function. The present procedure was based on a task used by Pearce et al. (2005) to investigate foraging behaviour in pigeons. In this task, pigeons were placed in a large open field area and presented with eight food-baited pots, each in different spatial location. Pigeons had to forage the food reward from all eight pots and any return visits to depleted pots during the trial was considered a working memory (WM) error. We have adapted this task for mice using an open arena that contained six pots. Each pot was baited with a single liquid reward and mice were required to consume all six rewards in order to complete the task. Mice typically exhibit win-shift foraging behaviours, whereby they explore previously un-entered arms in favour of those already entered (Hyde, Hoplight, & Denenberg, 1998; Anagnostaras et al., 2003). Therefore, we hypothesised that wild type (WT) mice would quickly adopt a win-shift strategy and minimise the number of errors or return visits to previously depleted reward locations within a trial.

In order to characterise the effects of hippocampal (HPC) cell loss on the foraging, the first experiment examined the performance of male C57Bl/6 mice on the foraging task following excitotoxic lesions of the HPC (Experiment 1). We hypothesised that mice with hippocampal lesions would show increased working memory errors, i.e., return visits to depleted pots. In addition, based on evidence that rats with hippo- campal damage displayed an increased tendency to return to previously visited locations (Whishaw & Tomie, 1997; Honey, Marshall, Mcgregor, Futter, & Good, 2007), we also hypothesised that lesioned mice would display perseverative behaviour by returning immediately to locations recently visited and depleted of reward.

Previous studies have shown that the contribution of the hippo- campus to performance on the radial arm maze is related to the type of information (i.e., extramaze versus intra-maze cues) used to guide navigation. For example, Jarrard, Davidson, and Bowring (2004) showed that

rats with hippocampal lesion had severe deficits in spatial working and reference memory components of an 8 arm radial maze but lesioned rats were capable of acquiring a non-spatial version of the task to control levels of performance (see also, Jarrard, 1983; M'Harzi & Jarrard, 1992). To test the hypothesis that mice with HPC lesions would be able to forage efficiently using intra-maze cues, Experiment 2 assessed foraging when distal visual extramaze cues were obscured, by drawing a black curtain around the arena, and each pot was identified by a unique pattern on its external wall.

Finally, Experiment 3 examined whether foraging behaviour was disrupted in PDAPP mice over the course of ageing. The development of synaptic pathology caused by the accumulation of β-amyloid peptide in the brain is thought to be a key initial event in the development of memory loss, supported by the medial temporal lobe. Dodart and colleagues tested this hypothesis in PDAPP mice using a radial arm maze procedure (Dodart et al., 1999). Using an uninterrupted 3/8 reference and working memory task, PDAPP mice were impaired on both reference and working memory components at 3, 6 and 10 months of age. However, the nature of this impairment and whether search strategies change with age in PDAPP mice remains unclear. More recently, Clark et al. (2015) showed that the performance of 3xTg mice may depend on task requirements in the radial arm maze. More specifically, 3xTg developed age-dependent and age-independent deficits in a 4 from 8-arm radial arm procedure. Performance of 3xTg mice on an uninterrupted version of the radial arm maze was normal at both 3 and 8 months of age. In contrast, retention of a 4-baited arm procedure, that included a delay between choices, was impaired at both 3 and 8 months of age in transgenic mice. The main aim of Experiment 3 was therefore to determine whether the accumulation of Aβ pathology caused an age- related change in search or foraging strategy and working memory errors in PDAPP mice. The pattern of locations visited and the type of error made by control and mutant mice was assessed (within-subjects) during ageing using an uninterrupted foraging procedure. Based on results from previous studies with PDAPP mice, it was hypothesised that transgenic mice would show both age-independent and age-dependent impairments in performance.

2. Methods

2.1. Subjects

For experiments 1 and 2 a total of 26 male C57Bl/6 mice aged 6 months were used to assess HPC involvement in the foraging task. Thirteen mice received bilateral HPC excitotoxic lesions and 13 received control (SHAM) surgery (as described below). Three weeks prior to behavioural assessment. However, due to insufficient hippocampal damage, 2 mice were removed from Experiment 1 and 2 analysis. Therefore, a final number of 13 SHAM control mice and 11 HPC lesion mice were used to assess the role of the HPC in the foraging task. Experiment 3 used a total of 29 mice; 14 heterozygous male PDAPP mice (Games, Adams, Wolozin, & Zhao, 1995) expressing the hAPP^{V717F} genetic mutation and 15 wt littermate control mice (all maintained on a C57Bl/6 genetic background (Harlan) as previously described (Hartman et al., 2005). The same mice were tested at ages 6–8, 10–12 and 14–16 months of age to ascertain any age-dependent changes in performance in PDAPP mice.

All mice used throughout this study were housed in standard conditions in cages measuring L: 48 cm × W: 15 cm × H: 13 cm with an opaque plastic base and a wire top. The cage floors were covered in sawdust, approximately 1 cm deep, and contained a cardboard tube, wooden gnawing block and approved nesting material. Holding rooms were maintained at a stable temperature and relative humidity levels at around 21 °C ± 2 °C and 60 ± 10% respectively. Mice were given ad libitum access to food and water, unless otherwise stated as part of a behavioural test, and were kept on a 12hr light/dark cycle. All behavioural testing was carried out during the light hours. All animals were health-checked weekly and maintained according to UK Home Office and EU regulations and the Animal Scientific Procedures Act (1986).

2.2. Surgery

Mice were anaesthetised with Isoflurane [2-chloro-2- (di- fluromethoxy)-1, 1, 1-trifluoro-(ethane)] in O2 during stereotaxic surgery. The skull was exposed by a scalp incision. A bone flap was removed overlying the infusion sites in each hemisphere (see Table 1a). Infusions of 0.09 mM N-Methyl D-Aspartic Acid (NMDA, Sigma-Aldrich, UK) in sterile phosphate were delivered at a rate of 0.3 μl per minute into each hemisphere using a 30G cannulae microinjection 2μl Hamilton #75 syringe (Hamilton Company, Reno, USA). Following each infusion, the needle was left in place for 2 min before being retracted slowly. Upon completion, the wound was sutured and the animal was given a subcutaneous injection of gluco-saline to aid rehydration. In SHAM-operated mice, 2 holes were drilled in accordance to the stereotaxic coordinates in Table 1 before being sutured. Each mouse was then placed in a 30 °C temperature controlled recovery chamber with monitoring until the mouse was deemed alert and mobile. Following this, mice were returned to a new home cage containing a sawdust bedding, covered in tissue paper to reduce sawdust entering the wound. Mice were also provided with sweetened porridge (ReadyBrek) for 24 h. post-surgery to encourage eating and subsequently were given ad libitum access to standard mouse chow and water.

2.3. Perfusion

Mice were given an intraperitoneal (IP) injection of 0.2 ml 200 mg/ ml pentobarbitol (Euthetal, Merial, Harlow, UK) to induce terminal anaesthesia. The heart was exposed and a cannula inserted into the left ventricle. Approximately 50 ml of 0.1 M PBS (pH 7.4) was then pumped through the circulatory system. Following this, approximately 100 ml of 4% paraformaldehyde in 0.1M PBS (PFA) was infused through the circulatory system to initially fix brain tissue. The brain was then extracted and post-fixed in 4% PFA at room temperature (RTP) for 6 h before being transferred to 30% reagent grade sucrose in dH$_2$O. The brain remained in sucrose until it sank, indicating it was fully saturated (approximately 48 h). Brains were then sliced using a freezing micro- tome. 40 μm coronal sections were mounted on gelatinised slides in 0.1 M PBS. Slides were left to dry for 48 h prior to staining.

2.4. Cresyl violet staining

Staining of coronal sections was carried out by immersing slides in xylene for 4 min before immersion into descending concentrations of ethanol (100% → 90% → 70%) for 2 min per ethanol concentration. Slides were then immersed in dH^{2}O for 2min before 0.005% Cresyl violet was applied for 3min. Slides were then further immersed in dH^{2}O for 30s before dehydrated in an ascending concentration of ethanol (70% → 90% → 100% → 100%) for 3 min per immersion. Slides were given two final exposures to xylene, each for 5 min. Finally, slides were cover-slipped with DPX Mounting media and allowed to dry for 48 h. Sections were then imaged using a Leica DMRB microscope and images were captured using an Olympus DP70 camera and assessed using the programme analySIS-D.

Lesion size. Although the main intention was to produce a set of homogeneous lesions across animals, the% of hippocampus damaged was estimated for each animal was estimated. Recreation of the lesion from each animal was drawn on images obtained from Paxinos and Franklin (2004) stereotaxic atlas and printed onto 1 mm square graph paper. A total of 7 schematic sections were used. The first section used was 1.82 mm posterior to bregma followed by 2.06 mm, 2.30 mm, 2.54 mm, 2.80 mm, 3.08 mm, and the final section 3.28 mm from bregma. Lesioned areas were drawn onto 1 × 1 mm graph paper from lesioned images and lesion size calculated as follows: percentage lesioned area was calculated as (hippocampal area lesioned/total hippocampal area) ∗ 100. Dorsal hippocampus was defined as 3 mm ventral from the horizontal plane passing through bregma and lamda on the surface of the skull. Ventral hippocampus was defined as starting from 2.54 mm posterior to bregma as described by Paxinos and Franklin (2004). The% of area occupied by the lesion across the

7 coronal section templates was then calculated.

