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Research report

Hippocampal lesions in rats impair learning and memory for locations on a touch-sensitive computer screen: The "ASAT" task

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ABSTRACT

It has been repeatedly demonstrated across species that the hippocampus is critical for spatial learning and memory. Consequently, numerous paradigms have been created to study spatial learning in the rodent. Most of these tasks, such as the Morris water maze, 8-arm radial maze, and T-maze, are non-automated procedures. It was our goal to create an automated task in the rodent that is quickly learned, hippocampal-dependent, and minimizes the confounding variables present in most tests measuring hippocampal-dependent learning and memory. To accomplish this, we created a novel search task using a standard operant box fitted with a touch-sensitive computer monitor. Subjects were required to locate an S+ "hidden" amongst other identical stimuli on the monitor. In two versions of the task the S+ stayed in the same location within a session but shifted location between sessions. In a third version of the task the S+ was moved to a new location after every 10 trials. It was found that the location of the S+ was quickly acquired each day (within 10 trials), and that the hippocampal-lesion group was impaired when compared to their control cohort. With the benefits inherent in automation, these tasks confer significant advantages over traditional tasks used to study spatial learning and memory in the rodent. When combined with previously developed non-spatial cognitive tests that can also be run in the touch-screen apparatus, the result is a powerful cognitive test battery for the rodent.

1. Introduction

It is now well understood that animal models are an essential component in research into the neural basis of learning and memory. As the hippocampus is thought to be one of the most important structures for learning and memory in humans [1–6], behavioural models of learning and memory in animals have focussed on tasks that depend on this structure. The most popular means of studying spatial memory in the rodent have been tasks using apparatus such as the Morris water maze (MWM; [7,58,59,8,9]), the Olton 8-Arm radial maze [7,10–17], and the T-maze [7,18–21].

Researchers are increasingly employing automated testing methods. These protocols have numerous advantages over non-automated methods, such as greater ease of testing, minimized experimenter contact with the subjects, and consistency and accuracy in task parameters such as stimuli, responses, inter-trial intervals, and delays. One such reportedly hippocampal-sensitive automated test of learning and memory is the delayed non-matching to position (DNMTP) task, in which rodents are required to remember, across a variable delay, the location of one of two levers. DNMTP has been shown to be vulnerable to non-spatial mediating strategies [22–24], and recently it has been suggested that this task is not sensitive to lesions of the hippocampus at all [25]. Accordingly, operant DNMTP has fallen from favour with many researchers as a test of spatial learning. Thus, there is a dearth of viable automated hippocampal-dependent tests of spatial memory.

The aim of the present study was to develop an automated, and quickly acquired, test of spatial memory that is sensitive to lesions of the hippocampus. Operant testing with a touch-sensitive monitor is ideally suited to this purpose [26–28,31]. In this method, inspired by automated cognitive test batteries for the human and monkey, rodents respond to abstract graphic stimuli presented on a computer monitor and their responses (nose-pokes to the screen) are automatically recorded. In addition to the advantages conferred by automation, numerous added advantages are inherent in this technique. For example, stimuli are presented on a computer screen, allowing many more spatial locations than are possible with standard lever-based automated procedures. Tasks carried out

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using this method are more similar to those used with human participants who often perform cognitive tests on a similar computerized apparatus. Furthermore, spatial tasks carried out using this method can be compared to non-spatial tasks performed in the same apparatus, eliminating the influence of many confounding variables in assessment of effects between behaviour tasks. This method has been successfully used to administer non-spatial and visuospatial tests of learning and memory to both rats and mice [28–33].

In the automated spatial array task (ASAT) developed in the present study, the subject is presented with an array of locations, each indicated by an identical white square. "Hidden" within this array is a correct location (S+). If the animal nose-pokes this location it receives a food reward. Retrieval of the food reward initiates a short inter-trial interval (ITI; 5 s), after which the next trial can be initiated. Responses at the other locations within the display result in no programmed consequences; they are neither punished, nor rewarded. However, responses at the S− are recorded and serve as a measure of performance. Although our intention is not to present ASAT as a direct analogue of the MWM or 8-arm radial maze, ASAT does possess superficial similarities to these tasks, and comparison to them may help to illustrate how this new task is run. For example, one can think of the array of locations in ASAT as the pool in the MWM, or the array of arms in the 8-arm radial maze. In maze-based assays where the subject searches for a hidden platform or a baited arm, in ASAT the subject searches for a single rewarded location on the computer screen. As in the MWM or 8-arm radial maze, this location must initially be found by chance (although a search strategy could be used to aid this process) but across trials, the animal becomes more proficient at locating the S+, resulting in a shorter latency or swim distance (in the MWM), or errors made to locate the S+ (in the radial maze or ASAT). Although the neural mechanisms behind this learning may prove to be different in ASAT, the result is very much like what is seen in the MWM or 8-arm radial maze, an increased efficiency in locating the S+. This improvement in performance in ASAT may be due to the use of hippocampal-dependent learning and memory for the S+ location. If so, then like the MWM or 8-arm radial maze a lesion of the hippocampus would be expected to impair acquisition of the task. As such rats with lesions to the hippocampus were tested on three versions of ASAT, and their performance compared to that of a sham-lesion control group.

2. Materials and methods

2.1. Apparatus

Animals were trained in operant boxes (Med Associates, VT, USA; *h* 23 cm, *w* 30 cm, 25 cm) made of a metal frame with a wall constructed of clear Perspex and metal (see Fig. 1). The floor consisted of grid bars spaced approximately 1 cm apart, situated 3 cm above a sawdust-filled waste bin. One end of the chamber was equipped with a touch-sensitive, flat-screen, LCD computer monitor (24 cm × 29 cm viewable area, Craft Data Ltd., Chesham, UK). An infrared touch detection system was placed 4 mm from the glass of the screen, and recorded a "touch" once an object was within approximately 4 mm of the screen. This monitor was then covered with one of two "masks", a piece of black Perspex (38 cm × 28 cm) with response windows cut into it. A spring-hinged "shelf" was attached 16 cm above the grid floor. This shelf was at a 90° angle to the mask and had a depth of 6 cm with a width of 20.5 cm. This mask was attached to the screen leaving a space of 0.5 cm between the mask and monitor to ensure that it would not trigger the touch-screen area. On the wall opposite from the monitor was a food magazine equipped with a light, and an infrared beam and beam detector. Above the food magazine was a house light (3 W), and a small speaker. Each operant box was housed within a sound-attenuating chamber equipped with a small fan. The boxes and monitors were controlled using IBM Netvista computers running programs written in-house in Microsoft Visual Basic.

The first mask, used only during the initial training and Experiment 1 (see below), consisted of 12 square response windows arranged in three rows and four columns (only the bottom two rows were used for a total of eight response locations, see Fig. 1). Each response window was 4.25 cm × 4.25 cm. The lowest row was 17 cm above the floor with 1 cm separating the 1st and 2nd row, as well as the 2nd and 3rd row. Squares were equally spaced within the rows, with each row being 23 cm wide, allowing approximately 2.25 cm between columns. The second mask (used for Experiments 2 and 3, see below) contained three rows and seven columns for a total of 21 response windows. However only the bottom two rows were used for a total of 14 response locations. The total length of a row was 20 cm, whereas the total length of a column was 14 cm. All response windows were 2 cm × 2 cm equally spaced allowing a separation of 1 cm between locations.

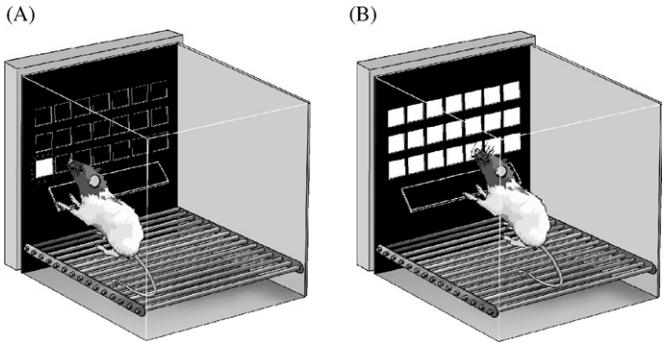

Fig. 1. The touch-screen apparatus. A flat-screen monitor with an integrated touch-screen assembly was placed as the end of a standard operant chamber. A food magazine, speaker, and house light were attached to the rear of the chamber (not shown). The figure shows the presentation of the start location (A; ASAT2 and ASAT3), as well as the search for the S+ (B; ASAT1–3). The mask shown is the same as that used in ASAT2 and ASAT3, and all possible locations are illuminated for illustration purposes.

