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En route to delineating hippocampal roles in spatial learning

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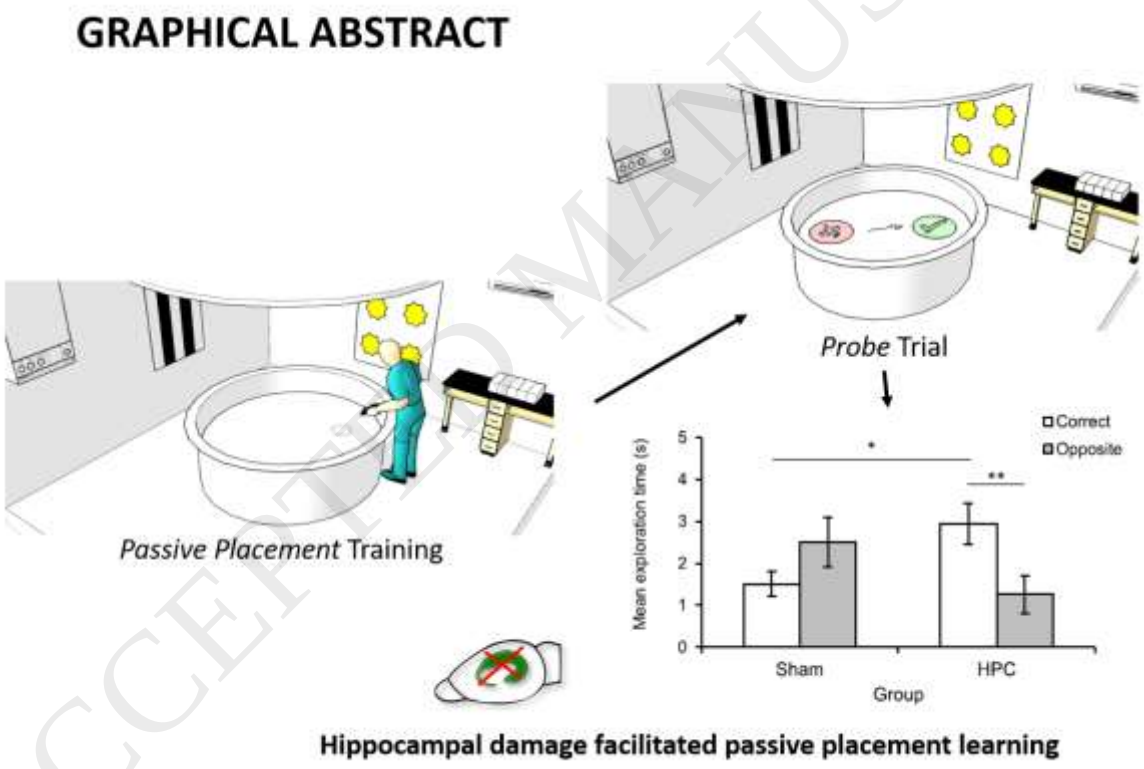

Abstract

The precise role played by the hippocampus in spatial learning tasks, such as the Morris Water Maze (MWM), is not fully understood. One theory is that the hippocampus is not required for 'knowing where' but rather is crucial in 'getting there'. To explore this idea in

the MWM, we manipulated 'getting there' variables, such as passive transport or active swimming towards the hidden platform, in rats with and without hippocampal lesions.

Our results suggested that for intact rats, self-motion cues enroute to the hidden goal were a necessary component for 'place learning' to progress. Specifically, intact rats could not learn the hidden goal location, when passively transported to it, despite extensive training. However, when rats were either given hippocampal lesions, or placed in a light-tight box during transportation to the hidden goal, passive-placement spatial learning was facilitated. In a subsequent experiment, the 'getting there' component of place navigation was simplified, via the placement of two overhead landmarks, one of which served as a beacon. When 'getting there' was made easier in this way, hippocampal lesions did not induce deficits in 'knowing where' the goal was. In fact, similar to the facilitation observed in passive-placement spatial learning, hippocampal lesions improved landmark learning relative to controls. Finally, demonstrating that our lesions were sufficiently deleterious, hippocampal-lesioned rats were impaired, as predicted, in an environmental-boundary based learning task. We interpret these results in terms of competition between multiple memory systems, and the importance of self-generated motion cues in hippocampal spatial mapping.

[248 words]

Keywords

spatial learning, hippocampus, passive learning, cognitive map, getting there, knowing where, self-generated motion, multiple memory systems

1. Introduction

The Morris Water Maze (MWM) requires animals, usually rats or mice, to learn to escape from a tank of opaque water by swimming to a small hidden platform beneath the water surface [1]. Crucially, the platform cannot be identified by local olfactory, visual or auditory cues, and can only be identified by tactile cues when the animal bumps into it. Accordingly,

the animal first typically swims quasi-randomly until it happens upon the hidden platform, then gradually learns to use available visual cues and its self-motion to navigate to the platform location. After a period of training, when the hidden platform is removed, the animal typically heads in the appropriate direction, from a distant location, and exhibits a strong search bias to the former goal location [2]. Thus, a hidden goal location can be recognised in close proximity to, as well as some distance from, where it was originally encountered, raising the possibility that spatial learning can occur enroute to a goal ('getting there') as well as directly at the goal ('knowing where'). Evidence has also shown that lesions of the hippocampus severely impair performance when rats are required to navigate to a hidden goal in the MWM [2], but it is not entirely clear whether the observed deficits are confined to 'getting there', 'knowing where' or whether hippocampal damage disrupts both navigational components in tandem.

Several studies have shown that rats with hippocampal damage are capable of learning the location of a hidden goal in the MWM task when the training protocol is adapted to facilitate acquisition of the task. Such measures include additional training [3-5], cueing or shaping of the target location [6-8] or starting with a large target area and gradually reducing its size over training [9]. This evidence, in conjunction with additional studies supporting the notion that the hippocampus is particularly important for self-generated motion (SGM), or path integration, during navigation [7, 10, 11, 12, but note that 12 induced only fimbria-fornix lesions], implies that the 'getting there' component of navigation plays a crucial role in 'knowing where' following damage to the hippocampus. Key support for the importance of self-generated motion in hippocampal spatial mapping comes from electrophysiological studies recording spatial neurons within the rodent hippocampal formation [13-15]. For example: a) passive transport degrades spatial signals in place cells, greatly increasing place field size [16]; b) in rodent virtual environments, only 25% of place cells are modulated primarily by visual cues while most of the remainder receive significant self-generated motion inputs in combination with visual information [17]; and c) passive transport greatly degrades the spatiality of grid cells and speed cells in parahippocampal cortex [18, 19]. Taken together, such studies clearly show that passive transport greatly degrades spatial signals in the extended hippocampal spatial mapping system.