2.5. Apparatus

All training and testing was carried out in a quiet testing room. The room contained a variety of extra-maze visual cues (e.g., wall posters, shelving, equipment, etc.) around the walls of the test room at a height observable from inside the arena. The position of the extra-maze cues, the experimenter and recording equipment remained constant throughout the study. Initial training on consuming rewards from the pots was carried out in identical home cages (L 48 cm × W 15 cm × H 13 cm) with a 1 cm deep bed of sawdust covering the floor. White ceramic pots (Lakeland, UK) with a diameter of 6.5 cm and a depth of 3.5 cm were mounted on a wooden cube base measuring 3 × 3 × 6 cm. Pots were secured to the floor of the cage/arena with blue-tac. Following initial training, mice were exposed to the same pots in the test arena measuring 60 cm × 60 cm with 40 cm high walls. The same arena was used for all experiments in this study. The walls were made of clear Perspex and covered externally with white card. The arena was placed on a stand and elevated 50 cm above floor level in the centre of the test area. The floor of the arena was also covered in sawdust, approximately 1 cm in depth. In the arena, the reward pots were arranged approximately 20 cm apart. Each trial was recorded using a camera (VM-904K, Shiba Electrics Ltd, Hong Kong) suspended above the centre point of the arena connected to a DVD recorder (Panasonic DMR E50EBS), and time taken to complete the task was measured with an electronic stopwatch (Fischer Scientific, UK) by the experimenter.

2.6. Procedure

Training (homecage): Throughout the training and test phase, mice were water-deprived to approximately 90% of their pre-training weight. Water was given for 4 h immediately after training or testing each day. The first stage of training encouraged mice to associate a liquid reward (1:3 sweetened condensed milk (Nestle) solution, prepared in water; H_2O) with a ceramic pot. During initial training, mice were removed from their home cage and placed into an identical home cage with sawdust bedding together with one ceramic pot placed in the centre of the cage, for three successive trials separated by a 5-min intertrial-interval. Between each mouse, pots were wiped clean with 70% ethanol wipes to remove any odour cues, and the milk solution replenished. On the first day, the ceramic pot was baited with lowering volumes of milk solution (50, 10 and 5 ml). Once a mouse began to consume the reward, it was removed immediately from the cage and returned to its home cage. Mice were given no more than 10 min per trial to consume the liquid reward. When mice had successfully demonstrated drinking behaviour with the volumes described above, the volume was reduced to 30υL, which was pipetted into the centre of the pot. This volume was used for the remainder of training and testing. This procedure was repeated until each mouse had consumed the 30uL reward on each trial for 2 consecutive days.

Training (Test Arena): Mice continued reward training in the test arena. Mice were initially exposed to an empty arena with sawdust covering the base for 10 min to allow free exploration. For the following 3–4 consecutive days of training, 2 baited pots were placed diagonal across from one another in the arena, 40 cm apart, 10 cm from the arena walls (Fig. 1A). On each day, the location of the pots was moved to a new location to prevent the development of any systematic search bias in the test phase. Mice were placed into the centre of the arena and allowed to explore until they had consumed both rewards or a 10-min time limit was reached. After this the mouse was returned to its home cage. This process was repeated until all mice foraged in both pots in < 3 min (3–4 consecutive days).

Testing: Mice were then tested over the next 4 consecutive days with one session per day. During these sessions the arena was set up with six pots arranged in a circular shape, each 20 cm apart (Fig. 1B). Each pot contained 30uL of milk solution. The order of testing was

counter- balanced and each mouse in turn was taken from their home cage and placed in the centre of the arena facing away from the experimenter. The mouse was then allowed to explore the arena and forage pots until they had consumed all 6 rewards or until 10 min had elapsed from when the first pot was foraged. Following the trial, mice were returned to their home cage. The pots were then wiped clean with 70% ethanol wipes and the milk solution replenished before the next mouse was tested. All test sessions were recorded onto a DVD player using an overhead camera. All training and test protocols remained identical across all experiments. However, in Experiment 2, a black curtain was drawn around the test arena to remove the extramaze visual cues. The pots were also now individually designed and patterned distinctively from one another (Fig. 1C). To prevent any consistency between the relative spatial locations of the pots across the 4 days of testing, pots were swapped their location each day, so that no individual pot was neighbouring the same 2 pots on any test day.

2.7. Scoring

A score of foraging behaviour was defined as a mouse jumping onto the rim of a pot and directing its nose in towards the bottom to consume a reward. A number of error scores were taken from this task to assess performance. They are detailed in Table 2. In this scoring procedure, "total error" acted as a measure of efficient foraging behaviour. Other measures were used to assess within-trial behaviours, such as, perse- verative and repeat errors. Perseverative errors were defined as immediate return to a pot that was just foraged, with no intervening visits to other pots. Such perseverative behaviours have been observed in HPC lesioned animals and patients with AD and mouse models of AD pathology (Lamar et al., 1997; Huitrón-Reséndiz et al., 2002; Yoon, Okada, Jung, & Kim, 2008). In contrast, a repeat error was defined as a mouse returning to a pot where an error had already been made during the trial. This error was independent of the perseverative error measure, as the latter reflected the mouse's immediate return to a pot that had just been foraged. As total error incorporated all types of errors made within the trial, the repeat error was able to provide a measure of within-trial memory for foraged pots that was independent of perse- verative approach behaviours.

The order in which the pots were visited was recorded. This measure assessed the extent to which chaining responses (such as circling behaviour) mediated task performance. Response chaining was defined as foraging in pots that lie adjacent to each other in a sequence, e.g., foraging successively in pot 1, 2, 3, etc. The adoption of circling behaviour has been noted in a number of rodent navigation studies and is thought to reflect a strategy to reduce working memory load. The spatial distribution of errors across the arena was also recorded. More specifically, errors made in pots neighbouring a pot that had just been foraged or errors made to pots distal to one just foraged were recorded. This score was calculated as a ratio against total errors made by each mouse to provide a measure of response bias that was independent of the total number of errors.

The total time taken to complete the task and the time taken to initiate pot exploration after being released was recorded. These measures provided an index of changes in motivation and/or anxiety on task performance.

In Experiment 3, PDAPP and WT mice were tested using the same procedure described above. The same mice were tested longitudinally at 6–8, 10–12 and 16–18 months of age.

2.8. Statistical analysis

Data was analysed using Microsoft Excel for calculation of mean number of errors, times and standard error of the mean. IBM SPSS Statistics software was used to analyse all data statistically. An α-level of 0.05 was used for all analyses. Effect sizes were reported for all statistics: Cohen's d (d) was calculated for independent sample t-tests, partial eta-squared

($\eta_p{}^2$) for ANOVA analysis, Cohen's r value for Mann- Whitney U tests (r) and Kendall's W for Friedman tests (Cohen, 1973, 1988; Fritz, Morris, Richler, 2012; Tomczak & TomcZak, 2014). The data were checked for violations of distribution and homogeneity of variance by Shapiro-Wilk test and Levene's test respectively. Due to high levels of variability in data sets, and the frequency of zero scores in the error measures, violations of these tests were observed ($p < 0.05$). Therefore, data that violated these tests were subjected to transformation (i.e. square root, log-10) based on the level of positive/negative skew and reassessed. Data that then showed no further violations of distribution were analysed by repeat measures ANOVA and independent samples t-test. Data that could not be transformed due to the presence of zeros in the data were analysed using non-parametric statistics. Mann-Whitney U Tests were used to compare between group factors and Wilcoxon Signed-Rank Tests or Friedman's test with Bon- ferroni correction, to adjust for multiple post hoc comparisons, were used to compare within subject factors.

3. Results

3.1. Histology

An example of the maximum and minimum tissue damage obtained as a result of excitotoxic lesions are displayed in Fig. 2 respectively. Two lesioned animals were removed from the study following histological analysis due to complete sparing of the ventral hippocampus. Eight mice showed a complete lesion of the HPC with the exception of the most posterior ventral DG (often observed unilaterally) and small sparing of the ventral CA1 and CA3 of the HPC. Three mice showed complete removal of the dorsal HPC with further bilateral ventral hippocampal damage. As observed in Fig. 2A, an acceptable minimal lesion showed intact ventral HPC structure unilaterally at the most posterior reference. Nevertheless, damage was observed in the ventral DG. Cortical damage around the infusion site was observed in all le- sioned animals, predominantly in the parietal association cortex and visual cortex. No cell loss was detectable in the SHAM control mice, except for two mice that displayed restricted unilateral damage to the visual cortex.

The mean% area of the hippocampus damaged in the lesion group across both hemispheres and dorsal–ventral extent of the structure is shown in Table 1b. The lesion size was relatively homogeneous between animals. None of the behavioural measures showed a correlation with lesion size (data not shown), which presumably reflected the lack of systematic variation in lesion size.

3.1.1. Experiment 1: Hippocampal lesions disrupt spatial working memory and alter foraging strategies

The latency measures across all 4 trials can be observed in Table 4. Mice with hippocampal lesions showed very similar times to SHAM controls with respect to the total time taken to complete the task as well as the time taken to engage with the task, $t(22) = -0.15$, $p = 0.89$, d $= 0.06$ and $t(22) = 0.83$, $p = 0.42$, $d = 0.34$ respectively. These results indicate that any changes in foraging performance between these two groups were not an effect of gross motor and/or motivational differences.