2.2. Subjects

16 male Lister hooded rats were used for this study (Harlan, UK). They were housed four per cage and maintained on a reverse day/night light cycle (lights on from 7 p.m. to 7 a.m.). Rats were maintained on *ad libitum* food (standard rat chow, Purina) and water prior to surgery and during the 1 week following surgery. Once training began, food was restricted to maintain animals at 85–90% of normal weight; however *ad libitum* access to water was still allowed. All behavioural testing occurred during the subjects' dark phase. All experimentation was conducted in accordance with the UK animals (Scientific Procedures) Act, 1986.

2.3. Surgery

The surgical procedure used here is adapted from Ito et al. [34]. Prior to surgery, animals were randomly allocated to a lesion or a sham control group. All animals were anaesthetized with Avertin during the course of surgery. Once anaesthetized, rats were placed in a sterotaxic frame with the incisor bar set at 3.3 mm below the interaural line (Kopf, USA). Small holes were drilled into the skull above the injection sites. Lesioned animals then had a bevelled-tipped micro-syringe lowered into the hippocampus to deliver *N*-methyl-D-aspartic acid (0.09 M in sterile PBS) into the hippocampus at a rate of 0.1 μl per min (see Table 1 for additional details). Anterior–posterior, as well as medial–lateral coordinates were calculated relative to bregma, whereas dorsal–ventral coordinates were calculated relative to dura. Immediately after surgery animals were placed in heated chambers in a darkened room and allowed to recover with free access to food and water. Diazepam was administered (5 mg/ml; volume 0.1 ml/kg, i.m.) if seizures occurred during, or immediately after surgery.

2.4. Histology

When all behavioural testing was completed, animals were anaesthetized with Dolethal (2 ml, i.p.) and transcardially perfused with 100 ml of PBS, followed by 250 ml of 4% paraformaldehyde. Brains were then removed and further fixed in paraformaldehyde at 4 °C for 24 h. Prior to cutting, samples were soaked in 20% sucrose and PBS for 24 h. 60 μm sections were cut using a cryo-stat, and every fifth section was mounted on a gelatin-coated glass slide, and then stained with cresyl violet. When completed, lesion and control subjects were examined under a light microscope to determine the extent of damage.

Reconstructions of lesions are shown in Fig. 2. The actual lesion size and placement was very close to the intended lesion. All animals showed slight sparing of the posterior CA1 region (bregma −6.30 and posterior), and the most anterior portion of the CA3 region [35]. Two subjects showed significantly more sparing. One subject had sparing to the right dentate gyrus and the dorsal sections of the CA1 region throughout the whole structure. Furthermore in the same animal some unilateral sparing (right) of the ventral CA1 region was seen anterior to bregma −6.30. The second subject showed sparing to the dorsal and ventral ends of the CA1 region (left side) beginning at bregma −5.20. This sparing increases until most of the CA1

Table 1
NMDA injection co-ordinates for hippocampal lesions

Anterior/posterior	Medial/lateral	Dorsal/ventral	Volume per site (μl)	Post injection diffusion time (min)
−2.8	±1.6	−3.3	0.4	4
−4.2	±2.6	−3	0.4	4
−4.8	±4.8	−6	0.2	2
−5.3	±4.6	−4.2	0.2	2
−5.3	±4.6	−6	0.2	2
−5.8	±4.6	−4.2	0.2	2

region is spared by bregma −6.30. Three rats had bilateral cortical damage and one had unilateral cortical damage; there was no cortical damage in any of the remaining rats. ANOVAs performed on the acquisition data of rats with and without cortical damage (bilateral or unilateral) revealed no significant differences between lesion groups on the basis of cortical damage in any of the experiments (Experiment 1: $P = 0.35$; Experiment 2: $P = 0.13$; Experiment 3: $P = 0.25$). Thus all eight rats were included in a single lesion group for subsequent analyses.

2.5. Training

Prior to training, animals were placed in the operant chambers for 20 min to habituate to the environment. To encourage exploration food pellets were placed in the food receptacle as well as on the shelf and mask attached to the touch-screen. During this time the touch-screen was not activated. After habituation, animals were trained to respond to a tone associated with food reward (0.045 g formula "*P*", Noyes). This was accomplished by delivering a 0.5 s tone, followed by a food pellet, every 30 s. During this time, white squares were presented in all of the response windows. If an animal touched the monitor it was rewarded with three food pellets, and the beginning of the next trial was initiated. However, no response also resulted in the tone and a one-pellet reward after 30 s. This procedure was followed for one session (100 trials) to ensure the rat had learned the association between a tone and food. Next, rats were required to touch any area of the monitor while all squares were displayed to earn a reward. The screen remained active until a response occurred. Once a response occurred a tone was sounded, a food pellet was delivered, and the touch-screen was deactivated. The next trial began 5 s after the pellet was collected. A session was complete when either 30 min passed, or after the rat had completed 100 trials. Animals were trained in this fashion until they successfully completed 100 trials within 30 min. This typically took 3 sessions. A final stage of training was used to ensure that animals did not develop a bias to one area of the screen. In this stage one of the eight response locations would be randomly illuminated and the rat was required to poke at this location to earn a reward. Pokes at other locations were not punished or rewarded. When successful, a reward pellet was delivered. Eating this food pellet would trigger the inter-trial interval (5 s) and the next trial, with a new location, would begin after a nose-poke into the food magazine. This

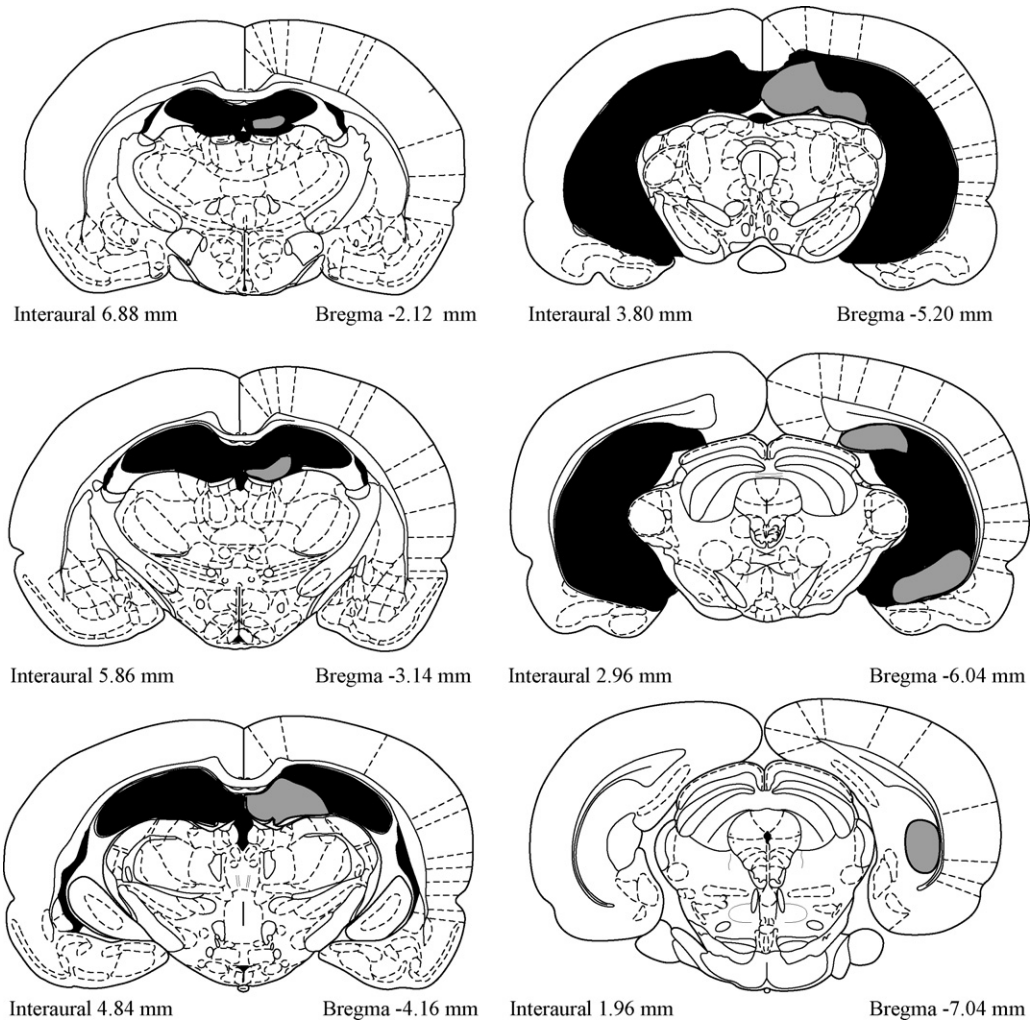

Fig. 2. Reconstruction of the extent of lesions to the hippocampus (adapted from [35]). Using just black, the extent of the smallest lesion is illustrated, whereas the largest lesion is illustrated in black and gray.