The following series of experiments investigated the role of the hippocampus in 'knowing where' when 'getting there' was either not under the control of the animal (*Experiment 1; passive placement to the goal*) or was made easier by the presence of a homing beacon (*Experiment 3*). *Experiment 2* attempted to prevent the animal from performing spatial mapping prior to being placed at the goal location. Specifically, the aim was to use a light-tight box to prevent the rat from combining visual cues with highly-degraded motion cues (i.e. in the absence of self-generated motion). *Experiment 4* tested the rats with hippocampal damage used in *Experiments 1* and *3* in a task requiring learning based on environmental shape. This final experiment was intended to demonstrate the effectiveness of the lesions by replicating robust hippocampal-dependent behavioural deficits observed in previous studies [20-22].

Experiments 1 and *2* employed a passive placement version of the classic MWM task [2]. As opposed to rats being released from various start locations at the edge of the pool in order to locate a submerged platform, the experimenter passively transported rats (from the various start locations) and placed them onto the hidden platform. This allowed for the encoding of information at the goal and its relationship to surrounding room cues but not when the rat made its own way to the goal. Several experiments have investigated this type of passive learning in intact rats, but the findings have been equivocal, with a number of studies reporting modest learning abilities [23-26] and another series of experiments failing to demonstrate passive spatial learning at all [27]. It has also been shown that rats are capable of passively learning the location of a hidden goal based on cues provided by the walls forming the pool, e.g. brightness [28], local geometric properties [29, 30] or the arrangement of patterns [30]. Moreover, Kosaki et al. [31] used a similar design to reveal that hippocampal lesions impaired passive spatial learning based on the relative positions of distinctively patterned walls in a square pool. Importantly, however, the current study is the first to investigate the role of the hippocampus in a passive learning version of the classic hippocampal-dependent MWM task [2].

The strategies employed and sensory mechanisms engaged during mammalian navigation can be varied [14], making it difficult to dissect out the cause/s of any observable navigational deficits following hippocampal damage. As opposed to the hippocampus being essential for representing a 'place', one argument is that it is in fact crucial for the flexible

integration of non-spatial information during navigation tasks [7, 9, 32, 33]. For example, animals with hippocampal damage may struggle to elucidate the purpose of a specific task and perseverate with inappropriate behaviours, which would emerge as an acquisition deficit. A second, somewhat related argument, discussed above, is that the hippocampus is not essential for place learning *per se* but is critical for processing self-generated motion cues used enroute to getting to a place. For either argument, one would predict hippocampal damage should not markedly impair performance on a passive spatial learning task during which any non-spatial memory demands and/or self-generated motion cues are rendered irrelevant. The following studies provide an examination of the role of the hippocampus (CA1-CA4 pyramidal fields and dentate gyrus) during both passive and active navigation tasks.

2. Material and methods

2.1 Experiment 1 Hippocampal Lesion: Passive and Active Navigation

2.1.1 Subjects

The subjects were 25 experimentally naive male Lister hooded rats (*Rattus norvegicus*) supplied by Harlan Olac (Bicester, Oxon, England). At the start of the experiment rats were approximately 4 months of age. 13 animals underwent surgery to create bilateral lesions of the hippocampus (*HPC*) and 12 animals underwent sham operations. Rats were provided with ad libitum access to food and water, and housed in pairs in a light-proof, temperature-controlled room in which the lights were turned on at 0700 hours and off at 2100. Testing was conducted at the same time each day, during the light phase. All experiments were performed under the Animals (Scientific Procedures) Act 1986 and according to Home Office and institutional guidelines.

2.1.2 Surgical Procedure

Each animal was placed into a Perspex anaesthetic chamber, which was filled with a mixture of isoflurane (5%) and oxygen (2L/min). Once deeply anaesthetised, the experimenter removed the animal from the chamber, shaved its head and then secured it into a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). A plastic pipe was positioned close

to the rat's snout, which fed a constant supply of isoflurane and oxygen. At this stage, the anaesthetic was reduced to a maintenance concentration (1-2% isoflurane at 0.8L/min) and it was ensured that the animal's heart rate and reflexes were closely monitored throughout to make sure the rat remained at the appropriate level of anaesthesia.

During surgery the rat was wrapped in a sterile drape and placed on a heat mat. A digital thermometer probe was placed under the animal's body so that the experimenter could monitor its temperature. Eye ointment was placed over the eyes of the rat and an incision was made, with a scalpel, along the midline of the scalp. Saline solution was constantly applied to the surface of the brain to retain moisture. Sections of bone covering the neocortex on each hemisphere were removed using a dental drill and burr cutter. An arm comprising of a 2-µl Hamilton syringe and electronic microdrive (model KDS 310, KD Scientific, New Hope, PA) was then mounted on to the stereotaxic frame. Once attached, it was possible to manoeuvre the needle of the syringe to the appropriate coordinates and, with the electronic microdrive, administer the desired quantity (.05 - .10 µl) and rate of infusion (.03 µl/min) of excitotoxin (Ibotenic acid).

There were 28 injection sites for each bilateral hippocampal lesion (the coordinates and volume of infusions used followed the protocol described by [34]. Ibotenic acid (Biosearch Technologies, San Rafael, CA), dissolved in phosphate-buffered saline (pH 7.4) to produce a 63-mM solution, was infused at each injection site with the needle left in place for 2 minutes to permit thorough diffusion of the ibotenic acid into surrounding tissue. Prior to penetrating the dura with the Hamilton syringe needle, a finer gauge needle was used to create a small surface slit at the point of entry to facilitate passage. Each time the Hamilton syringe needle was removed from the brain it was thoroughly cleaned using two cotton buds soaked with 70 % alcohol. Sham animals underwent a similar surgical procedure, except that after having the dura perforated with a standard needle, the subsequent insertion of the Hamilton syringe needle was not performed.

After surgery, sutures (Mersilk 3-0, Ethicon Inc.) bound the wound of the animal, which was allowed to recover in a warm chamber until conscious. All animals were administered subcutaneously with Buprenorphine (.01 mg/kg, pre and post operation) to provide analgesia, and a saline and glucose solution (10-ml, post operation) to facilitate rehydration.

Once the rat had sufficiently recovered, it was placed, alone for the first two days, back into its home cage where it was provided with soaked chow and a hydrogel pack. All animals were given a minimum of 14 days postoperative recovery time prior to commencement of training.