However, lesioned mice did show changes in foraging behaviours. The mean number of total errors and errors acquired during training are shown in Table 3 and Fig. 3A, respectively, A repeated measures ANOVA showed no significant main effect of trial, $F(3, 66)=0.48$, $p = 0.69$, $\eta_p{}^2 = 0.02$ and no significant trial × group interaction, $F(3, 66) = 0.03$, $p = 0.99$, $\eta_p{}^2 = 0.001$. However, there was a main effect of group, $F(1, 22) = 154.2$, $p = 0.007$, $\eta_p{}^2 = 0.29$, which confirmed that HPC lesioned mice made more errors across acquisition. In addition, mice with hippocampal lesions showed a greater number of perse- verative errors across

training (see Table 5), Mann-Whitney U Test, U = 24.5, z = −2.77, p = 0.006, r = −0.56. HPC lesioned mice also showed a greater number of chaining responses compared to SHAM controls, Mann-Whitney U test, U = 30.00, z = −2.48, p = 0.013, r = −0.49. Thus, hippocampal cell increased search errors, especially perseverative and chaining responses.

The number of errors in neighbouring versus distal pots are presented as a ratio of total errors, corrected for perseverative errors, is presented in Fig. 3C. An analysis of these scores revealed that HPC lesioned mice had a significantly higher ratio of error scores in neighbouring pots compared to SHAM controls, $t(22) = -2.14$, p = 0.044, d = 0.13. Furthermore, HPC lesion mice made fewer errors in distal pots compared to SHAM control mice, $t(22) = 2.14$, p = 0.044, d = 0.13. An analysis of repeat error scores (excluding perseverative errors (see Fig. 3B) indicated that HPC lesioned mice displayed more repeat errors, $t(12.15) = -3.24$, p = 0.007, d = 1.37. Hippocampal Lesions in mice increased visits to depleted pots and increased local search behaviours.

3.1.2. Experiment 2: Hippocampal lesions disrupt foraging behaviour based on intramaze cues

Similar to the results observed in Experiment 1, HPC lesioned mice showed very similar times in both total time and latency to engage relative to SHAM control mice (see Table 4), $t(22) = -0.29$, p = 0.77, d = 0.12, and $t(22) = 0.20$, p = 0.84, d = 0.083 respectively.

Inspection of Table 5 shows that mice with HPC lesions continued to display a greater number of perseverative errors compared to control mice. However, this difference was not statistically different, $t(22) = -2.28$, p = 0.056, d = 0.89. HPC lesioned mice also showed a greater chaining response ratio when compared to SHAM control mice, $t(22) = -4.05$, p = 0.001, d = 1.70 (Table 5). This chaining strategy was further accompanied by a greater number of errors in neighbouring pots (corrected for perseverative errors), $t(22) = -3.28$, p = 0.003, d = 1.32 and less in distal pots, $t(22) = -3.28$, p = 0.003, d = 1.322 (Fig. 4C). Thus, similar to Experiment 1, mice with hippocampal lesions continued to show altered search behaviours when pots were identified by individual patterns.

The total number of errors, shown in Fig. 4A and Table 3, were analysed by a repeated measures ANOVA. Results showed a non-significant difference between lesion group, $F(1, 22) = 131.4$, p = 0.31, $\eta_p^2 = 0.05$, a non-significant within-subjects main effect of trial, $F(3, 66) = 2.44$, p = 0.072, $\eta_p^2 = 0.10$, and a non-significant trial × group interaction, $F(3, 66) = 2.42$, p = 0.074, $\eta_p^2 = 0.01$. No between group difference was evident for the mean number of repeat errors (corrected for perseverative errors; Fig. 4B), U = 52.0, z = 1.02, p = 0.25, r = −0.23. The introduction of intramze cues on the pots reduced the errors displayed by mice with hippocampal lesions to a level shown by control mice.

3.1.3. Experiment 3: PDAPP mice display age-independent changes in foraging strategy, but age-related impairment in working memory performance

Motor and engagement performance of PDAPP mice in the foraging task are reported in Table 4. A repeat measures ANOVA of total time with a between subject factor of genotype and within subject factor of age revealed a non-significant main effect of genotype, $F(1, 27) = 3.48$, p = 0.073, $\eta_p^2 = 0.11$, a non-significant main effect of age, $F(1.56, 42.13) = 2.62$, p = 0.096, $\eta_p^2 = 0.09$ and no significant age ∗ genotype interaction, $F(1.56, 42.13) = 0.19$, p = 0.78, $\eta_p^2 = 0.01$. A similar analysis of engagement time revealed no significant main effect of genotype, $F(1, 27) = 3.88$, p = 0.059, $\eta_p^2 = 0.13$, a significant main effect of age, $F(1.64, 44.37) = 3.57$, p = 0.045, $\eta_p^2 = 0.12$, and no significant age ∗ genotype interaction, $F(2, 54) = 0.67$, p = 0.67, $\eta_p^2 = 0.024$. Thus, engagement time decreased with age and this effect was

comparable across genotype.

Perseverative behaviours (see Table 5) were analysed with non- parametric Mann-Whitney U Tests for between-subject comparisons and Friedman Test for within subject effects of age. The Man-Whitney U Tests revealed no significant main effect of genotype at 6–8 months, U = 104.5, z = −0.2, p = 0.98, r = 0.004, 10–12 months, U = 129.5, z = 1.09, p = 0.29, r = 0.20, but a significant main effect of genotype at 14–16months of age, U=174.5, z=3.09, p=0.002, r=0.57. A Friedman test reported that there was a significant within-subject effect of age in WT mice, $X^2(2) = 6.70$, p = 0.041, Kendall's W = 0.22. Pairwise comparisons were then performed with a Bonferroni correction for multiple comparisons and revealed no significant differences between any individual age ranges in WT mice (6–8M vs 10–12M p = 0.25, 6–8 M vs 14–16 M p = 0.99, 10–12 M vs 14–16 M p = 0.053). A significant within-subject effect of age was also obtained in PDAPP mice, $X^2(2) = 6.37$, p < 0.035, Kendall's W = 0.23. Pairwise comparisons further revealed a significant difference between perseverative errors made at 6–8months and 14–16months of age, p=0.023 (no significant differences were reported when comparing 6–8M vs 10–12M, p=0.09, or 10–12M vs 14–16M, p=0.57). Therefore, PDAPP mice showed an age-dependent increase in perseverative errors at 14–16 months of age.

An analysis of chaining responses (Table 5) and error location ratios (Fig. 5C) showed that PDAPP mice exhibited more chaining responses overall. The ANOVA revealed a main effect of group, $F(1, 27) = 39.77$, p < 0.001, $\eta_p^2 = 0.59$ and age, $F(2, 54) = 5.18$, p = 0.009, $\eta_p^2 = 0.16$, but there was no significant interaction between these factors, $F(2, 54) = 1.09$, p = 0.35, $\eta_p^2 = 0.04$. A similar pattern of results was observed with the ratio of neighbouring and distal errors (corrected for perseverative errors; Fig. 5C). An analysis of errors in neighbouring pots revealed a significant main effect of genotype, $F(1, 27) = 17.44$, p < 0.001, $\eta_p^2 = 0.39$, a significant main effect of age, $F(2, 54) = 4.53$, p = 0.015, $\eta_p^2 = 0.14$, and no significant age × genotype interaction, $F(2, 54) = 0.19$, p0.82, $\eta_p^2 = 0.01$. Analysis of the ratio of distal errors revealed a main effect of genotype, $F(1, 27) = 17.44$, p < 0.001, $\eta_p^2 = 0.39$, a main effect of age, $F(2, 54) = 4.53$, p = 0.015, $\eta_p^2 = 0.14$, and no significant age × genotype interaction, $F(2, 54) = 0.19$, p0.82, $\eta_p^2 = 0.01$. Thus, although ageing was associated with increases in the error ratio and chaining response, this effect was similar for both WT and PDAPP mice. Consequently, PDAPP mice showed an age-independent difference in foraging strategy.

Inspection of total errors and repeat errors (corrected for perseverative errors; see Table 3 and Fig. 5A) suggested a trend for PDAPP mice to show a greater number of total errors with age. A repeat measures ANOVA with genotype as the between-subject factor and age and trial as the within-subject factors revealed a non-significant main effect of genotype, $F(1, 27) = 1.84$, p = 0.18, $\eta_p^2 = 0.06$, a non-significant main effect of age, $F(2, 54) = 0.13$, p = 0.88, $\eta_p^2 = 0.005$, a non-significant age × genotype interaction, $F(2, 54) = 2.50$, p = 0.09, $\eta_p^2 = 0.09$, a non-significant main effect of trial, $F(2.1, 54.56) = 0.23$, p = 0.81, $\eta_p^2 = 0.01$ (Greenhouse-Geisser corrected), a non-significant trial × genotype, $F(3, 81) = 0.71$, p = 0.55, $\eta_p^2 = 0.03$, a non-significant age × trial interaction, $F(6, 162) = 0.60$, p = 0.73, $\eta_p^2 = 0.02$, and a non-significant age × trial × genotype interaction, $F(6, 162) = 0.46$, p = 0.84, $\eta_p^2 = 0.02$.