Table 2
Testing parameters used in each version of ASAT

	Array size (locations used)	Total trials per session	Possible start locations	S+ locations per session
ASAT1	8	100	0	1
ASAT2	14	50	4	1
ASAT3	14	50	4	5

was repeated until each rat could complete 100 trials in 1 h. In all cases this took three sessions or less.

2.6. Data pre-processing and statistical analysis

Prior to statistical analysis all data were pre-processed using a macro generated in Microsoft Excel. Rats had a tendency to make several rapid responses within centiseconds of each other at a single location before trying a new location. This filter removed all responses after the first that occurred consecutively at a single location within one sec. If an animal was to respond at location 1, and then again 0.5 s later, this would be scored as a single visit. If the delay between responses was greater then a second, or if activation of another location occurred during that second, then the responses would be considered multiple visits. These processed data were analysed using repeated measures ANOVAs, or independent sample *T*-tests. Total errors were used as the dependent variable, lesion as the between-subjects independent variable, and trial block or individual trial as the repeated measure. When appropriate, *post hoc* tests were performed using the Student–Newman–Keuls (SNK) test for multiple dependent samples. Prior to analysis, individual animal means were calculated by taking the average of several sessions of testing. This was done to avoid any confounding variables associated with the day of testing, or testing location used. All statistics were generated with Statistica 6.1 (StatSoft, Tulsa, USA).

2.7. ASAT general methods

Within this study, three different versions of the ASAT task were used (see Table 2). The first version of the task (ASAT1) consisted of an array of 8 locations and 100 trials per session. The S+ remained in the same location within a session, but moved between days. The second version (ASAT2) was the same in all regards except that the second mask was used to allow more locations, four start locations were introduced, and the total number of trials per session was reduced to 50. This was done to mimic the effect of the start locations used in the MWM. The final version of the task (ASAT3) was the same as ASAT2 except that the S+ location moved after every 10 trials within a testing session. This shift was not cued. The number of non-rewarded locations visited was used as the main dependent variable for all phases of the study. Locations visited were chosen over latency to locate the S+ for two reasons. (1) Motivational factors or motor impairments should have less of a confounding effect on locations visited then on search latency, especially considering the self-paced nature of the tasks. (2) Most researchers use an error measure for appetitive maze tasks, or total distance travelled for the MWM. Where significant effects of lesion were seen on errors made, additional analyses were performed to determine if the difference in accuracy was caused by errors at the previous S+ (the S+ from the previous session in ASAT1 and ASAT2, or the S+ from the previous trial block in ASAT3) or errors at locations not previously rewarded (all locations other than the one last rewarded).

3. Experiment 1: ASAT1

3.1. Behavioural testing methods

On each trial in this first version of ASAT, rats were presented with an array of eight adjacent locations on the touch-sensitive monitor (search phase), each illuminated with an identical white square. Each location was identical to the others except that one served as an S+. A poke at this location triggered the delivery of a reward pellet and a tone. Pokes at the other locations had no behavioural consequences, although these responses were recorded. Retrieval of the reward resulted in the onset of a 5 s intertrial interval. Once this interval had passed the food magazine was illuminated indicating that the subject could now initiate the next trial by nose poking in the food delivery magazine. This sequence was repeated until the rat had finished a session of 100 trials, or until an hour had passed, whichever occurred first. The S+ location was the same between trials, but was changed between sessions. In ASAT1 and ASAT2 data was analysed in 10 trial blocks, as well as individually for the first 10 trials (constituent components of trial block 1). To minimize confusion "trial block" will be used to denote blocked data (e.g. trials 1–10, 11–20, ...) whereas "trial" will be used in reference to single trial analysis (e.g. trial 1, 2, 3, ...).

3.2. Results

The data presented here is from sessions 17–24, of 24 total training sessions. Trials 1–16 are not included in this analysis because behaviour had not yet stabilized and all animals were not consistently completing 100 trials. The data from a single session were first averaged in blocks of 10 trials. These 10, 10-*trial blocks* were then averaged with the equivalent block from 7 other testing sessions (data were examined in blocks of eight sessions, so a session at each possible S+ location could be included in analysis). Statistics were performed on the resulting averaged data. When only the *first 10 trials of a session* were considered for statistical analysis trials were simply averaged across sessions.

Main effects were seen of lesion ($F(1,14) = 12.03$, $P = 0.0037$), indicating that the lesion group made significantly more errors than the control group, and *trial block* ($F(9,126) = 212.0$, $P < 0.0001$), where errors decreased across the testing session. Furthermore, an interaction was found between *trial block* and lesion ($F(9,126) = 5.61$, $P < 0.0001$). *Post hoc* analysis indicated a significant difference from the lesion group only during the *first trial block* (SNK, $P = 0.0006$). Accordingly, the *first trial block* was broken into its *10 constituent trials* and then analysed with a second ANOVA (Fig. 3).

Main effects of lesion ($F(1,14) = 10.05$, $P = 0.0068$) and trial were seen ($F(9,126) = 21.6$, $P = 0.0001$), but no interaction between the two was observed ($F(9,126) = 3.33$, $P = 0.85$). Again, the lesion group made more errors to locate the S+. When errors were divided into those made at the former S+ or previously non-rewarded locations an effect of lesion was seen according to both measures (Fig. 4). The lesion group made more errors at the formerly rewarded location ($F(1,14) = 16.12$, $P = 0.001$) and more errors at locations pre-

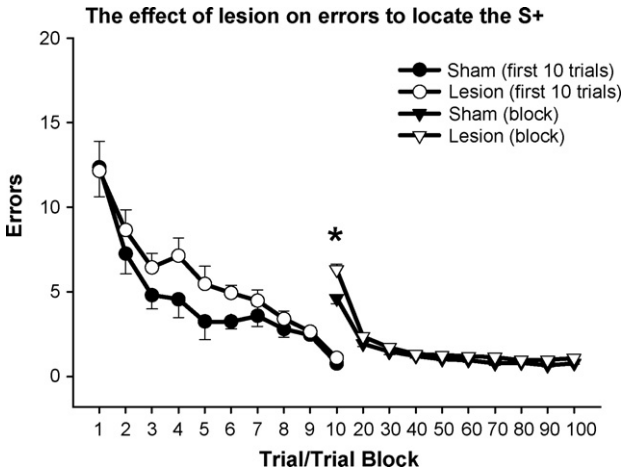

Fig. 3. Performance of control and hippocampal-lesioned rats in ASAT1. The figure shows the mean errors made in locating the S+ during the first 10 trials, as well 10-trial blocks 1–10. Trial blocks are labelled as the last trial in that block (i.e. trial block 20 consists of trials 11–20). A significant effect of lesion is seen within the first 10 trials ($P < 0.01$), as well as across trial block ($P < 0.01$). Error bars represent 1 S.E.M.