Upon completion of behavioural procedures, rats were injected with a lethal dose of sodium pentobarbitone (Euthatal) and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde solution (0.1M phosphate-buffered). Each brain was removed from the animal, placed in a jar filled with 4% paraformaldehyde solution (0.1M phosphate-buffered solution) for several days and then transferred to a second jar filled with 25% sucrose (in 0.1M phosphate buffered saline) for another day. Using a cryostat set to -19^{o}C the brains were frozen and sliced into coronal sections (40-µm thick), which were placed onto positively charged slides (Thermo Scientific Superfrost Plus). The sections were stained with cresyl violet and analysed using a microscope and brain atlas [35]. Reconstructions of the brain sections were created and these images were processed in Matlab® to determine the percentage of hippocampal tissue damage.

2.1.3 Apparatus

The experiment took place in a white, circular, fibre glass pool with a diameter of 200 cm and a depth of 60 cm. The pool was filled to a depth of 30 cm with water, which was warmed to a temperature of 25°C (± 2°C), rendered opaque with the addition of 500 ml of white opacifier (OP303B, supplied by Rohm and Haas, UK) and changed daily. The pool was elevated 40 cm off the ground on a secure platform positioned in the centre of a laboratory room (465 x 395 x 230 cm high). A white, circular, Perspex ceiling (200 cm in diameter and 0.5 cm thick) was suspended directly above the pool at a distance of 108 cm from the uppermost edge of the pool walls. Recessed into this suspended ceiling were eight 45-W spotlights, each 18.5 cm in diameter and arranged equidistantly from one another in a 100 cm-diameter, centred circle. These spotlights, as well as two 35-W, 150 cm strip lights individually placed on the east and west walls (68 cm above and parallel to the floor with the midpoint on the east-west axis of the pool) and four 50 x 50 cm ceiling lights each housing four 14-W tubes 50 cm in length and positioned in each corner of the room (60 cm from each wall comprising the corner) illuminated the testing room during the experimental

period. There was a hole, 35 cm in diameter, cut out of the centre of the suspended ceiling which allowed a wide-angled video camera to be positioned centrally on a tripod 5 cm above. A HDD DVD recorder (Sony RDR-HXD890) and monitor (Ganz ZM-CR114NP-II) were located on a table in the southwest corner of the room where images from the video camera were transmitted. The recorded video files were subsequently analysed using Ethovision software (EthoVision, Noldus, NL) to measure the swim path of each rat.

The walls and ceiling of the laboratory comprised of white PVC. The north wall was covered with black wallpaper except for a vertical white stripe (25 cm wide) positioned horizontally central and spanning the height of the wall. A free-standing white board (122 cm long x 81 cm wide) was positioned 54 cm outward from the southwest edge of the circular pool, which acted as a screen to conceal the experimenter who sat in the southwest corner of the lab during trials. Various posters were situated on the walls around the room.

The escape platform, which stood 2 cm below the surface of the opacified water, was constructed of clear Perspex and comprised of a circular disc (10 cm in diameter, 1 cm thick), with concentric grooves machined into it, sat atop a cylindrical rod (1.5 cm in diameter x 26 cm long) which was itself attached to a square base (25 x 25 x 1 cm thick).

For *Pre-Training* (described below), a white rectangular pool (90 x 180 x 58 cm high) was manufactured by suspending polyurethane boards inside the curtained, circular pool. Hollow, square aluminium rail (1.5 x 1.5 cm) sat on the top lip of the circular pool from which the boards were suspended vertically. Velcro was used on the outer facing corners of the rectangle to hold the boards together. A visible beacon, attached to the hidden platform, moved around the rectangular pool between trials. The beacon was a stick painted with black and white horizontal stripes (band width 1 cm) that stood vertically to a height of 15 cm above the surface of the water.

2.1.4 General Procedure

Rats were transported into the test laboratory, five at a time, in an opaque carrying box, which housed each animal in a separate compartment. The trial commenced with the experimenter placing the rat gently into the pool, ensuring that the rat's head faced the wall, and ended when the hidden platform was located. If the animal failed to find the

platform within sixty seconds, the experimenter guided the rat to the platform by holding out a hand in front of its nose. The rats were left on the platform for 30 seconds before the experimenter removed the animal from the pool, dried it with a towel and placed it back into the holding box, where it remained until the remaining four animals had each completed a trial. This cycle was repeated until all five rats had received four training trials (one session). For test trials, conducted at the end of training, the escape platform was removed and animals were released from a novel location, equidistant from where the goal site had been during training and an arbitrary opposite zone (see *Performance Measures*), and allowed to swim for a specified period. At the end of each day the pool walls were cleaned with disinfectant spray and thoroughly rinsed with clean water.

2.1.5 Assignment of groups

HPC lesioned animals were split into two groups: *Group Active Swim* (n=6) or *Group Passive Placement* (n = 7) with 6 sham-operated controls assigned to each of these groups. Platform position during the *Extra-Maze* task was counterbalanced so that for each group, half the lesioned animals were trained to locate the platform in the northwest quadrant of the pool and half with the platform in the southeast quadrant (for *HPC* rats in the *Passive Placement* condition, 4 were assigned to southeast and 3 to northwest). The same applied for sham-operated controls.

2.1.6 Pre-Training

All rats received two sessions (4 trials per session) of *Pre-Training* in a rectangular pool. In each trial, rats were trained to locate a visible escape platform. The purpose of *Pre-Training* was to familiarise animals with climbing onto the escape platform and encourage them to avoid adopting a strategy of repeatedly circling around the edge of the pool. It also provided those animals assigned to the subsequent *Passive Placement* condition the aversive experience of swimming in the pool and the reinforcement of finding the escape platform, both intended to discourage any jumping off the platform during subsequent *Passive Placement* training (described below). The midpoints of each wall were designated as the points of release into the pool. These were assigned randomly for each trial with the constraint that each of the four different release points were used within a session. The orientation of the pool, fully enclosed by curtains, remained constant across all trials of *Pre-*

Training. The escape platform was moved pseudo-randomly across trials with the constraint that its position varied according to where the rat was released from, i.e. rats could not adopt a fixed motor response after release, and its centre was a minimum of 25cm from the edge of the pool.