Repeat errors (see Fig. 5B) were analysed using non-parametric Mann-Whitney U test (between-subjects) and Friedman's test (multiple within-subjects comparison) due to violations of Shapiro-Wilk test, p < 0.05 and inability to transform data due to a number of zero scores in the data set. Results from Mann-Whitney U test revealed no significant differences between genotypes at 6–8months of age, U = 104.5, z = -0.22, p = 0.98, r = 0.004, 10–12 months of age, U = 139.5, z = 1.52, p = 0.13, r = 0.28, but a significant difference

between WT and PDAPP mice at 14–16 months of age, $U = 150.5$, $z = 1.99$, $p = 0.046$, $r = 0.37$. Within-subjects analysis revealed no significant effect of age in WT mice, $X^2(2) = 1.2$, $p = 0.55$, Kendall's $W = 0.04$, or in PDAPP mice, $X^2(2) = 2.33$, $p = 0.31$, Kendall's $W = 0.08$. This analysis shows that PDAPP mice were impaired relative to WT control animals only at 14–16 months of age.

4. Discussion

This study used a procedurally simple open-field uninterrupted foraging task to evaluate the role of the hippocampus (HPC) in both spatial and non-spatial working memory (SWM). Experiments 1 and 2 tested the hypothesis that mice with excitotoxic lesion of the HPC would show a selective deficit in the foraging task when extramaze cues guided performance compared to a similar task in which intramaze cues guided performance.

The results from Experiments 1 showed that HPC cell loss impaired foraging behaviour when the pots were distinguished by their spatial location. Under these conditions, mice with hippocampal lesions displayed more total, perseverative, and repeat errors and a bias towards searching in previously rewarded locations relative to control mice. In contrast, when the distal extramaze cues were obscured and the pots identified by unique patterns, mice with hippocampal lesions displayed similar total errors and repeat errors relative to control mice. Nevertheless, the lesioned mice continued to display a tendency to wards perseverative behaviour and a bias towards searching in the vicinity of previously rewarded pots. Thus, both tasks revealed that mice with hippocampal lesions showed marked perseveration in returning to either locations or distinctive pots previously visited on a trial. This sensitization of approach responses parallels observation reported by Honey et al. (2007). In the latter study, rats with hippocampal lesions showed a greater tendency to revisit locations in an open field that had recently been visited. The present study confirmed that observation in mice with hippocampal lesions and also showed that sensitization of exploratory behaviour was less evident when intramaze cues (patterned pots) were used; although there was a numerical tendency for lesioned mice to show greater perseverative responding. A similar tendency for rats to return to intramaze cues that were previously associated with reward in a radial arm maze was also noted by Jarrard et al. (2004); although this effect was ameliorated with additional training.

To summarise thus far, the presence of intramaze cues to locate rewards mitigated the strategy of returning to previously foraged pots adopted by mice with hippocampal lesions. In other words, working memory errors in mice with hippocampal lesions was comparable to control mice when behaviour reflected the use of intramaze cues. Nevertheless, mice with hippocampal lesions continued to show a trend to sensitization of approach responses to previously baited pots and a significant bias to visiting adjacent pots. This general tendency to show sensitization of approach responses has been noted previously in a simple open-field exploration task by Honey and colleagues (Honey & Good, 2000; Honey et al., 2007) and was interpreted as evidence for disrupted short-term memory processes. The present study has therefore extended this observation to the use of a rewarded foraging task using extra-maze and intra-maze cues.

It is worth noting that, in contrast to the current study, Olton and Werz reported that rats with HPC lesions showed a preference for foraging in arms far away from the arm where they had just received a reward (Olton & Werz, 1978). There are several important differences between the current study and that conducted by Olton and Werz (1978), not least is the use of a different apparatus, species and very different lesion procedures. The latter difference may be paramount in accounting for changes in exploratory behaviour of lesioned animals between the two studies. Although mice show comparable levels of performance on many navigation tasks developed with rats; there is increasing evidence for subtle differences between species. For example, mice tend to show less stable place fields and subtle

differences in navigational strategies to solve similar tasks, such as the watermaze (see review by Hok et al., 2016). It is important therefore to characterise the pattern of normal mouse behaviour and their neural substrates to facilitate interpretation of genetic modifications.

Experiment 1 and 2 provided evidence that disruption to hippocampal function impaired foraging behaviour. Experiment 3 was designed to test that the hypothesis that APP overexpression and or progressive build-up of amyloid in the brain of transgenic mice expressing the human APP V717F mutation would lead to impairments in foraging behaviour. The experiment focussed on the use of extramaze cues by PDAPP mice in order to (1) assess parallels with foraging impairments induced by hippocampal cell loss; and (2) because prior evidence indicated that spatial working memory was disrupted in both an age-independent and age-dependent manner using an uninterrupted radial arm task. One novel feature of Experiment 3 was the within-subject longitudinal design to assess changes in spatial information processing in PDAPP mice; other studies to date have used cross-sectional designs to assess changes in spatial working memory in PDAPP mice (Dodart et al., 1999). Experiment 3 revealed there was an age-independent deficit in chaining behaviour and increased tendency to visit near as opposed to distal pots. This indicates that overexpression of human APP was sufficient to disrupt foraging behaviours independently of an interaction with age and amyloid accumulation. Although the precise mechanism for this change is not clear, recent work has highlighted that APP (and PS1) overexpression can cause chronic elevation of cytoplasmic calcium and the calpain-calpastatin system (Saito et al., 2016), which may disrupt the dynamics of synaptic plasticity processes (Baudry and Bi, 2016).

It is worth commenting on the adoption of chaining or circling responses by HPC lesioned and transgenic PDAPP mice. This strategy was accompanied by an increase in the ratio of errors made to adjacent pots. Altered foraging responses and escape strategies have previously been reported in HPC lesioned mice and transgenic models of amyloid pathology, including PDAPP mice (Olton & Werz, 1978; Chen et al., 2000; Huitrón-Reséndiz et al., 2002; Janus, 2004). In PDAPP and TgCRND8 models, altered search strategies in the Barnes maze and MWM have been associated with age and with age-related increases in amyloid pathology (Johnson-Wood et al., 1997; Chishti et al., 2001; Huitrón- Reséndiz et al., 2002; Janus, 2004). PDAPP mice also displayed an age- independent deficit in non-spatial search strategies in the Barnes maze from 3 to 5 months of age (Huitrón-Reséndiz et al., 2002). Similar to this, chaining strategies were observed in the foraging taging task in PDAPP mice from 6 to 8 months of age. This behaviour likely refected a strategy designed to reduce the load on short-term or working memory. With PDAPP mice, the adoption of this strategy was age-independent and be a compensatory mechanism to overcome deficits potentially caused by APP overexpression.

There is an alternative explanation for the increase in errors made to adjacent pots and that is these errors may result from a deficit in discriminating similar locations with overlapping elements or features. Spatial pattern separation is a process in which memory components containing similar or overlapping features are separated to form independent memories (Rolls, 2013). The DG and CA3 regions of the hippocampus have been identified as key components of a spatial pattern separation system (Gold & Kesner, 2005; Yassa and Stark, 2011). Gracian and colleagues used a radial arm maze procedure and showed that aged rats made significantly more errors than young rats when performance relied on discriminating adjacent arms when spatial interference was high (Gracian, Shelley, Morris, & Gilbert, 2013). In contrast, when the discrimination was based on more distal arms, aged and young rats were comparable. Spatial pattern separation is therefore affected by age. Lesions of the dentate gyrus similarly disrupt discrimination of adjacent spatial locations (Morris, Churchwell, Kesner, & Gilbert, 2012) and PDAPP mice show early disruption of dentate gyrus volume and neurogenesis (Redwine et al., 2003; Wu et al., 2004; Donovan et al., 2006). Thus, APP overexpression and dentate gyrus morphological abnormalities may contribute to the early onset of adjacent location errors by PDAPP mice. To explore this issue more directly, further

experiments assessing the ability of PDAPP mice to discriminate adjacent and distal pots would be required. An alternative strategy would be to interrupt chaining responses, for example, by returning the animal to the central zone after reward retrieval; although this may introduce other complications.

Finally, two behavioural measures did reveal an age-related change in PDAPP mice. Fourteen-to-sixteen month-old transgenic mice showed increased perseverative responding and an increase in the number of repeat errors relative to WT mice. The increase in perseverative responding and repeat errors is consistent with a disruption to short-term or working memory processes. Indeed, a similar pattern of age-dependent and independent spatial memory deficits in PDAPP mice has also been reported using a serial position watermaze task (Chen et al., 2000; Daumas et al., 2008). In these experiments, mice were required to learn multiple platform locations and the deficit in PDAPP mice was thought to reflect sensitivity to proactive interference. There was no report of a tendency to re-visit previously reinforced locations in the Daumas et al. (2008) study. Nevertheless, Huitron-Resendiz et al. (2002; see also Daumas et al., 2008) reported that young PDAPP mice (3–5 months of age) showed a tendency to display repeat visits to previously located areas in an open field escape task. However, it is unclear whether this "perseverative" measure reflected immediate returns to a location (as specified in the present study) or errors distributed across the session – what we refer to as repeat errors. In addition, the onset of the water- maze navigation deficit was much earlier than that reported in the present study. Of course, there are several procedural differences that may account for this, including the use of a water escape task that may encourage different strategies, and differences in background strain (Swiss Webster × C57BL/6 × DBA/2). Nevertheless, taken together, the current pattern of results along with the results of previous experiments are consistent with an age-related disruption to short-term or working memory processes in PDAPP mice. There is increased sensitivity of working memory and executive function to decline across both normal ageing and during pathological changes associated with pre- clinical and clinical stages of dementia in patients (Kirova, Bays, & Lagalwar, 2015). The present results are consistent with the view that the accumulation of β-amyloid is a contributing factor to the disruption of spatial working memory processes and flexible behaviour.