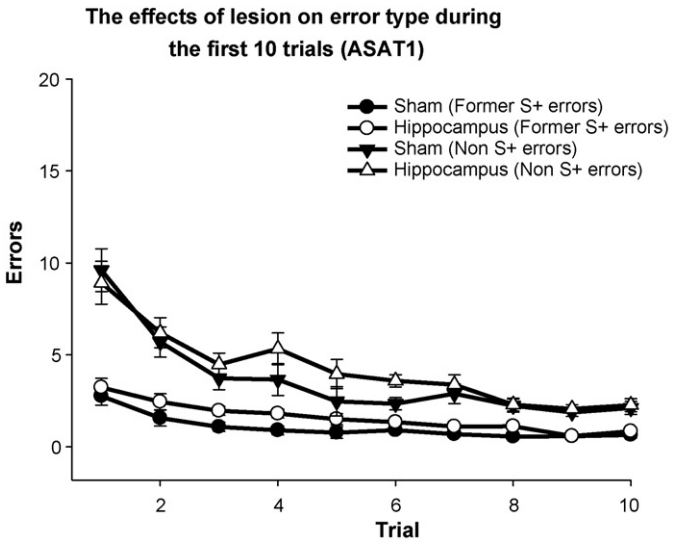

Fig. 4. Performance of control and hippocampal-lesioned rats in ASAT1 on former S+ errors and non-S+ errors during the first 10 trials. A main effect of lesion was seen ($P = 0.047$) on non-S+ errors, and on former S+ errors ($P = 0.001$. Error bars represent 1 S.E.M.

viously not rewarded ($F(1,14) = 4.69$, $P = 0.047$). An effect of trial was seen in both instances (former S+ $F(9,126) = 13.96$, $P = 0.001$); not rewarded locations $F(9,126) = 21.69$, $P < 0.0001$), while no interaction between lesion and trial was seen (former S+ $F(9,126) = 0.61$, $P = 0.77$); not rewarded locations $F(9,126) = 0.57$, $P = 0.81$). When expressed as a proportion of total errors lesioned animals were found to make more errors at the former S+ than their sham-lesioned counterparts ($F(1,14) = 5.27$, $P = 0.03$). This finding indicates that as well as making more non-selective memory errors, lesioned rats remember and perseverate at the previously rewarded S+ location more then the control group. However no effect was seen of trial ($F(9,126) = 1.23$, $P = 0.28$), although a near significant trial by lesion interaction did exist $F(9,126) = 0.91$, $P = 0.056$). The increased proportion of responses made by the lesion groups towards the former S+ decreased across trials. Moreover on the first trial of a session a significant preference was seen for the previous session's S+, with both groups making more than the expected chance level proportion of responses at the former S+ location (Chance = 0.125; Sham mean = 0.20 $T(1,7) = 6.69$, $P = 0.000$; Hippocampus mean = 0.29 $T(1,7) = 8.32$, $P > 0.0001$). Thus both hippocampus-lesioned and control rats displayed memory for the previous S+ location.

The same overall pattern of results was seen during training trials. Analysis of total errors to locate the S+ on trials 1–8 revealed that the effect of lesion nearly reached significance ($P = 0.07$; data not presented), while an analysis of trials 9–16 revealed a significant effect of lesion ($P < 0.05$; data not presented).

3.3. Discussion

The initial findings from this experiment indicate that lesions of the hippocampus impair performance of a simple search task using a touch-sensitive monitor. Moreover, evidence of memory across days is also seen, with both experimental groups making proportionally more errors at the previous session's S+ during the first trial of a newly commenced session, than would be expected by chance levels of performance alone. To our knowledge, this is the first example of a deficit induced by lesions of the hippocampus in rodents using a touch-screen equipped operant box. Other automated spatial search tasks have been developed, some using novel 'virtual' environments, but it is unclear whether these are sensitive to neurobiological manipulations such as lesions of the hippocampus [36–39]. An interaction was also seen between trial block and lesion, with a significant difference in the first block of 10 trials, but not in subsequent trials. Analysis of the number of errors made on each of the ten trials during the first trial block showed that the sham and hippocampus lesion groups made the same number of errors in locating the S+ during the first trial. Once the first trial was complete the sham-lesioned animals acquired the rewarded location more quickly than did the lesion animals. Interestingly, the initially impairment was short-lived and by the 10th trial both groups showed a marked improvement in their accuracy in searching for the S+. Hippocampal lesions are known to causes behavioural changes that are not purely cognitive in nature, such as changes in stereotyped and appetitive behaviours [40,41]. Indeed, such behavioural changes might explain why hippocampal-lesioned animals made more errors at the previously rewarded location, suggesting an increase in perseverative responding. Alternatively, increased responses to the previous S+ could reflect enhanced memory in the lesioned animals. However, the observed increase in errors at non-rewarded locations, indicating memory impairment, seems to conflict with this hypothesis.

Thus, performance was impaired in ASAT1 following hippocampal lesions because of a combination of increased perseverative responding and memory impairment. Interestingly, the memory deficit (increased errors at previously non-rewarded locations) was transient. One possible explanation for this short-lived impairment is that within the first ten trials the rats had shifted from a working memory solution (in this instance defined as a memory that is quickly acquired, highly flexible, and dependent upon the hippocampus), to one that is based on reference or motor memory. Indeed, it has been shown that two-choice discriminations can be independent of the hippocampus when acquired in as few as ten trials [42]. As such, a switch from a hippocampal-based strategy to one dependent upon other brain regions may explain the short-term nature of the deficits seen. If so, it might be expected that the performance of animals with hippocampal lesions would be similar to that of the sham-lesioned animals as long as solving the task with a motor strategy was effective, and as long as the task was not subject to the confounding effects of perseveration. However, if the ability to solve the task with a motor strategy were prevented, then it might be hypothesized that a larger deficit would be seen, as is the case with other spatial tasks like the MWM. For instance, it has been shown that in the MWM, rats with a fornix lesion can complete the task if they are allowed to start from the same position, which may allow the use of a motor strategy, but if the start position is moved, then these same rats are impaired compared to a sham-lesion group [43]. Therefore in Experiment 2, we redesigned ASAT to reduce the utility of any motor strategy that may have been assisting the subjects in locating the S+.

4. Experiment 2: ASAT with start locations (ASAT2)

4.1. Behavioural testing methods

This version of the task was the same as ASAT1 in most regards. However, on the screen two rows of seven locations each were used (mask 2), instead of two rows of four locations (ASAT1). Each corner location (i.e. upper right corner, lower right corner, upper left corner, lower left corner) served as a "start location". We hoped insertion of a start location would decrease the lesioned rats' ability to locate the S+ via motor memory. Prior to the search phase of each trial, one of the 4 start locations was randomly illuminated (these changed between trials). The rat had to nose-poke at this location. Once successful, this response initiated the search phase

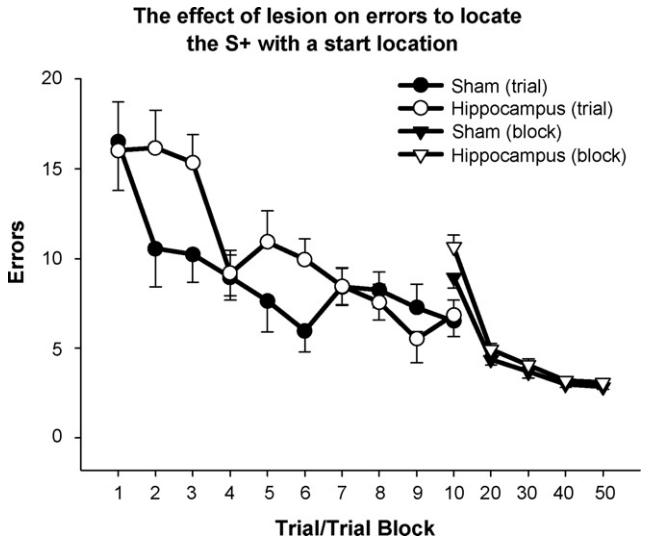

Fig. 5. Performance of control and hippocampal-lesioned rats in ASAT2. The figure shows the mean errors made in locating the S+ during the first 10 trials as well 10-trial blocks 1–5. Trial blocks are labelled as the last trial in that block (i.e. trial block 20 consists of trials 11–20). Error bars represent 1 S.E.M.

of the trial that was identical to that in Experiment 1. The array was illuminated, and a poke at the S+ location resulted in delivery of reward. Pokes at locations other than the S+ were recorded and scored for errors, but they did not have any impact on the progression of the trial. During the search phase, the location that had been used as a start location would be illuminated and responses to it were counted as errors, but these locations were not used as correct locations. A session was ended after 50 trials were completed, or 1 h passed, whichever occurred first. The session length was reduced, as in Experiment 1 rats showed little change in behaviour after the 50th trial. Rats learned this protocol by simple trial and error learning, a response at the start location to initiate the search phase was not rewarded.