2.1.7 Extra-Maze Training

Following *Pre-Training*, rats received 14 sessions of *Extra-Maze* training conducted in the circular pool with unrestricted access to ambient room cues. Animals were trained to swim to (*Group Active Swim*) or were placed on (*Group Passive Placement*) an invisible escape platform occupying a fixed location. The platform was positioned so that its centre was 50 cm from the edge of the pool along a northwest-southeast axis, either in the northwest or southeast quadrant of the pool depending on which position had been assigned to that particular animal. For *Group Active Swim*, the trial commenced and terminated as described in the *Procedure* section above. For rats in *Group Passive Placement*, however, the experimenter carried the animal to the appropriate release point, held it just above the surface of the water, and moved it from the point of release to the escape platform on a straight-line trajectory, where it was placed (*Figure 1B*). Each rat was left on the platform for 30 seconds before being removed from the pool. Once removed and quickly dried, the animal was given a 30 second inter-trial interval, starting immediately after removal from the platform, before commencing the second trial. This cycle was repeated until the animal had completed four trials after which it was dried and placed back into the holding box so that the next rat could begin its four trials. The experimenter continued in this fashion until all five rats had received four trials. Eight points, evenly spaced around the circumference of the pool, were used as the points of release from the edge of the circular pool. The release points were assigned randomly for each trial with the constraint that eight different release points were used across two sessions (8 trials). This manipulation ensured rats could not learn a fixed strategy from a constant release point.

2.1.8 Extra-Maze Test

After 14 sessions of *Extra-Maze* training, rats received the probe trial on day 15 (Figure 1C), which was preceded by two additional training trials (using the south and north release

points). For the test trial, the platform was removed and animals were placed in the centre of the pool from a south-westerly direction and allowed to swim for 120 seconds.

2.1.9 Performance Measures

For each training trial, acquisition rate was measured by recording escape latency, which was the time taken for a rat to find the escape platform. This was recorded live by the experimenter using a stopwatch whilst watching images of the test arena on a monitor.

For the probe trials, the recorded footage of each rat's swim path was tracked using Ethovision (3.1) software. With this program it was possible to overlay zones onto the recorded images so that the time a rat spent in a designated area could be objectively measured. Exploration was considered to have taken place if the rat's head entered a target zone. Two circular zones measuring 33 cm in diameter were created: one centred on the area where the escape platform was formerly placed during training (*Correct Zone*) and the other occupying the equivalent position in the opposite quadrant of the pool (*Opposite Zone*) (see Figure 1C).

In order to establish if any lesion differences in performance were attributable to differences in motor function (swim speed) or thigmotaxis, which has been shown through pharmacological [36] and hormonal [37] studies to be a reliable indicator of anxiety, Ethovision was used to record the mean velocity (cm / s) and the amount of time rats spent close to (≤ 20 cm) the walls of the pool. These measures were recorded for all probe trials. Additionally, any lesion differences in thigmotaxis during the first training trials, when it could be argued rats were at their most anxious in a novel, aversive task, were also investigated. This would offer more insight into whether thigmotaxis, and therefore anxiety, could have influenced performance during acquisition. For such training trials, during which the escape platform was present, time spent close to the walls of the pool as a percentage of total time taken to locate the hidden escape platform was calculated for the first session.

2.1.10 Data Analyses

A two-way ANOVA of escape latencies with *Lesion* group (*Sham* and *HPC*) as the between-subject variable and session as the repeated measure was conducted to analyse acquisition. For the test trial, a two-way ANOVA of exploration times was conducted with *Lesion* group

as the between-subjects variable and *Zone* (*Correct* and *Opposite*) as the repeated measure. For all post hoc analyses the *Bonferroni* correction was applied. The effect of lesion on swim velocity and thigmotaxis was analysed using independent-samples t-tests.

2.2 Experiment 2 In-Box Passive Placement

2.2.1 Subjects

The subjects were 30 experimentally naive male Lister hooded rats (*Rattus norvegicus*) supplied by Harlan Olac (Bicester, Oxon, England). At the start of the experiment, rats were approximately 2.5 months of age and housed in identical conditions to *Experiment 1*.

The *Apparatus*, *General Procedure*, *Pre-Training, Performance Measures and Data Analyses* were identical to *Experiment 1*.

2.2.2 Extra-Maze Training

Extra-Maze training and the use of a *Passive Placement* group (n=10; using a new group of intact animals) was the same as that described for *Experiment 1* (Figure 2A). However, there was no *Group Active Swim* but two new groups: *Group Passive Box* (*n*=10) and *Group Passive/Swim* (*n*=10). *Group Passive Box* training was identical to *Group Passive Placement's* except that instead of each rat being placed on to the hidden escape platform by the experimenter's hand, a light tight box (15 x 22 x 11 cm high) was used to transport the rat from the holding area to the escape platform (Figure 2B). For *Group Passive/Swim*, rats were trained identically to *Group Passive Placement* but training was interspersed with a series of 30 second swim trials with the hidden escape platform removed. In total, four swim trials were conducted, each in place of the fourth trial at the end of sessions 3, 6, 9 and 12.

2.2.3 Extra-Maze Test

The test trial was identical to *Experiment 1* (section *2.1.8* and Figure 2C).

2.3 Experiment 3 Extra-Maze + Landmark Task

2.3.1 Subjects

The subjects were 20 male Lister hooded rats (*Rattus norvegicus*) supplied by Charles River (UK), which were approximately 8 months of age at the start of the experiment. All animals had previously participated in unrelated object recognition and colour discrimination tasks, and it was ensured that this prior experience was counterbalanced. At the start of the experiment there were 12 animals with bilateral lesions of the hippocampus (HPC) and 8 sham-operated animals. However, following histological analysis (see section *3.6.2*), one lesioned animal was excluded from the experiment, so henceforth *Group HPC* contained 11 rats.

2.3.2 Surgical Procedure

Refer to *Experiment 1* (section 2.1.2) for the surgical procedure.

2.3.3 Apparatus

The room cues used were identical to those described in *Experiment 1* except that there was no black wall paper covering the north wall. The black wall was introduced for the passive learning tasks in *Experiment 1 & 2* to heighten the salience of the room cues to facilitate what was a very difficult task. In the current experiment, all animals could actively swim to the escape platform and were guided by overhead landmarks (one serving as a beacon), so the addition of the black wall was not necessary.

Two types of landmark were used, a sponge ball, 9.5 cm in diameter and painted matt black, and a hollow, octagonal prism, constructed of white polystyrene with each rectangular panel measuring 9.5 x 4 x 1 cm thick. The prism also had two centred, horizontal black stripes (2.5 cm band width) with a gap of 2.5 cm between them, painted around the entire perimeter of its outer surface. Each landmark was suspended 27cm above the surface of the water from their lowest vertical point using thin soldering wire attached to hooks affixed to the ceiling above.