Before concluding, it is worth considering the pros and cons of the foraging task. First of all, the task is (relative to the radial arm maze task) quick to acquire by mice. The use of an appetite reward as opposed to an escape/avoidance may mitigate issues associated with use of aversive stimuli, e.g., anxiety. Furthermore, the task allows for repeat testing and is clearly sensitive to both age-dependent and age-independent changes in brain function associated with genetic manipulations. The task also allows investigation of foraging behaviour based on extramaze and intramaze information. The analysis provides measures similar to that obtained from the all arms baited uninterrupted radial arm maze task, including perseverative responding and repeat or working memory error. Other adaptations of the task include the use of baited and non-baited pots to investigate reference and working memory performance in mice. The apparatus can also be easily moved to new contexts to examine context-dependent aspects of performance. One of the main limitations of the task, in its current form, is that the experimenter cannot directly manipulate animal's choice behaviour without directly interacting with the animal. For example, interpolating delays between choices would mean physically interacting with the mouse. This may not be of major concern as other working memory procedures, such as the T-maze forced choice alternation task, also involve interacting with the animal.

In summary, the main aim of this study was to investigate the effects of hippocampal cell loss and APP overexpression on foraging behaviour in an unrestricted choice procedure. Hippocampal cell loss in mice disrupted efficient foraging when pots were discriminated on the basis of their location (using extra maze cues) but less so when the pots were identified by unique patterns. Changes in the behaviour of lesioned mice that were common to both

versions of the task were increased number of perseverative errors, increased preference for searching in adjacent pots and the adoption of a chaining/circling strategy. PDAPP mice showed an age-independent deficit in adoption of a circling strategy, and an age-dependent increase in perseverative responding and repeat or working memory errors. Whilst the former is likely an effect of APP overexpression (Saito et al., 2016), the latter effects are likely caused by an accumulation of amyloid pathology (Games et al., 1995; Johnson-Wood et al., 1997; Hartman et al., 2005). In conclusion, mice with hippocampal lesions and mice expressing an APP mutation showed disruption of spontaneous foraging behaviour and the nature of the strategies adopted to maximise reward compared to WT control mice. In the latter case, APP mice showed both age-dependent and –independent effects of the mutation on performance. This highlights the potential utility of foraging tasks to assess components of navigation behaviour and working memory processes in animals with compromised hippocampal function.

References

Anagnostaras, S. G., Murphy, G. G., Hamilton, S. E., Mitchell, S. L., Rahnama, N. P., Nathanson, N. M., & Silva, A. J. (2003). Selective cognitive dysfunction in acet- ylcholine M1 muscarinic receptor mutant mice. NatureNeuroscience, 6(1), 51–58.

Baudry, M., & Bi, X. (2016). Calpain-1 and Calpain-2: The Yin and Yang of synaptic plasticity and neurodegeneration. Trends in Neurosciences, 39(4), 235–245.

Benjamini, Y., Fonio, E., Galili, T., Havkin, G. Z., & Golani, I. (2011). Quantifying the buildup in extent and complexity of free exploration in mice. Proceedings of the National Academy of Sciences, 108(Suppl 3), 15580–15587.

Brunel, N., & Trullier, O. (1998). Plasticity of directional place fields in a model of rodent CA3. Hippocampus, 8(6), 651–665.

Cacucci, F., Yi, M., Wills, T. J., Chapman, P., & O'Keefe, J. (2008). Place cell firing cor- relates with memory deficits and amyloid plaque burden in Tg2576 Alzheimer mouse model. Proceedings of the National Academy of Sciences, 105(22), 7863–7868.

Chen, G., Chen, K. S., Knox, J., Inglis, J., Bernard, A., Martin, S. J., ... Morris, R. G. M. (2000b). A learning deficit related to age and b -amyloid plaques in a mouse model of Alzheimer's disease. Nature, 2940(1998), 975–979.

Chen, G., Chen, K. S., Knox, J., Inglis, J., Bernard, A., Martin, S. J., ... Morris, R. G. M. (2000a). A learning deficit related to age and [beta]-amyloid plaques in a mouse model of Alzheimer's disease. Nature, 408(6815), 975–979.

Chishti, M. A., Yang, D. S., Janus, C., Phinney, A. L., Horne, P., Pearson, J., ... Westaway,

D. (2001). Early-onset amyloid deposition and cognitive deficits in transgenic mice expressing a double mutant form of amyloid precursor protein 695. The Journal of Biological Chemistry, 276(24), 21562–21570.

Clark, J. K., Furgerson, M., Crystal, J. D., Fechheimer, M., Furukawa, R., & Wagner, J. J. (2015). Alterations in synaptic plasticity coincide with deficits in spatial working memory in presymptomatic 3xTg-AD mice. Neurobiology of Learning and Memory, 125, 152–162.

Cohen, J. (1973). Eta-squared and partial eta-squared in fixed factor ANOVA designs. Educational and Psychological Measurement, 33, 107–112.

Cohen, J. (1988). Statistical power analysis for the behavioural sciences (2nd ed.). New York: Academic Press.

Daumas, S., Sandin, J., Chen, K. S., Kobayashi, D., Tulloch, J., Martin, S. J., ... Morris, R. G. M. (2008). Faster forgetting contributes to impaired spatial memory in the PDAPP mouse: Deficit in memory retrieval associated with increased sensitivity to inter- ference ? Learning & Memory, 625–632.

Derdikman, D., Whitlock, J. R., Tsao, A., Fyhn, M., Hafting, T., Moser, M.-B., & Moser, E. I. (2009). Fragmentation of grid cell maps in a multicompartment environment. Nature Neuroscience, 12(10), 1325–1332.

Dodart, J. C., Meziane, H., Mathis, C., Bales, K. R., Paul, S. M.a., & Ungerer (1999). Behavioral disturbances in transgenic mice overexpressing the V717F beta-amyloid precursor

protein. Behavioral Neuroscience, 113(5), 982–990.

Donovan, M. H., Yazdani, U., Norris, R. D., Games, D., German, D. C., & Eisch, A. J. (2006). Decreased adult hippocampal neurogenesis in the PDAPP mouse model of Alzheimer's disease. The Journal of Comparative Neurology, 495(1), 70–83.

Fonio, E., Benjamini, Y., & Golani, I. (2009). Freedom of movement and the stability of its unfolding in free exploration of mice. Proceedings of the National Academy of Sciences USA, 106(50), 21335–21340.

Fritz, C., Morris, P., & Richler, J. (2012). Effect size estimates: Current use, calculations, and interpretation. Journal of Experimental Psychology, (141), 2–18.

Fu, H., Rodriguez, G.A., Herman, M., Emrani, S., Nahmani, E., Barrett, G., ..., Duff, K.E. (2017) Tau pathology induces excitatory neuron loss, grid cell dysfunction, and spatial memory deficits reminiscent of early Alzheimer's disease. Neuron 93(3), 533–541.

Games, D., Adams, D., Wolozin, B., & Zhao, J. (1995). Alzheimer-type neuropathology in transgenic mice overexpressing V717F APP. Letters to Nature, 373(9), 523–527. Gold, A. E., & Kesner, R. P. (2005). The role of the CA3 subregion of the dorsal hippo- campus in spatial pattern completion in the rat. Hippocampus, 15(6), 808–814. Gordon, G., Fonio, E., & Ahissar, E. (2014). Emergent exploration via novelty manage-

ment. Journal of Neuroscience, 34(38), 12646–12661. Gracian, E. I., Shelley, L. E., Morris, A. M., & Gilbert, P. E. (2013). Age-related changes in

place learning for adjacent and separate locations. Neurobiology of Aging, 34(10),

2304–2309. Graham, M. E. (2015). From wandering to wayfaring: Reconsidering movement in people

with dementia in long-term care. Dementia, 16(6), 732–749. Hartman, R. E., Izumi, Y., Bales, K. R., Paul, S. M., Wozniak, D. F., & Holtzman, D. M.

(2005). Treatment with an amyloid-beta antibody ameliorates plaque load, learning deficits, and hippocampal long-term potentiation in a mouse model of Alzheimer's disease. The Journal of Neuroscience, 25(26), 6213–6220.

Hok, V., Poucet, B., Duvelle, É., Save, É., & Sargolini, F. (2016). Spatial cognition in mice and rats: Similarities and differences in brain and behavior. Wiley Interdisciplinary Reviews: Cognitive Science, 7(6), 406–421.

Honey, R. C., & Good, M. (2000). Associative modulation of the orienting response: distinct effects revealed by hippocampal lesions. Journal of Experimental Psychology. Animal Behavior Processes, 26(1), 3–14.

Honey, R. C., Marshall, V. J., Mcgregor, A., Futter, J., & Good, M. (2007). Revisiting places passed: Sensitization of exploratory activity in rats with hippocampal lesions. The Quarterly Journal of Experimental Psychology, 60(5), 625–634.

Huitrón-Reséndiz, S., Sánchez-Alavez, M., Gallegos, R., Berg, G., Crawford, E., Giacchino, J. L., ... Criado, J. R. (2002). Age-independent and age-related deficits in visuospatial learning, sleep-wake states, thermoregulation and motor activity in PDAPP mice. Brain Research, 928(1–2), 126–137.

Hyde, L. A., Hoplight, B. J., & Denenberg, V. H. (1998). Water version of the radial-arm maze: Learning in three inbred strains of mice. Brain Research, 785(2), 236–244.