4.2. Results

Once all animals had been successfully trained on the new protocol with the new mask, data were collected from 12 sessions and then used for statistical analysis. This stage of the study was also designed to allow investigation of the effects of the previous day's S+ location on the following day's performance. Specifically conditions were balanced so effects of moving the S+ up or down a row, as well as one, two, or three columns away, between days could be assessed. As such, 12 days worth of testing was required to properly counterbalance these conditions. No clear effects of these manipulations were seen; as a result data from these manipulations are not presented.

The lesion group tended to make more errors than the sham group, but this effect did not reach significance. A main effect of *trial block* was observed ($F(4,56) = 156.0$, $P < 0.0001$), while the effect of lesion approached, but just failed to reach, statistical significance ($F(1,14) = 3.84$, $P = 0.07$). The interaction between *trial block* and lesion approached, but did not reach significance ($F(4,56) = 2.46$, $P = 0.055$; Fig. 5). Analysis of the ten *trials* comprising the first block indicated a main effect of trial ($F(9,126) = 168.0$, $P < 0.0001$), but not lesion ($F(1,14) = 3.51$, $P = 0.082$). Again, no interaction was indicated between trial and lesion ($F(9,126) = 1.69$, $P = 0.097$; Fig. 5). However, when testing our a priori hypothesis, that animals with lesions of the hippocampus will commit more errors, with a one-tailed ANOVA, a significant effect of lesion was seen on both total errors ($P = 0.035$) as well as errors during the first 10 trials ($P = 0.041$).

4.3. Discussion

Our primary findings were that the introduction of a start location did not produce a larger effect of hippocampus lesion. The lesion group did require more visits to find the S+ location; however this effect only approached, but never reached, significance. In the MWM, solving the task with a non-spatial strategy is minimized by the use of multiple start locations [43]. Eichenbaum et al. found that rats with fornix lesions were able to learn the location of the hidden platform in the MWM when a fixed location was used, but were not able to complete the task in a similar amount of time with multiple start locations. The introduction of multiple start locations did not have a similar effect in our paradigm. We suggest that the addition of start locations did not have a larger effect on accuracy because they were not suitably disruptive to prevent strategies that may have been aiding in completion of the task. Perhaps a greater effect of the start locations would have been seen if the total search area was greater, or if more start locations were used. It is also possible that later trials are being solved by a non-motor-based strategy, such as one based on the use of local cues. If this were the case then no effect of start location would be expected.

The largest effect of lesion appeared to manifest itself during the first 10 trials in both Experiment 1 and 2 (although not significant in Experiment 2). We hypothesized that the procedure could be made more sensitive by creating a testing method that focused on the first 10 trials alone. Therefore, in our next experiment we shifted the location of the S+ every 10 trials during a testing session of 50 trials.

5. Experiment 3: ASAT with shifting S+ locations (ASAT3)

5.1. Behavioural testing methods

This version of the task was the same as that in Experiment 2 (ASAT2) in all respects except one. In Experiments 1 and 2 the S+ stayed in the same location throughout the session. Now, in Experiment 3 the S+ changed locations after every 10 trials. 5 different locations were used for a total of 50 trials per session. All 50 trials were used to calculate total errors. However only trials 11–50 were included in analysis of errors made at the former S+ and those made at previously non-rewarded locations. Thus, each day's testing resulted in three different measures of learning (total errors, errors made at the former S+, and those errors made at previously non-rewarded locations). Since multiple locations were used within a single day's testing fewer locations were needed for counter-balancing. Therefore, each averaged learning curve was again averaged across six sessions of testing prior to analysis.

5.2. Results

The lesion group made significantly more errors than did sham-lesioned control rats. As a new S+ location was used every 10 trials, a new learning curve was produced for each location (4 or 5 learning curves per session depending upon the measure). These learning curves were then averaged to create one, 10 trial learning curve per rat. Main effects were seen of both averaged trial ($F(9,126) = 151.0$, $P < 0.0001$) and lesion ($F(1,14) = 7.18$, $P = 0.018$; Fig. 6). However no interaction was seen between averaged trial and lesion ($F(9,126) = 0.532$, $P = 0.85$). In ASAT1 and ASAT2 *post hoc* analysis indicated that a significant effect of lesion was no longer seen at the end of 10 trials. However, the main effect of

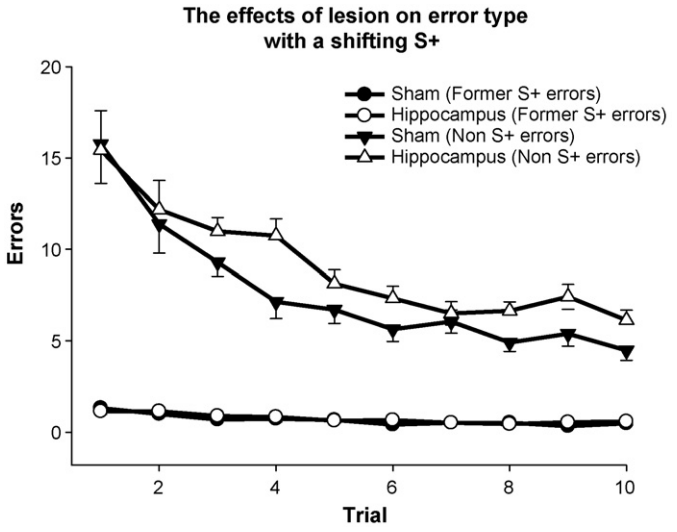

Fig. 6. Performance of control and hippocampal-lesioned rats in ASAT3 on former S+ errors and non-S+ errors. A main effect of lesion was seen ($P < 0.02$) on non-S+ errors, but not on former S+ errors. Error bars represent 1 S.E.M.

lesions seen here suggested that a difference might still exist using this paradigm. If so, this would indicate that ASAT3 might be a more suitable measure of hippocampal-dependent learning and memory. Therefore data from the 8th, 9th, and 10th trials were averaged together to test whether a significant effect of lesion could still be found at the end of the block of ten trials. An ANOVA showed a main effect of lesion ($F(1,14) = 8.49$, $P = .011$; Fig. 7) indicating that a significant effect of lesion was still present at the end of the testing block. An analysis of the error type indicated that the hippocampal-lesioned animals made more errors at previously non-rewarded locations than the sham group ($F(1,14) = 6.67$, $P = 0.021$, Fig. 6). An effect was also seen of trial ($F(9,126) = 23.7$, $P < 0.0001$), while there was no interaction between trial and lesion ($F(9,126) = 0.6$, $P = 0.7$). Moreover, no effect of lesion was seen in errors made at the former S+ ($F(1,14) = 0.58$, $P = 0.45$), and there was no interaction between lesion and trial ($F(9,126) = 0.84$, $P = 0.57$), although there was an effect of trial ($F(9,126) = 12.31$, $P < 0.0001$).

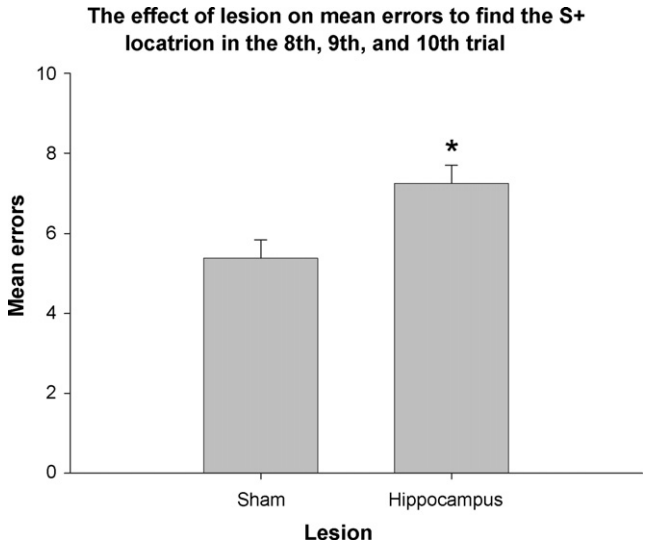

Fig. 7. Performance of control and hippocampal-lesioned rats in ASAT3. The figure shows the effect of lesion on mean errors made during the final 3 trials. A main effect of lesion is seen ($P = 0.01$).