2.3.4 Procedure

Refer to *Experiment 1*'s *General Procedure* (section 2.1.4)

2.3.5 Extra-Maze + Landmark Training

Animals received 15 sessions of *Extra-Maze + Landmark* training. Each session consisted of four trials, except sessions 10, 13 and 15, which consisted of two training trials followed by one test trial. Two discrete, visually distinct landmarks were each positioned 110 cm apart and 35 cm from the edge of the pool along a northwest-southeast axis. For each animal the array of room cues and the location of each landmark remained constant. The centre of the escape platform was positioned directly below one of the landmarks and remained in the same position throughout training. Thus, both distal room cues (*Extra-Maze*) and landmark identity (*Landmark*) were informative in signalling the location of the escape platform (dual solution task; see Figure 3A). Landmark identity and platform position were counterbalanced so that within each lesion group (*Sham* and *HPC*), half the animals were trained with the platform under the black ball and half under the striped prism. These landmark subgroups were split further so that half the animals were trained with the platform in the northwest quadrant of the pool and the remaining half with the platform in the southeast quadrant. The four release points (north, south, east and west) were randomised with the constraint that rats were released once from each point within a session. This was to discourage animals using a fixed motor response from a constant release point.

2.3.6 Test Trials

Rats received three test, or probe, trials each lasting 60 seconds. The first test (*Extra-Maze + Landmark Test*), which took place on trial 3 of session 10, was conducted in the circular pool with the landmarks and room cues arranged identically as they were during training. This test was conducted to provide a behavioural measure, alongside the training data, of how much rats had learned about the landmark and room cues in compound. Rats were then provided with two and a half sessions of retraining before receiving their second test trial on trial 3 of session 13, the type of which was counterbalanced so that half the animals from each group were given the *Extra-Maze Test* and the other half were given the *Landmark Test*. For the *Extra-Maze Test*, the room cues remained identical to training but the landmarks were removed. For the *Landmark Test*, a curtain was drawn around the full circumference of the pool, so that animals were denied access to extra-maze room cues. The landmarks were positioned the same distance from each other and the edge of the pool as during training, but on a northeast-southwest axis as opposed to a northwest-southeast

axis (see Figure 3Bi). Finally, rats received a further one and a half sessions of *Extra-Maze + Landmark* retraining before being presented with their third test on trial 3 of session 15. Those animals that received the *Extra-Maze Test* previously were now given a *Landmark Test* and for the remaining animals the reverse applied.

2.4 Experiment 4 Shape Task

After completion of *Experiments 1* and *3*, the same rats were subsequently tested in a task to assess the effect of hippocampal damage on learning based on the shape of the environment. As such, the current experiment was divided into two parts: *Experiment 4A* (using the rats from *Experiment 1*) and *Experiment 4B* (using the rats from *Experiment 3*).

2.4.1 Subjects

Refer to *Experiment 1* for the subjects used in *Experiment 4A*, and *Experiment 3* for those used in *4B*.

2.4.2 Apparatus

The experiment was conducted in a white rectangular pool (90 x 180 x 58 cm high) described in *Experiment 1* (section *2.1.6 Pre-Training*).

2.4.3 Assignment of groups

For this task in which all animals actively swam during training and test, approximately half the lesioned animals found the escape platform in a corner of the rectangular pool where the short wall was to the right of a long wall and the remaining half experienced the platform in a corner where the short wall was to the left of a long wall. The same counterbalancing applied for sham-operated controls.

2.4.4 Procedure

General procedures were identical to those described in *Experiment 1*.

2.4.5 Shape Training

Animals received 6 sessions (*Experiment 4A*) and 4 sessions (*Experiment 4B*) of *Shape* training conducted in a white rectangular pool (note the rats in *Experiment 4A* required

slightly more training than the animals in *Experiment 4B*, presumably because the rats (both lesioned and sham) in the latter group had different prior swimming experience to the former group. *Shape* training involved rats having to locate the escape platform in one corner of the pool, e.g. the platform was always found in a corner where a short wall was to the left of a long wall. Throughout this training and the subsequent test trial, the curtains were drawn around the pool so that rats could only use shape-based information provided by the walls of the pool. The escape platform was placed in the designated corner with its centre 25 cm from the point at which the two walls of the corner met on a trajectory which split this corner in half (see Figure 4A).

Within a session the platform was located in one corner of the rectangular pool for a randomly selected two trials and in the diametric opposite corner for the remaining two trials. Technically, the diametrically opposite corners of the white rectangular pool should look identical to a rat but the escape platform was oscillated between these corners to minimise the chance rats could use some local cue, odour or otherwise, to aid their search for the platform. The midpoints of each wall were designated as the points of release into the pool. The arena was rotated between each trial and could be oriented in four positions through a north-south or east-west axis. The release points and arena positions were assigned randomly for each trial with the constraint that the four different release points and orientations were used within a session.

2.4.6 Shape Test

After the final session of *Shape Training*, rats received the test trial (*Shape Test*), which lasted 30 seconds (Figure 4B). Note that this arena was a lot smaller than the circular arena used in Experiments 1, 2 and 3, so the test trial was shorter. The escape platform was removed and rats were placed in the centre of the white rectangular pool. The orientation of the pool was novel (now diagonal relative to the training orientations).

2.4.7 Performance Measures

For the *Shape Test*, four circular search zones each measuring 33 cm in diameter were individually positioned so that the centre of each zone corresponded to where the centre of the escape platform would have been if it had been paired with that corner. For this test,

the time rats spent in the two correct corner zones (*Correct Zone*), according to the geometric layout, was calculated and compared to time spent in the remaining two corner zones (*Incorrect Zone*).

3. Results

3.1 Lesions

For ease of exposition, histological analyses are presented at the end of the results section. Please refer to the *Histology* section for the extent of induced lesion damage in the animals used in *Experiments* 1, 3 and 4.

3.2 Experiment 1: Hippocampal lesions facilitated passive spatial Learning

In *Experiment 1*, we explored the hippocampal role in a standard and passive placement MWM hidden platform task. Overall, hippocampal-lesioned rats were not significantly impaired during acquisition of the *Active Swim* task ($F(1, 10) = 2.39$, $p = .153$), consistent with a minor/non-obligatory role for the hippocampus in highly-repetitive place learning [4, 5]. After this extensive training, the probe trial revealed that for rats trained in the *Active Swim* condition, sham rats did, while hippocampal rats did not, show significantly higher exploration in the *Correct* than *Opposite Zone* (Figure 1D**;** *Sham*: *Correct* > *Opposite* zone time, $F(1, 10) = 13.7$, $p = .004$, $\eta_p^2 = .58$, 95% CI [.09, .76], power = .90; *HPC*: *Correct* = *Opposite* zone time, $F(1, 10) = 3.71$, $p = .083$ $\eta_p^2 = .27$, 95% CI [.00, .57], power = .35), however, the lesion difference was not very marked (with no significant *Zone* x *Lesion* interaction, $F(1, 10) = 1.57$, $p = 0.239$; main effect of lesion $F(1, 10) = 2.24$, $p = 0.166$). In summary, hippocampal lesions induced, if anything, only a mild deficit for *Group Active Swim*.