Janus, C. (2004). Search Strategies Used by APP Transgenic Mice During Navigation in the Morris Water Maze. Learning & memory (Cold Spring Harbor, N.Y.), pp.337–346. Jarrard, L. E. (1983). Selective hippocampal lesions and behavior: Effects of kainic acid lesions on performance of place and cue tasks. Behavioral Neuroscience, 197, 873–889. Jarrard, L. E., Davidson, T. L., & Bowring, B. (2004). Functional differentiation within the

medial temporal lobe in the rat. Hippocampus, 14, 434–439.   Johnson-Wood, K., Lee, M., Motter, R., Hu, K., Gordon, G., Barbour, R., ... Games, D.

(1997a). Amyloid precursor protein processing and Aβ42 deposition in a transgenic mouse model of Alzheimer disease. Proceedings of the National Academy of Sciences, 94(4), 1550–1555.

Johnson-Wood, K., Lee, M., Motter, R., Hu, K., Gordon, G., Barbour, R., ... McConlogue, L. (1997b). Amyloid precursor protein processing and A beta42 deposition in a trans- genic mouse model of Alzheimer disease. Proceedings of the National Academy of Sciences of the United States of America, 94(4), 1550–1555.

Kirova, A. M., Bays, R. B., & Lagalwar, S. (2015). Working memory and executive function decline across normal aging, mild cognitive impairment, and Alzheimer's disease. Biomed Research International, 2015, 748212.

Kunz, L., Schröder, T. N., Lee, H., Montag, C., Lachmann, B., Sariyska, R., Reuter, M., Stirnberg, R., Stöcker, T., Messing-Floeter, P. C., Fell, J., Doeller, C. F., & Axmacher, N. (2015). Reduced grid-cell-like representations in adults at genetic risk for Alzheimer's disease. Science, 350(6259), 430–433.

Laczó, J., Andel, R., Vyhnalek, M., Vlcek, K., Magerova, H., Varjassyova, A., ... Hort, J. (2010). Human Analogue of the Morris Water Maze for Testing Subjects at Risk of Alzheimer's Disease. Neurodegenerative Diseases, 7(1–3), 148–152.   Lamar, M., Podell, K., Carew, T. G., Cloud, B. S., Resh, R., Kennedy, C., Goldberg, E.,

Kaplan, E., & Libon, D. J. (1997). Perseverative behavior in Alzheimer's disease and

subcortical ischemic vascular dementia. Neuropsychology, 11(4), 523.   Lavenex, P. B., Bostelmann, M., Brandner, C., Costanzo, F., Fragnière, E., Klencklen, G.,

Lavenex, P., Menghini, D., & Vicari, S. (2015). Allocentric spatial learning and

memory deficits in Down syndrome. Frontiers in Psychology, 6, 62.   Lee, J., Kho, S., Yoo, Bin, H., Park, S., & Choi, J. (2014). Spatial memory impairments in

amnestic mild cognitive impairment in a virtual radial arm maze. Neuropsychiatric

Disease and Treatment, 10, 653–660.   M'Harzi, M., & Jarrard, L. E. (1992). Strategy selection in a task with spatial and non-

spatial components: Effects of fimbria–fornix lesions in rats. Behavioral and Neural

Biology, 58(3), 171–179.   Mokrisova, I., Laczo, J., Andel, R., Gazova, I., Vyhnalek, M., Nedelska, Z., ... Hort, J.

(2016). Real-space path integration is impaired in Alzheimer's disease and mild

cognitive impairment. Behavioural Brain Research, 307, 150–158. Morris, A. M., Churchwell, J. C., Kesner, R. P., & Gilbert, P. E. (2012). Selective lesions of the dentate gyrus produce disruptions in place learning for adjacent spatial locations.

Neurobiology of Learning and Memory, 97(3), 326–331. Olton, D. S. (1987). The radial arm maze as a tool in behavioral pharmacology. Physiology

& Behavior, 40(6), 793–797. Olton, D. S., Walker, J. A., & Wolf, W. A. (1982). A disconnection analysis of hippocampal

function. Brain Research, 233(2), 241–253. Olton, D. S., & Werz, M. A. (1978). Hippocampal function and behavior: Spatial dis-

crimination and response inhibition. Physiology & Behavior, 20(5), 597–605. Paxinos, G., & Franklin, K. B. J. (2004). The mouse brain in sereotaxic coordinates. Gulf

Professional Publishing. Pearce, J. M., George, D. N., Haselgrove, M., Erichsen, J. T., & Good, M. A. (2005). The

influence of hippocampal lesions on the discrimination of structure and on spatial

memory in pigeons (Columba livia). Behavioral Neuroscience, 119(5), 1316–1330. Redwine, J. M., Kosofsky, B., Jacobs, R. E., Games, D., Reilly, J. F., Morrison, J. H., ...

Bloom, F. E. (2003). Dentate gyrus volume is reduced before onset of plaque

formation in PDAPP mice: A magnetic resonance microscopy and stereologic analysis. Proceedings of the National Academy of Sciences of the United States of America, 100(3), 1381–1386.

Rolls, E. T. (2013). The mechanisms for pattern completion and pattern separation in the hippocampus. Frontiers in Systems Neuroscience, 7(74), 10–3389.

Saito, T., Matsuba, Y., Yamazaki, N., Hashimoto, S., & Saido, T. C. (2016). Calpain acti-vation in Alzheimer's model mice is an artifact of APP and presenilin overexpression. The Journal of Neuroscience, 36(38) 9933–9936.

Shapiro, M.L., Tanila, H. & Eichenbaum, H. (1997) Cues that hippocampal place cells encode: dynamic and hierarchical representation of local and distal stimuli. Hippocampus, 7 (6), pp.624–642.e pathology. Proceedings of the National Academy of Sciences 94(24), 13287–13292.

Tomczak, M., & Tomczak, E. (2014). The need to report effect size estimates revisited. Trends in Sport Sciences, 1(21), 19–25.

Walker, J. A., & Olton, D. S. (1979). Spatial memory deficit following fimbria-fornix le-sions: Independent of time for stimulus processing. Physiology & Behavior, 23(1), 11–15.

Whishaw, I. Q., & Tomie, J.-A. (1997). Perseveration on place reversals in spatial swimming pool tasks: Further evidence for place learning in hippocampal rats. Hippocampus, 7(4), 361–370.

Wu, C.-C., Chawla, F., Games, D., Rydel, R. E., Freedman, S., Schenk, D., ... Bloom, F. E. (2004). Selective vulnerability of dentate granule cells prior to amyloid deposition in PDAPP mice: Digital morphometric analyses. Proceedings of the National Academy of Sciences of the United States of America, 101(18), 7141–7146.

Yassa, M.a., & Stark, C. E. L. (2011). Pattern separation in the hippocampus. Trends in Neurosciences, 34(10), 515–525.

Yoon, T., Okada, J., Jung, M. W., & Kim, J. J. (2008). Prefrontal cortex and hippocampus subserve different components of working memory in rats. Learning & Memory, 15, 97–105.