No effect of lesion ($F(1,14) = 0.76$, $P = 0.39$), trial ($F(9,126) = 1.01$, $P = 0.43$), or lesion × trial interaction ($F(9,126) = 1.12$, $P = 0.34$) was seen on the proportion of errors made at the previous S+. *T*-tests indicated that whatever the type of error measured, no differences existed between the sham and lesion group on averaged first trial performance (total errors $T(1,14) = 0.182$, $P = 0.85$; errors at the former S+ $T(1,14) = 0.74$, $P = 0.46$); errors not at the former S+ $T(1,14) = 0.128$, $P = 0.89$). *T*-tests were also performed to examine the proportion of errors made at the former S+ on the trial after a switch of S+. Both experimental groups were found not to differ significantly from chance level performance (Chance = 0.0714; Sham average = 0.0762, $T(1,7) = 0.977$, $P = 0.36$; Hippocampus average = 0.071, $T(1,7) = -0.027$, $P = 0.979$).

5.3. Discussion

After changing the procedure so that the S+ shifted locations after every ten trials, a significant effect of lesion was seen. Importantly, an effect of lesion was still evident after 10 trials of testing. This finding is in contrast to that in Experiments 1 and 2 where behavioural deficits were short-lived and seen only in early acquisition of the S+ location. This suggests that ASAT3 is the most robust version of ASAT tested.

After ten trials, control rats are still making roughly 5 errors to locate the S+. That the rats do not make zero errors locating the S+ might suggest that they had not yet completely learned the location of the S+, and instead are using a search strategy that allows them to locate the S+ in about 5 trials. However, if such a search strategy can be used to locate the S+, then rats would always make about 5 errors, and not the roughly 15 errors they make when a new S+ is introduced. The error rate decreased by 300% across the ten trials, indicating rapid acquisition of the S+ location.

A remaining question, then, is why rats' performance asymptotes at about 5 errors (this error rate is consistent across at least the final three blocks of testing). One explanation may be the effect of interference: with each block of 10 trials during an ASAT3 session the task becomes progressively more difficult because of growing interference from previous trials. Although it has been demonstrated that perseveration to the former S+ is not occurring in ASAT3, this does not mean that the memory of the former S+ is not still affecting behaviour. This influence would be expected to increase as the number of formerly correct locations increases. Indeed, analysis of the effect of number of pervious S+s on accuracy did indicate a numerical increase in errors as testing progressed, but this increase did not reach significance (analysis not reported).

In general, in ASAT rats make more repeated errors to a particular location than they do in other assays such as the 8-arm radial maze. A potential reason for this difference is that in assays such as the radial maze there is cost associated with exploration of incorrect arms (energy spent, delay in reward retrieval). In ASAT, however, very little time or effort is required to make any single response. This may be why rats make multiple responses at locations in general and especially on the first trial after an S+ shift.

Importantly, no effect of lesion was seen on errors at the formerly rewarded S+ location. This was not the case in ASAT1 where lesions of the hippocampus caused significant increases in errors at both the formerly rewarded S+ location, as well as at previously non-rewarded S+ locations. The results of ASAT1 suggest that much of the impairment caused by lesions of the hippocampus was not the result of the lesion's effect on working memory, but rather an increase in preference for the previously rewarded location, similar to an effect previously seen in the MWM [40]. However the deficit seen here is likely to be one of working memory as no effect of lesion was seen on errors at the previously rewarded location, or in the proportion of errors at the previously rewarded location. It appears

that the switching of the S+ every 10 trials discourages perseverative behaviour, or the formation of a reference memory-based S+ representation, by rats.

6. General discussion

An automated method for studying hippocampally mediated spatial memory has great potential for researchers interested in the mechanisms of memory, for the modelling of diseases affecting such memory and for development of therapies. Unfortunately, once-popular methods such as lever-based DNMTP have been shown to suffer from confounding mediating strategies [22], and indeed may not even be sensitive to hippocampal lesions [25]. New paradigms, sensitive to lesions of the hippocampus, such as conditional DNMP have been created. Although effects of hippocampal lesions are seen in these paradigms they cannot be attributed unequivocally to a specific failure in spatial cognition [44]. Therefore the aim of the present investigation was to develop a new automated method for studying hippocampally mediated spatial memory using operant boxes equipped with touch-sensitive computer monitors. In the resulting task, the automated spatial array task, an array of identical white squares was displayed on a monitor, each square denoting a unique spatial location. One of these locations acted as an S+. In Experiment 1 (ASAT1) the rat was required to locate the S+ (in one of eight possible locations), and then return to it on subsequent trials to earn a food reward. We found that rats with lesions of the hippocampus made more errors in this task, but the impairment was short-lived with proportionally more errors made towards the former S+. This suggested that while an impairment in memory was observed, the primary cause for the deficit in acquisition was an increase in perseverative responding. In Experiment 2 (ASAT2), the rat was required to start its search from a specific location within the array, which shifted from trial to trial (similar to the starting locations used in the MWM procedure), and the total number of search locations was increased to 10. In this instance the lesion group again made more errors; however this effect narrowly missed statistical significance. Analysis of both data sets indicated that the largest effect of lesion was seen during the first 10 trials of the session, and little additional change in performance in either group was seen after the first 10 trials. Therefore in Experiment 3 (ASAT3) we focused on the first 10 trials of the session. This was done by changing the S+ location every 10 trials (5 different locations per session of 50 trials) within a session. Further statistical analysis indicated that rats with hippocampal lesions were significantly impaired even in the final trials of the 10-trial blocks. Performance of both groups appeared to have stabilized at this point, indicating robust performance impairment in the hippocampal-lesioned rats. Unlike in ASAT1, the impairment in ASAT3 cannot be attributed to an increase in perseverative responding to the formally rewarded location, and thus the impairment seen is due solely to impaired acquisition of the new S+ location.

Although this task is automated, it has similarities to other well-established, non-automated tests of spatial cognition. The obvious comparison is with the MWM, a task that inspired (along with the human self-ordered search task; [45]) the development of ASAT. In both the MWM and ASAT, the subject is required to search for, and then remember, a spatial location. Unlike the MWM, which requires rats to swim and is aversively motivated (rats escape from water), ASAT is a "dry" method that is appetitively motivated (rats work for reward). These features might be seen as advantages in cases where researchers wish to avoid the effects of fatigue or stress. In these respects ASAT shares more in common with the "cheese-board" task, developed by Kesner et al. as a dry-land version of the MWM [46,47]. In the cheese-board task the subject is required to search a board filled with dozens of small food wells. Hidden in one of these wells is a food reward. Once the reward is found, the rat is removed from the course, and the well re-baited. The rat is then reintroduced into the course and again allowed to search for a food reward. As the number of trials increases, the latency to find the correct location decreases, much like the learning curve seen in the MWM, and in ASAT. Furthermore, lesions of the hippocampus impair performance in the MWM [8,9], cheese-board task [46,48], and ASAT. Impairments following hippocampal lesions in ASAT are likely to occur for the same reasons they are observed in the MWM task or the cheese-board task, namely all of these tasks require hippocampal input to solve the spatial component of the task—although the nature of this input could be different across tasks.