For the *Passive Placement* condition, hippocampal rats spent *more* time than *Sham* rats searching in the *Correct Zone* (Figure 1E; *Zone* x *Lesion* interaction, $F(1, 11) = 5.95$, $p = .033$: *HPC* > *Sham* for *Correct Zone* time, $F(1, 11) = 5.73$, $p = .036$, $\eta_p^2 = .34$, 95% CI [.00, .61], power = .53). Sham rats did *not*, while hippocampal rats *did*, show significantly higher exploration in the *Correct* than *Opposite Zone* (Figure 1E: *Sham*: $F(1, 11) = 1.53$, $p = .242$;

HPC: $F(1, 11) = 5.08$, $p = .046$, $\eta_p^2 = .32$, 95% CI [.00, .60], power = .47). In summary, hippocampal-damaged rats exhibited a robust facilitation in the *Passive Placement* condition, relative to controls.

Note the scale of the y axes for Figure 1D (probe trial following active swim training) and Figure 1E (probe trial following passive placement training) are very different reflecting far superior performance in those rats that were able to make their own way to the goal during training. The implications of this finding are considered in the discussion.

Finally, there was no significant effect of hippocampal lesions upon swimming velocity (*Active Swim*: $t(10) = 2.15$, $p = .057$; *Passive Placement*: $t(11) = -.918$, $p = .378$) or thigmotaxis (*Active Swim*: $t(10) = .348$, $p = .735$; *Passive Placement*: $t(11) = -1.78$, $p = .102$) during the test trial. There was also no significant effect of lesion on thigmotaxis during the first trial of *Pre-Training* ($t(23) = -.874$, $p = .391$) or during the first trial of *Extra Maze Training* for *Group Active Swim* ($t(10) = -.206$, $p = .841$).

<<<<< FIGURE 1 ABOUT HERE >>>>>

3.3 Experiment 2: Degrading sensory cues en route to the hidden goal facilitated passive spatial learning

Overall, the set-up for *Experiment 2* was very similar to *Experiment 1*'s *Passive Placement* condition, with two key changes. One, instead of a group deprived of their hippocampus, there was a group sensorily deprived of much of the 'en route' information by being placed inside a light tight box on the way to the platform (*Group Passive Box*). Two, in addition to a control group similar to the shams in *Experiment 1* (*Group Passive Placement*), there was a second control group that was additionally provided with intermittent swimming trials, with the platform removed, during training (*Passive/Swim*; see methods section *2.2.2*). This was to ensure that any failure to preferentially explore the correct zone in the final probe trial was not simply due to unfamiliarity with swimming in the pool. Figure 2D depicts the results of this passive placement spatial memory task. There was a significant *Group* x *Zone* interaction ($F(2, 27) = 3.38$, $p = .049$, $\eta_p^2 = .20$, 95% CI [.00, .41], power = .51). Subsequent analyses of the simple main effects revealed that, like the sham controls in *Experiment 1*'s *Passive Placement* condition, neither of the two control groups showed signs of learning the task (*Correct* vs *Opposite Zone*, $F(1, 27)$: *Group Passive Placement*: $p = .731$; *Group*

Passive/Swim: p = .281). Importantly, the *Passive/Swim* group's failure to learn the task suggested that lack of prior swimming experience *per se* was not a critical factor in impaired performance in the control groups. In contrast, the *en-route*-deprived rats did spend significantly more time searching in the *Correct Zone* when compared to the *Opposite Zone* (*Group Passive Box*: $F(1, 27) = 6.50$, p = .017, η_p^2 = .19, 95% CI [.01, .42], power = .61).

<<<<< FIGURE 2 ABOUT HERE >>>>>

3.4 Experiment 3: Hippocampal lesions did not impair place learning, but did facilitate landmark learning

Experiment 1 investigated the role of the hippocampus in a passive learning task, which was designed to remove non-spatial task demands and the need for self-generated motion cues when an animal finds its own way to a hidden goal. The results revealed that without being able to find the hidden goal using self-generated paths, intact animals were unable to learn the location of the goal. For rats with hippocampal lesions, however, rather than displaying a deficit in spatial learning, those animals performed better in the passive navigation task. *Experiment 3* took a similar approach in that rats were again guided to a specific goal location – this time by a beacon rather than the experimenter's hand as in *Experiment 1*, with the aim of facilitating the 'getting there' component of the task - but the animals were allowed to make their own way there. The hidden goal location is defined by various stable, distal room cues (place) as well as two 'overhead' landmarks suspended above the pool, one directly above the goal (beacon; Figure 3A). According to previous studies, rats with hippocampal damage are unimpaired at navigating to a visible beacon e.g. [2], but what *Experiment 3* sought to address was how much is learned about each cue type (place and landmark) in isolation following a training regimen in which both cue types signal the location of the hidden goal. If spatial deficits in hippocampal rats are primarily due to impaired performance during 'getting there', but these deficits are attenuated by the presence of a salient beacon, then one would expect no impairment in 'knowing where' based on distal room cues.

The results from the landmark probe trial will be equally insightful for a different line of enquiry. One theory predicts that the hippocampus is crucial for using memory representations in a flexible manner [38]. It is not that rats with hippocampal damage in the

MWM have no understanding of where they should be but rather they are unable to flexibly integrate novel spatial information [39]. This flexibility account of hippocampal function would predict a tendency towards *impairment* for the hippocampal-lesioned rats during the landmark probe trial, with hippocampals expected to be unable to flexibly derive the goal location when a) released from a novel start location at the start of the probe trial and b) unlike during training, the extra-maze room cues are occluded by drawing curtains around the watermaze (Figure 3B). An alternative prediction arises from ideas that posit different and competing neural substrates for landmark-dependent and landmark-independent spatial learning, with suggested substrates being the striatum and hippocampus respectively [40-42]. Competition-based theory would predict a tendency towards *improvement* for hippocampals in using landmark cues, in that the critical neural region/s using landmarks (e.g. striatum) would face no competition, in terms of controlling behaviour, from the hippocampus (see [43] for a recent example of the removal of cue-competition effects in the MWM following lesions).