Zhao, R., Fowler, S. W., Chiang, A. C. A., Ji, D., & Jankowsky, J. L. (2014). Impairments in experience-dependent scaling and stability of hippocampal place fields limit spatial learning in a mouse model of Alzheimer's disease. Hippocampus, 24(8), 963–978.
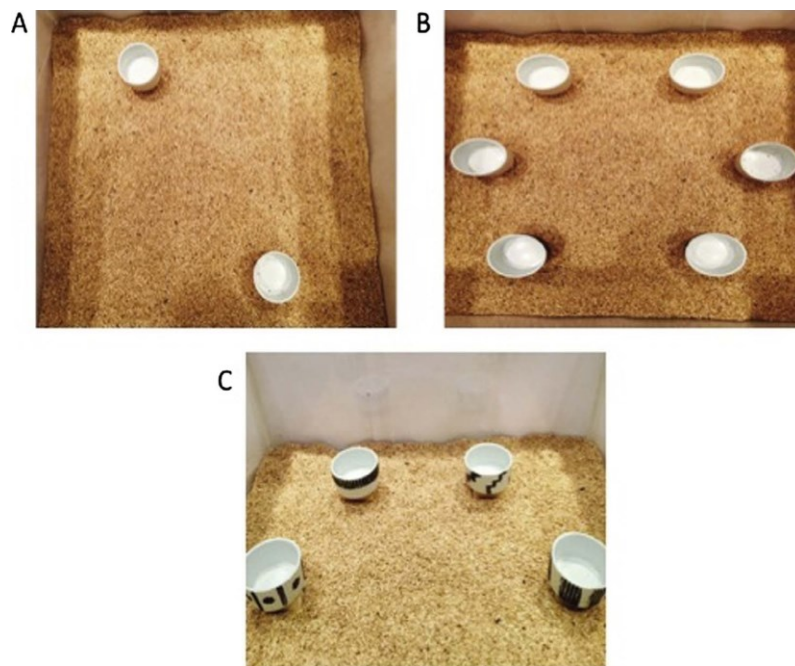
Fig. 1. Illustration of the pot locations during training and test periods. (A) Two pots placed opposite each other in the arena-training phase. (B) Six pots are placed in a radial formation for the test phase of the foraging task. (C) Novel pot designs used in experi- ment 2. All 6 pots were given a novel design (4 are shown in this figure). Position of the pots was changed each day, but the radial formation remained.
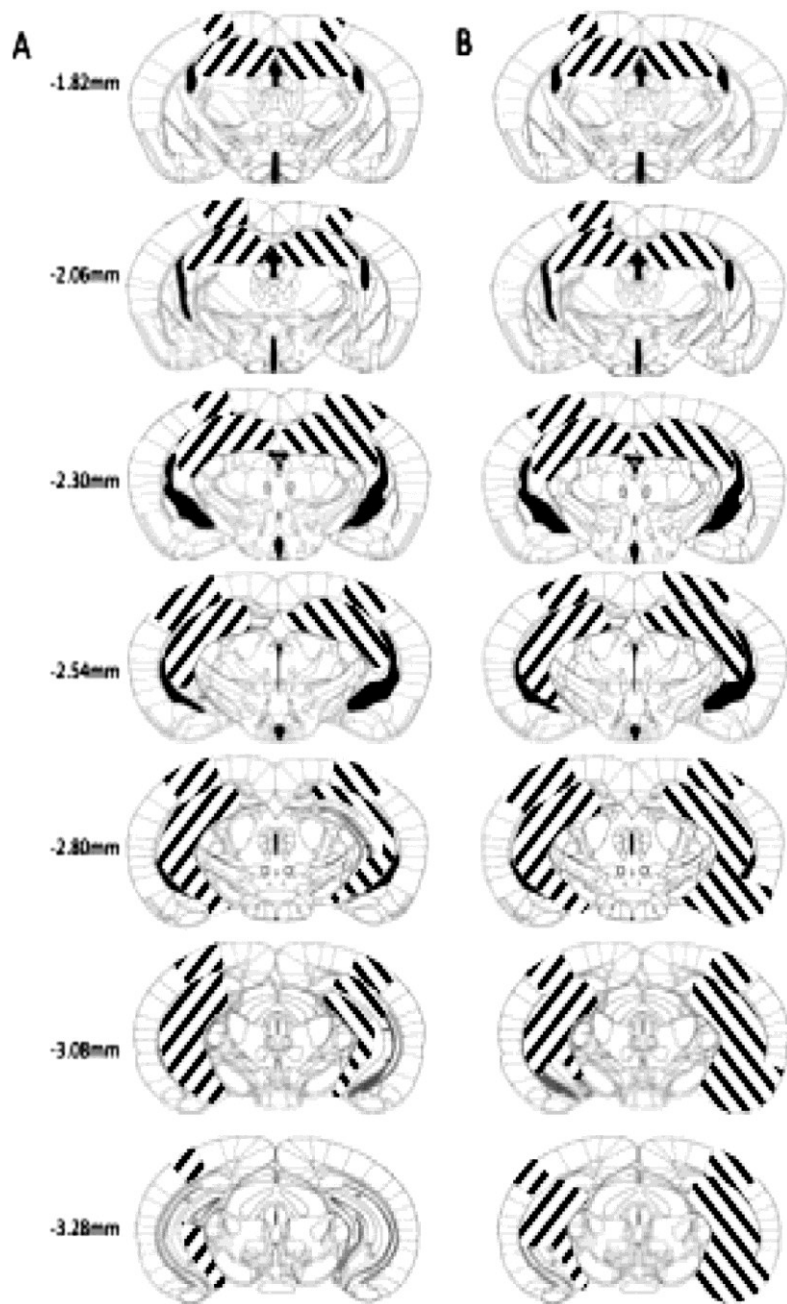
Fig. 2. Reconstruction of the minimal (A) and maximal (B) extent of bilateral hippocampal lesions through coronal sections through the brain. Coordinates represent distance from bregma in mm.
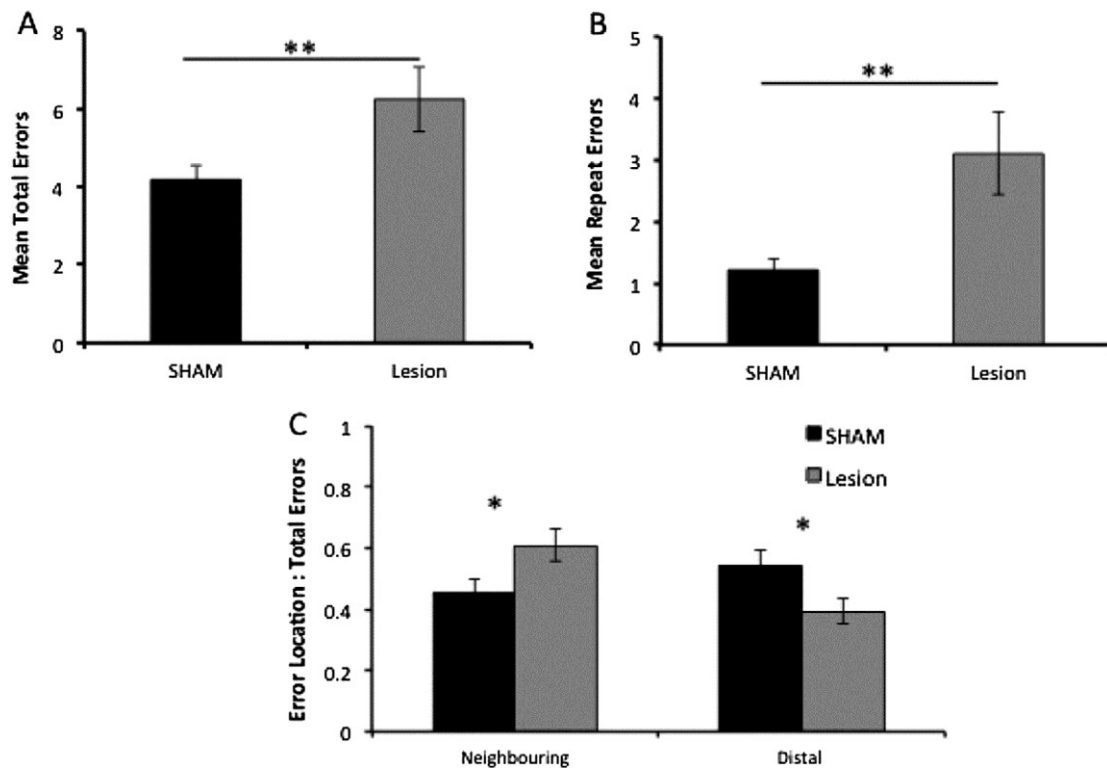
Fig. 3. Foraging behaviour in control mice and mice with HPC lesions. Measures of SWM in SHAM control (n = 13) and HPC lesion mice (n = 11). (A) Total number of errors. (B) Total number of repeat errors. (C) The ratio of neighbouring and distal errors to total errors made. *p < 0.05. Data were averaged across four trials for each mouse and mean score for each group is reported. Error bars represent the S.E.M.