Despite some similarities with the MWM and cheese-board, it is clear that ASAT differs from these other tasks in several ways. (1) The testing environment is much smaller than that used in the MWM, cheese-board, and other maze-based tasks. (2) The cues rats must use to judge locations are more impoverished and might be regard as "proximal", rather than "distal" cues. (3) The search behaviour required of the rat is unlike the "navigation" featured in most hippocampal-sensitive spatial tasks. For this reason, the deficits following hippocampal lesions seen in this paradigm may seem counterintuitive or surprising. However ASAT does, in a sense, require navigation, as the rat rears and "navigates" its snout through the array until it discovers the S+. Furthermore the search area, while considerably smaller than the MWM, is still large when compared to the size of the subject. One might predict that a larger search area would give rise to a larger, and longer lasting, deficit caused by dysfunction of the hippocampus. The influence of search area parameters on performance was well demonstrated in a mouse strain comparison study using the MWM where pool size was varied [49]. In that study latent deficits between strains were only apparent when the pool size was increased. If the non-rewarded locations in ASAT are seen as the pool, and the S+ as the platform (for comparison purposes only), then a more robust deficit would be expected if a larger 'pool' (array) were used. We hope to address this issue in future studies.

Alternatively, it may be that the comparatively impoverished environment and the small spatial array, with locations very close together, are the very factors that make ASAT hippocampal-sensitive. Using a delayed match-to-position paradigm in a cheese-board apparatus it was demonstrated that the hippocampus is important for remembering a location [50,51]. Then, by changing the distance between the S+ and S− (indicated by a pair of identical landmarks) these authors were able to improve or impair performance, without adjusting the delay between the sample and choice phase of the task. As the distance between the S+ and S− was decreased, so was the overall performance. Interestingly, animals with lesions of the hippocampus were more vulnerable to the effects of decreasing the distance between the S+ and S−, showing a greater decrease in accuracy. Kesner et al. later localised this effect to the dentate gyrus. Lesions of the dentate gyrus caused impairments in memory in the low separation condition, but not in the high separation condition [50–52]. Thus, dentate gyrus-lesioned rats were able to remember the S+ location only when there was little spatial ambiguity. It is likely that in ASAT the impoverished environment within the spatial chamber creates a high degree of stimulus overlap, thus taxing the hippocampus.

We have successfully demonstrated that spatial cognition can be measured in an automated manner in an operant box equipped with a touch-sensitive monitor. The deficits measured in ASAT may not be numerically as large as those observed using other methods, but they are consistent. A significant, or nearly statistically significant effect of hippocampal lesion was seen in every stage of training and testing. This is best illustrated in ASAT3 where lesioned animals

are still clearly impaired at the end of testing, making numerically about 25% more errors than control animals. Indeed, of the three versions of ASAT tested, ASAT3 appears to have the most potential as an automated task of hippocampus-dependent learning and memory.

This study was designed to explore novel methods for studying hippocampal function. In doing so, it also contributes to a growing body of literature on successful formation of reference memory in the presence of working memory impairments. If working memory is defined simply as short-term memory, then rats with hippocampal lesions do have short-term memory impairment. In Experiment 1 (ASAT1), hippocampal-lesioned rats are impaired only early in training, but not after a few trials of acquisition, suggesting a working (short-term) memory impairment, with relatively preserved reference memory. Indeed, the lesion group is able to eventually acquire the location of the S+, and can remember this location 24 h later. Such an interpretation is consistent with studies showing working, but not reference memory impairments following manipulations of the hippocampus. For example, in a recent study Niewoehner et al. [53] showed impaired working memory, but intact reference memory in a transgenic mouse that has functional loss of NMDA receptors within the dentate gyrus. Bannerman et al. argue for a neurobiological dissociation between reference memory and working memory, a position that is well supported by a recent review on the topic [54]. The results from ASAT1 seem to add credence to this argument. However this interpretation is dependent upon how one defines working memory in this context.

If the rats with hippocampal lesion can be thought of as having an impairment in short-term or working memory, it is possible that such an impairment could lead to ineffective spatial search strategies during the task. It may be that short-term working (or episodic) memory is used to help the rat remember where it has already searched. This might explain why a larger preference for the formerly correct locations was not seen in ASAT3 in the sham animals: development of such a spatial search strategy might have lessened the requirement for the animal to remember accurately the S+, and therefore would lead to fewer preservative responses to the previously rewarded S+. Whatever the precise nature of the processes used to solve the task, it is clear that some critical aspect depends on the integrity of the hippocampus.

Although numerous excellent paradigms exist for testing spatial learning and memory, we believe that automated tasks utilizing touch-screen technology possess unique advantages. For instance, automation allows more precise control over parameters such as delay and inter-trial interval, contact with the experimenter is minimized, and a single researcher can run many animals simultaneously. Perhaps most importantly, this assay helps to complement a cognitive test battery using the automated touch-screen apparatus for the rodent. It has already been shown that this apparatus can be used to study simple visual discriminations, visual conditional discriminations, attentional set-shifting, visual transverse patterning, as well as Pavlovian autoshaping and discrimination reversal learning [26–29,31,32,55–57]. The addition of an assay of spatial cognition to this already useful battery is a powerful improvement. Within the same apparatus numerous types of learning and memory, some with a hippocampal component and some without, can now be measured in an automated fashion. Not only does testing in this fashion offer a decrease in certain confounding variables, but also it also greatly increases the comparability between tasks. We hope that ASAT on its own, and also as part of a greater high-throughput rodent cognitive test battery, will serve as a useful tool for neurobiological investigations.

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References

[1] Burgess N, Maguire EA, O'Keefe J. The human hippocampus and spatial and episodic memory. Neuron 2002;35:625–41.
[2] Eichenbaum H, Cohen N. From conditioning to conscious recollection. New York: Oxford Press; 2001.
[3] Frisk V, Milner B. The role of the left hippocampal region in the acquisition and retention of story content. Neuropsychologia 1990;28:349–59.
[4] Scoville WB, Milner B. Loss of recent memory after bilateral hippocampal lesions. J Neurol Neurosurg Psychiatry 1957;20:11–21.
[5] Squire LR. Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychol Rev 1992;99:195–231.
[6] Vargha-Khadem F, Gadian DG, Watkins KE, Connelly A, Van Paesschen W, Mishkin M. Differential effects of early hippocampal pathology on episodic and semantic memory. Science 1997;277:376–80.
[7] Anger WK. Animal test systems to study behavioral dysfunctions of neurodegenerative disorders. Neurotoxicology 1991;12:403–13.
[8] Morris RG, Hagan JJ, Rawlins JN. Allocentric spatial learning by hippocampectomised rats: a further test of the "spatial mapping" and "working memory" theories of hippocampal function. Q J Exp Psychol B 1986;38:365–95.
[9] Morris RG, Garrud P, Rawlins JN, O'Keefe J. Place navigation impaired in rats with hippocampal lesions. Nature 1982;297:681–3.
[10] Barnes CA, Markowska AL, Ingram DK, Kametani H, Spangler EL, Lemken VJ, et al. Acetyl-1-carnitine. 2: Effects on learning and memory performance of aged rats in simple and complex mazes. Neurobiol Aging 1990;11:499–506.
[11] Bolhuis JJ, Strijkstra AM, Kramers RJ. Effects of scopolamine on performance of rats in a delayed-response radial maze task. Physiol Behav 1988;43:403–9.
[12] Lee I, Kesner RP. Time-dependent relationship between the dorsal hippocampus and the prefrontal cortex in spatial memory. J Neurosci 2003;23:1517–23.
[13] Levin ED, Rose JE. Nicotinic and muscarinic interactions and choice accuracy in the radial-arm maze. Brain Res Bull 1991;27:125–8.
[14] Olton DS. The radial arm maze as a tool in behavioral pharmacology. Physiol Behav 1987;40:793–7.
[15] Rawlins JN, Olton DS. The septo-hippocampal system and cognitive mapping. Behav Brain Res 1982;5:331–58.
[16] Schenk F, Contant B, Werffeli P. Intrahippocampal cholinergic grafts in aged rats compensate impairments in a radial maze and in a place learning task. Exp Brain Res 1990;82:641–50.
[17] White AM, Best PJ. The effects of MK-801 on spatial working memory and within-session spatial learning. Pharmacol Biochem Behav 1998;59:613–7.
[18] Brito GN, Thomas GJ. T-maze alternation, response patterning, and septo-hippocampal circuitry in rats. Behav Brain Res 1981;3:319–40.
[19] Ennaceur A, Neave N, Aggleton JP. Neurotoxic lesions of the perirhinal cortex do not mimic the behavioural effects of fornix transection in the rat. Behav Brain Res 1996;80:9–25.
[20] Stemmelin J, Cassel JC, Will B, Kelche C. Sensitivity to cholinergic drug treatments of aged rats with variable degrees of spatial memory impairment. Behav Brain Res 1999;98:53–66.
[21] Thomas GJ. Delayed alternation in rats after pre- or postcommissural fornicotomy. J Comp Physiol Psychol 1978;92:1128–36.
[22] Chudasama Y, Muir JL. A behavioural analysis of the delayed non-matching to position task: the effects of scopolamine, lesions of the fornix and of the prelimbic region on mediating behaviours by rats. Psychopharmacology (Berl) 1997;134:73–82.
[23] Dudchenko P, Sarter M. Behavioral microanalysis of spatial delayed alternation performance: rehearsal through overt behavior, and effects of scopolamine and chlordiazepoxide. Psychopharmacology (Berl) 1992;107:263–70.
[24] Herremans AH, Hijzen TH, Welborn PF, Olivier B, Slangen JL. Effects of infusion of cholinergic drugs into the prefrontal cortex area on delayed matching to position performance in the rat. Brain Res 1996;711:102–11.
[25] Sloan HL, Good M, Dunnett SB. Double dissociation between hippocampal and prefrontal lesions on an operant delayed matching task and a water maze reference memory task. Behav Brain Res 2006;171:116–26.
[26] Bussey TJ, Muir JL, Robbins TW. A novel automated touchscreen procedure for assessing learning in the rat using computer graphic stimuli. Neurosci Res Commun 1994;15:103–10.
[27] Bussey TJ, Everitt BJ, Robbins TW. Dissociable effects of cingulate and medial frontal cortex lesions on stimulus-reward learning using a novel Pavlovian autoshaping procedure for the rat: implications for the neurobiology of emotion. Behav Neurosci 1997;111:908–19.