In summary, *Experiment 3* set up opposing theoretical predictions for hippocampal rats in the critical *Landmark* probe trial (relative to control performance). Flexible relational accounts predicted a tendency towards impairment, while competitive spatial accounts predicted a tendency towards improvement.

Hippocampal lesions induced impairment during acquisition ($F(1,17) = 24$, $p < .001$, $\eta_p^2 = .59$, 95% CI [.22, .74], power = .99) and the *Extra Maze + LM Test* (*Zone*Lesion* $F(1, 17) = 7.8$, $p = .012$, $\eta_p^2 = .32$, 95% CI [.02, .56], power = .69; *HPC* < *Sham* for time in *Correct Zone*, $F(1, 17) = 6.2$, $p = .023$, $\eta_p^2 = .27$, 95% CI [.00, .53], power = .58; *HPC* > *Sham* for time in *Opposite Zone*, $F(1, 17) = 6.5$, $p = .021$, $\eta_p^2 = .28$, 95% CI [.00, .53], power = .60); nevertheless, hippocampal rats still learned the task both in terms of acquisition (Main effect of *Session*, $F(4.73, 80) = 30$, $p < .001$, $\eta_p^2 = .64$, 95% CI [.49, .71], power = 1.0 not interacting with *Lesion*, $F(4.73, 80) = 1.39$, $p = .239$) and during the *Extra Maze + LM Test* (*HPC* spent significantly more time in the correct versus opposite zone, $F(1, 17) = 24$, $p < .001$, $\eta_p^2 = .59$, 95% CI [.22, .74], power = .99; as was the case for sham animals $F(1, 17) = 61$, $p < .001$, $\eta_p^2 = .78$, 95% CI [.52, .87], power = 1.0)

Importantly, the results of the *Landmark* probe trial (Figure 3Bii) clearly showed that hippocampal lesions did not elicit impairment but rather an *improvement*. That is, while shams performed moderately (*Correct* Zone > *Opposite* Zone time: $F(1, 17) = 5.05$, $p = .038$, $\eta_p^2 = .23$, 95% CI [.00, .50], power = .48), the performance of the hippocampal-lesioned rats was superior to that of shams (Group *HPC: Correct Zone* > *Opposite Zone* time: $F(1, 17) = 24$, $p < .001$, $\eta_p^2 = .59$, 95% CI [.22, .74], power = .99; planned comparison: *HPC* > *Sham* for time in *Correct Zone*, $F(1, 17) = 4.50$, $p = .049$, $\eta_p^2 = .21$, 95% CI [.00, .48], power = .44). These results clearly favoured the competitive spatial account over the flexible relational account. Note that we did not predict a significant interaction here between lesion and zone, because there was no reason to suppose anything other than that sham rats would learn the task well.

There was no evidence of a lesion-induced impairment in the *Extra-Maze Test* (Figure 3Cii; *Lesion*Zone*, $F(1, 17) = .327$, $p = .575$) with both groups discriminating the *Correct* from *Opposite* zone (Fs$(1, 17) \geq 7.44$, ps $\leq .014$).

Finally, during each test trial there was no sign of any effects of hippocampal lesions upon swimming velocity (*Extra Maze + LM*: $p = .435$; *Landmark*: $p = .506$; *Extra Maze*: $p = .453$) or thigmotaxis (*Extra Maze + LM*: $p = .536$; *Landmark*: $p = .073$; *Extra Maze*: $p = .691$). There was also no significant effect of lesion on thigmotaxis during the first four *Extra Maze + Landmark* training trials (*Lesion*: $F(1, 17) = .285$, $p = .600$; *Trial*Lesion*: $F(3, 51) = .900$, $p = .448$).

<<<<< FIGURE 3 ABOUT HERE >>>>>

3.5 Experiment 4: Hippocampal lesions impaired learning based on the shape of the environment

Results from *Experiments 1* and *3* showed that impairments in hippocampal rats in highly-repetitive 'place' learning components were relatively mild/absent, in that hippocampal rats still showed clear signs of learning where the hidden platform was located in relation to extra-maze cues. Although a non-essential role for the hippocampus in highly-repetitive 'place' learning has been shown previously (e.g. [4]), it could conceivably be argued, despite

removal of 80-94% of the tissue of the hippocampus and dentate gyrus (see section *3.6 Histology*), that our lesions were somehow unusual or ineffective in terms of damaging hippocampal integrity. Accordingly, we felt it important to demonstrate the ability of our lesions to induce behavioural deficits in a task established to be hippocampus-dependent. We selected the *Environmental-Shape* task for this purpose, in which rats learn that the hidden platform is available at two rotationally-equivalent corners of a rectangular pool [20]. Neuronal recording and modelling studies strongly suggest that the hippocampus processes environmental geometry (e.g. [44-48]). Consistent with this work, several studies have shown that environmental shape learning is impaired by hippocampal lesions [20-22], and to our knowledge there are no lesion studies with evidence to the contrary. Accordingly, following the completion of *Experiments 1* and *3* in the circular pool, we ran the environmental shape task (here called *Experiment 4* for descriptive purposes).

For ease of illustration and given that the prior conditions experienced by rats in *Experiments 1* and *3* (assignment of training conditions in *Experiment 1* and order of tests in *Experiment 3*) were matched across sham and lesion groups, the analyses in *Experiment 4A* compared performance of all hippocampal rats (*n*=13) used in *Experiment 1* against all sham rats (*n*=12). Similarly, *Experiment 4B* combined all hippocampal rats used in *Experiment 3* (*n* = 11) and compared to all shams from the same experiment (*n*=8).

As expected, in *Experiment 4A*, following *Experiment 1*, hippocampal rats were clearly impaired in the *Shape* probe trial (Figure 4D; *Lesion*Zone*: $F(1,23) = 7.23$, $p = .013$, $\eta_p^2 = .24$, 95% CI [.01, .48], power = .66; *Sham* > *HPC* for time in *Correct* corners, $F(1, 23) = 15.09$, $p = .001$, $\eta_p^2 = .40$, 95% CI [.09, .60], power = .94; while both groups discriminated the *Correct* from *Incorrect* corners: *Sham*, $F(1, 23) = 33.7$, $p < .001$, $\eta_p^2 = .60$, 95% CI [.29, .74], power = 1.00; *HPC*, $F(1, 23) = 4.67$, $p = .041$, $\eta_p^2 = .59$, 95% CI [.29, .74], power = 1.00).