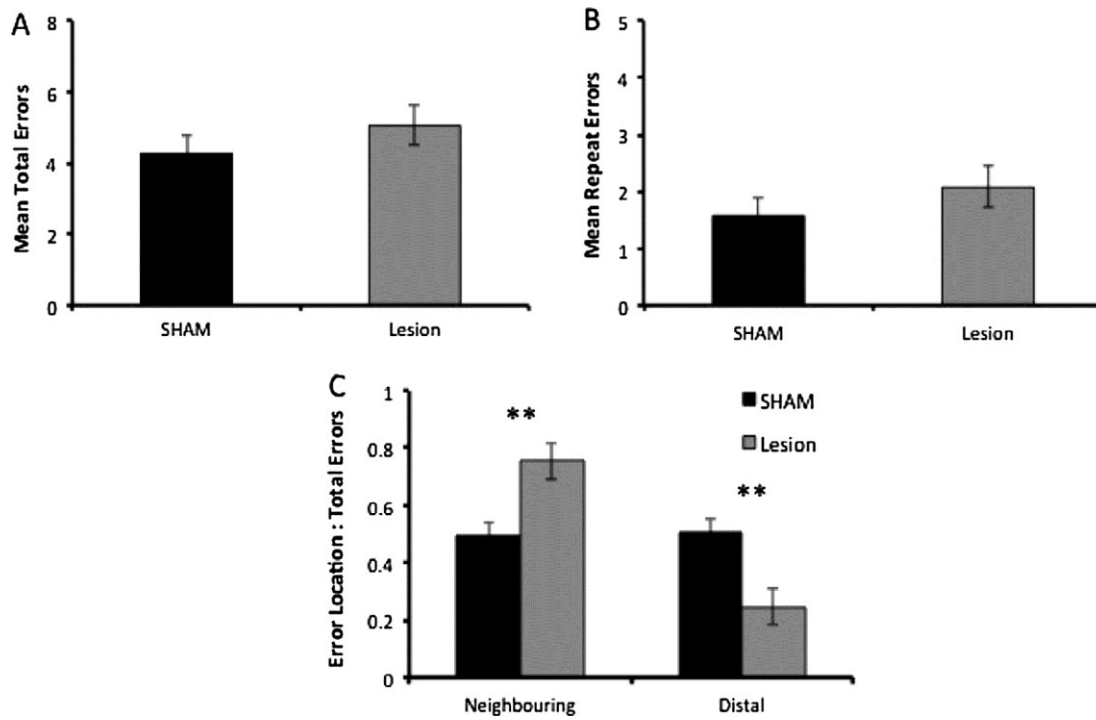Fig. 4. Foraging behaviour in mice with HPC lesions. Measures of SWM in SHAM control (n = 13) and HPC lesion mice (n = 11). (A) Total number of errors. (B) Total number of repeat errors. (C) The ratio of neighbouring and distal errors to total errors made. [**]p < 0.01. Data were averaged across four trials for each mouse and mean score for each group is reported. Error bars represent the S.E.M.
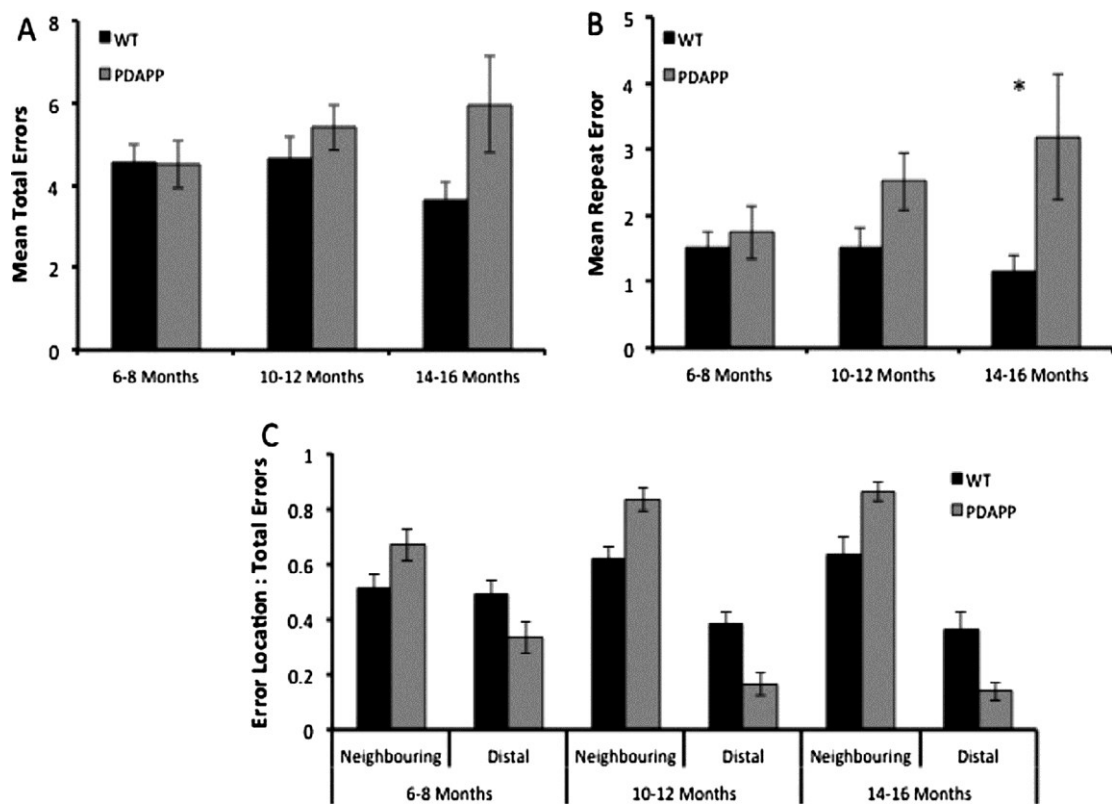
Fig. 5. Foraging behaviour and SWM performance in PDAPP (n = 14) and WT control mice (n = 15). Data were averaged across four trials for each mouse and mean score for each group is reported. Error bars represent the S.E.M. (A) Total number of errors. (B) Total number of repeat errors. (C) The ratio of neighbouring and distal errors to total errors made. *p < 0.05.

Table 1a
The stereotaxic coordinates for bilateral HPC lesions described as mm from bregma (anterior posterior), from the midline (lateral) and from the dura (ventral).

Site	Stereotaxic coordinates			
	Anterior/Posterior (−)	Lateral (±)	Ventral (−)	Volume (υL)
1	1.2	1.0	2.0	0.15
2	1.7	1.0	2.0	0.15
3	1.7	1.5	2.0	0.15
4	2.2	1.0	2.0	0.15
5	2.2	2.0	2.0	0.15
6	2.5	1.5	2.0	0.15
7	2.5	2.2	2.2	0.15
8	3.0	3.0	4.2, 3.0, 2.5	0.15
9	3.6	3.0	4.0, 3.0,	0.15

Table 1b
The mean % (+/−SEM) of regions showing cell loss in mice with hippocampal lesions across the two hemispheres and the dorsal- ventral extent of the structure.

Lesion area	Mean % area lesioned
Total	84.46 (2.12)
Left	85.63 (2.69)
Right	83.29 (5.40)
Dorsal	88.88 (2.34)
Ventral	66.92 (4.01)

Table 2
Overview of the types of errors scored to assess spatial memory and foraging behaviours in a foraging-based task. Errors are defined and examples of when these errors are scored are described.

Error measurement	Definition	Example of behaviour
Total error	A mouse returning to a pot where the reward was previously consumed	A mouse forages a reward from pot A and leaves pot A. The mouse then returns to pot A (Error)
Repeat error	A mouse returning to a pot where an error was already made. Hence, repeating the error	A mouse forages a reward from pot A, and leaves pot A. The mouse then returns to pot A (error). It leaves pot A again, forages in pot B before returning to pot A (repeat error)
Perseverative error	A mouse returning to a pot immediately after receiving a reward, or immediately after making an error	A mouse forages in pot A. The mouse leaves pot A and *immediately* returns to pot A
Chaining/circling response	When a mouse forages pots in a sequence of 3 or more pots immediately adjacent to one another	A mouse forages in pot A, B, and C etc. until the sequence is broken
Neighbouring pot error	A mouse making an error immediately in the same pot or the neighbouring pot to which it has just foraged (if this pot has already been foraged in)	A mouse forages a reward from pot A. The mouse then forages in pot B before foraging in pot A
Distal pot error	A mouse making an error in a pot one or more distant from a pot it has just foraged or made an error in	A mouse forages in pot A. The mouse then forages in pot C before returning to pot A

Table 3
Table showing the mean (and SEM) of the number of errors made in each trial by HPC lesion mice (n=11) and SHAM controls (n=13; Experiment 1 and 2) and WT (n=15) and PDAPP mice (n=14; Experiment 3). There were no significant group differences within trials.

Group		Mean number of errors by trial			
		Trial 1	Trial 2	Trial 3	Trial 4
		Experiment 1			
SHAM		4.38 (0.65)	4.69 (0.79)	4.15 (0.61)	3.54 (0.87)
Lesion		6.75 (1.91)	7.08 (1.15)	6.17 (1.21)	6.17 (1.09)
		Experiment 2			
SHAM		4.23 (0.87)	4.31 (0.70)	4.77 (1.37)	3.77 (0.43)
Lesion		7.92 (1.19)	4.23 (1.15)	3.92 (0.60)	4.15 (0.68)
Age		Experiment 3			
WT	6–8 Months	4.27 (0.64)	4.73 (0.57)	5.20 (0.91)	4.47 (0.59)
	10–12 Months	5.53 (0.71)	3.40 (0.67)	5.00 (0.70)	4.73 (0.79)
	14–16 Months	3.67 (0.56)	3.07 (0.55)	3.20 (0.75)	4.67 (1.02)
PDAPP	6–8 Months	4.29 (0.82)	4.64 (1.35)	4.57 (0.87)	4.57 (0.86)
	10–12 Months	5.00 (0.73)	5.64 (1.32)	4.86 (1.07)	4.79 (1.42)
	14–16 Months	7.07 (2.21)	6.14 (1.44)	4.71 (1.39)	5.93 (1.49)

Table 4
Table showing the mean (and SEM) of the times taken to complete and engage in the foraging task by HPC lesion mice (n=11) and SHAM controls (n=13; Experiment 1 and 2) and WT (n=15) and PDAPP mice (n=14; Experiment 3).

Group		Mean time scores (s)	
		Total time	Engagement time
		Experiment 1	
SHAM		190.8 (15.6)	43.1 (6.8)
Lesion		194.4 (19.4)	36.1 (5.4)
		Experiment 2	
SHAM		169.9 (19.7)	40.1 (4.6)
Lesion		178.1 (21.1)	38.8 (4.8)
Age	Experiment 3		
WT	6–8 Months	135.9 (11.9)	27.0 (5.2)
	10–12 Months	114.7 (17.7)	23.9 (7.8)
	14–16 Months	100.9 (10.9)	12.5 (3.5)
PDAPP	6–8 Months	175.1 (26.1)	38.4 (7.8)
	10–12 Months	134.9 (23.9)	40.3 (11.8)
	14–16 Months	141.9 (19.9)	32.1 (9.9)

Table 5
Table showing the mean (and SEM) of the number of perseverative errors and chaining responses in the foraging task made by HPC lesion mice (n=11) and SHAM controls (n=13; Experiment 1 and 2) and WT (n=15) and PDAPP mice (n=14; Experiment 3). Numbers in bold represent significant differences in between group comparisons.

Group		Foraging behaviours	
		Perseverative errors	Chaining response
		Experiment 1	
SHAM		0.73 (0.33)	0.69 (0.10)
Lesion		**2.80 (0.39)**	**1.11 (0.10)**
		Experiment 2	
SHAM		0.75 (0.14)	0.62 (0.13)
Lesion		**1.77 (0.46)**	**1.44 (0.14)**
Age	Experiment 3		
WT	6–8 Months	0.38 (0.06)	0.67 (0.09)
	10–12 Months	0.65 (0.12)	0.83 (0.11)
	14–16 Months	0.25 (0.07)	0.82 (0.13)
PDAPP	6–8 Months	0.39 (0.09)	1.08 (0.13)
	10–12 Months	0.91 (0.17)	1.45 (0.13)
	14–16 Months	**1.25 (0.32)**	**1.63 (0.12)**