[28] Bussey TJ, Saksida LM, Rothblat LA. Discrimination of computer-graphic stimuli by mice: a method for the behavioral characterization of transgenic and gene-knockout models. Behav Neurosci 2001;115:957–60.
[29] Brigman JL, Bussey TJ, Saksida LM, Rothblat LA. Discrimination of multidimensional visual stimuli by mice: intra- and extradimensional shifts. Behav Neurosci 2005;119:839–42.
[30] Bussey TJ, Muir JL, Everitt BJ, Robbins TW. Dissociable effects of anterior and posterior cingulate cortex lesions on the acquisition of a conditional visual discrimination: facilitation of early learning vs. impairment of late learning. Behav Brain Res 1996;82:45–56.
[31] Bussey TJ, Muir JL, Everitt BJ, Robbins TW. Triple dissociation of anterior cingulate, posterior cingulate, and medial frontal cortices on visual discrimination tasks using a touchscreen testing procedure for the rat. Behav Neurosci 1997;111:920–36.
[32] Chudasama Y, Robbins TW. Dissociable contributions of the orbitofrontal and infralimbic cortex to pavlovian autoshaping and discrimination reversal learning: further evidence for the functional heterogeneity of the rodent frontal cortex. J Neurosci 2003;23:8771–80.
[33] Janisiewicz AM, Baxter MG. Transfer effects and conditional learning in rats with selective lesions of medial septal/diagonal band cholinergic neurons. Behav Neurosci 2003;117:1342–52.
[34] Ito R, Everitt BJ, Robbins TW. The hippocampus and appetitive Pavlovian conditioning: effects of excitotoxic hippocampal lesions on conditioned locomotor activity and autoshaping. Hippocampus 2005;15:713–21.
[35] Paxinos G, Watson C. The rat brain. London: Academic Press; 1998.
[36] Nekovarova T, Bures J. Spatial decisions in rats based on the geometry of computer-generated patterns. Neurosci Lett 2006;394:211–5.
[37] Nekovarova T, Klement D. Operant behavior of the rat can be controlled by the configuration of objects in an animated scene displayed on a computer screen. Physiol Res 2006;55:105–13.
[38] Nekovarova T, Nedvidek J, Bures J. Spatial choices of rats based on abstract visual information: pattern- or configuration-discrimination? Behav Brain Res 2006;172:264–71.
[39] Holscher C, Schnee A, Dahmen H, Setia L, Mallot HA. Rats are able to navigate in virtual environments. J Exp Biol 2005;208:561–9.
[40] Whishaw IQ, Tomie JA. Perseveration on place reversals in spatial swimming pool tasks: further evidence for place learning in hippocampal rats. Hippocampus 1997;7:361–70.
[41] Tracy AL, Jarrard LE, Davidson TL. The hippocampus and motivation revisited: appetite and activity. Behav Brain Res 2001;127:13–23.
[42] Broadbent NJ, Squire LR, Clark RE. Rats depend on habit memory for discrimination learning and retention. Learn Mem 2007;14:145–51.
[43] Eichenbaum H, Stewart C, Morris RG. Hippocampal representation in place learning. J Neurosci 1990;10:3531–42.
[44] Sloan HL, Dobrossy M, Dunnett SB. Hippocampal lesions impair performance on a conditional delayed matching and non-matching to position task in the rat. Behav Brain Res 2006;171:240–50.
[45] Robbins TW, James M, Owen AM, Sahakian BJ, McInnes L, Rabbitt P. Cambridge Neuropsychological Test Automated Battery (CANTAB): a factor analytic study of a large sample of normal elderly volunteers. Dementia 1994;5:266–81.
[46] Kesner RP, Farnsworth G, DiMattia BV. Double dissociation of egocentric and allocentric space following medial prefrontal and parietal cortex lesions in the rat. Behav Neurosci 1989;103:956–61.
[47] Kesner RP, Farnsworth G, Kametani H. Role of parietal cortex and hippocampus in representing spatial information. Cereb Cortex 1991;1:367–73.
[48] Kirwan CB, Gilbert PE, Kesner RP. The role of the hippocampus in the retrieval of a spatial location. Neurobiol Learn Mem 2005;83:65–71.
[49] Van Dam D, Lenders G, De Deyn PP. Effect of Morris water maze diameter on visual-spatial learning in different mouse strains. Neurobiol Learn Mem 2006;85:164–72.
[50] Gilbert PE, Kesner RP. The role of the dorsal CA3 hippocampal subregion in spatial working memory and pattern separation. Behav Brain Res 2006;169:142–9.
[51] Gilbert PE, Kesner RP, DeCoteau WE. Memory for spatial location: role of the hippocampus in mediating spatial pattern separation. J Neurosci 1998;18:804–10.
[52] White NM. The role of stimulus ambiguity and movement in spatial navigation: a multiple memory systems analysis of location discrimination. Neurobiol Learn Mem 2004;82:216–29.
[53] Niewoehner B, Single FN, Hvalby Ø, Jensen V, Borgloh S, Seeburg PH, et al. Impaired spatial working memory but spared spatial reference memory following functional loss of NMDA receptors in the dentate gyrus. Eur J Neurosci 2007;25:837–46.
[54] Bannerman DM, Rawlins JN, Good MA. The drugs don't work-or do they? Pharmacological and transgenic studies of the contribution of NMDA and GluR-A containing AMPA receptors to hippocampal-dependent memory. Psychopharmacology (Berl) 2006;188:552–62.
[55] Bussey TJ, Clea Warburton E, Aggleton JP, Muir JL. Fornix lesions can facilitate acquisition of the transverse patterning task: a challenge for "configural" theories of hippocampal function. J Neurosci 1998;18:1622–31.
[56] Janisiewicz AM, Jackson 3rd O, Firoz EF, Baxter MG. Environment-spatial conditional learning in rats with selective lesions of medial septal cholinergic neurons. Hippocampus 2004;14:265–73.
[57] Morton AJ, Skillings E, Bussey TJ, Saksida LM. Measuring cognitive deficits in disabled mice using an automated interactive touchscreen system. Nat Methods 2006;3:767.
[58] D'Hooge R, De Deyn PP. Applications of the Morris water maze in the study of learning and memory. Brain Res Brain Res Rev 2001;36:60–90.
[59] McNamara RK, Skelton RW. The neuropharmacological and neurochemical basis of place learning in the Morris water maze. Brain Res Brain Res Rev 1993;18:33–49.