The same pattern of results was observed in *Experiment 4B* following *Experiment 3*, hippocampal rats were clearly impaired in the probe trial of the *Shape* task (Figure 4C; *Lesion*Zone*: $F(1, 17) = 19.1$, $p < .001$, $\eta_p^2 = .53$, 95% CI [.16, .71], power = 0.98; *Sham* > *HPC* for time in correct corners, $F(1, 17) = 16.16$, $p = .001$, $\eta_p^2 = .49$, 95% CI [.12, .68], power = 0.95; while both groups discriminated the correct from incorrect corners: *Sham*, $F(1, 17) =$

83.3, $p < .001$, $\eta_p^2 = .83$, 95% CI [.61, .90], power = 1.00; *HPC*, $F(1, 17) = 15.79$, $p < .001$, $\eta_p^2 = .48$, 95% CI [.12, .68], power = .95).

<<<<< FIGURE 4 ABOUT HERE >>>>>

In both *Experiments 4A* and *4B*, hippocampal lesions resulted in impaired learning in the environmental shape task, as exhibited during retrieval in the probe trial. We conclude that our lesions were effective in their intended destructive function, and that the unique pattern of *Experiment 1* and *3*'s results does not reflect any failure to damage hippocampal integrity. Taken together with post-mortem evidence of the extent and location of our lesions (see *3.6 Histology* section below), we conclude, consistent with previous studies, that the hippocampus plays a key role for normal performance in the environmental shape task.

3.6 Histology

In this section, we describe the lesions made by ibotenic acid injections in the rats that were used in *Experiments 1*, *3*, and *4*. We first describe the lesions in the rats used for both *Experiments 1* and *4A* (Figure 5A-B), and then the lesions in the rats used for both *Experiments 3* and *4B* (Figure 5C-D).

3.6.1 Experiments 1 & 4A's Histology

Figure 5A depicts reconstructions of the minimum (black shading) and maximum (grey shading) extent of hippocampal damage on a series of coronal sections (see also Figure 5B for photomicrographs of a representative hippocampal lesion). Rats in *Group HPC* all sustained bilateral damage to the dorsal and ventral hippocampus (CA fields 1-4), the dentate gyrus and the subicular cortices. Analysis of total hippocampal tissue loss revealed a mean loss of 86.4% (range 80.4% - 90.3%) with a median of 86.5%. The main sparing of hippocampal tissue was observed in the most medial areas of the dorsal hippocampus. Following histological examination, all 13 rats were considered acceptable for inclusion in subsequent behavioural analyses. In most rats there was damage to the cortical area overlying the dorsal hippocampus. This typically included partial damage to motor, visual, somatosensory, parietal and retrosplenial agranular cortices (for reports of similar

extrahippocampal damage in hippocamptomized rats see: [49, 50]). Similar to [49] the partial cortical damage described left plenty of sparing in each of these areas.

3.6.2 Experiments 3 & 4B's Histology

Figure 5C depicts reconstructions of the minimum (black shading) and maximum (grey shading) extent of hippocampal damage for rats in *Experiments 3* & *4B* (see also Figure 5D for representative photomicrographs). Again, all rats belonging to Group *HPC* sustained extensive bilateral damage to the dorsal and ventral hippocampus (CA fields 1-4), the dentate gyrus and the subicular cortices. One rat received lateral damage in both hemispheres that extended into the lateral entorhinal and perirhinal cortices, so this animal was excluded from the study. Analysis of total hippocampal tissue loss in the remaining 11 rats revealed a mean loss of 90.2% (range 85.7% - 93.6%) with a median of 90.4%. The main sparing of hippocampal tissue was observed in the most medial areas of the dorsal hippocampus. The pattern of damage and sparing was identical to that described above for rats with hippocampal damage in *Experiments 1* and *4A*.

<<<<< FIGURE 5 ABOUT HERE >>>>>

4. Discussion

Our study is the first to examine the effects of hippocampal lesions in a passive placement version of the classic Morris watermaze task [2]. We first describe the performance of the controls in this task. The findings from *Experiment 1*, replicated with a new group of animals in *Experiment 2*, showed that when rats were passively transported all the way to the hidden goal and later required to swim in the final probe trial, performance was no different from chance. As noted in the *Introduction*, previous evidence attempting to demonstrate rats are capable of learning the location of an escape platform in the MWM following passive placement training is controversial. A few studies reported modest learning [23-26]. However, there were failures to replicate such findings, including the work of [27] who retracted their earlier rats-are-capable-of-passive-learning stance [23] by showing that rats were *not* capable of such latent learning. The current results replicate the later findings of [27]: passive placement at the goal and thus exposure to the goal's surrounding cues was not sufficient, even after two weeks of training (56 trials), to enable learning. This strongly

suggests that self-generated motion cues were crucial for learning. Moreover, our study is the first to show that hippocampal lesions can facilitate passive placement learning.

A key question to emerge from the results of the current experiment is why were intact rats so poor at learning the location of the hidden goal following passive placement training? Given that the brain generates an internal map of space by integrating self-motion information with sensory landmarks [e.g. 51, 52] it is perhaps hardly surprising that the removal of multisensory self-motion inputs dramatically impairs spatial learning. The facilitation of spatial learning in Experiments 1 and 3 when rats were induced with hippocampal lesions, and in Experiment 2 when they were deprived of enroute sensory information while being transported to the hidden goal, offer a possible explanation for the inferior navigational performance in control rats: competition between different intact memory systems (see [53] for a review). Given that navigation emerges from several memory systems operating simultaneously, it is possible for the inactivation/deprivation of one system/cue-type to facilitate the processing by/learning of others (e.g. [41, 43]). The results of *Experiment 1* revealed that hippocampal lesions produced a mild impairment when rats were required to swim to a static, hidden platform, but they facilitated learning when rats were passively transported to the hidden platform. This pattern of results suggests the hippocampus plays a more crucial in navigation tasks requiring the integration of self-generated motion cues as opposed to passive navigation. A second, related question is why the rats with hippocampal lesions in *Experiment 1* were able to solve the passive placement spatial learning task, while sham animals performed at chance?

The results of *Experiment 2*, while not able to confirm the exact nature of the hippocampal deficits observed in *Experiment 1*, provide one intriguing explanation for the facilitatory role hippocampal damage had on passive placement learning. When rats in *Experiment 2* were transported to the hidden goal in a light tight box, and then tested for their ability to find that hidden goal when placed into the water, animals were able to learn this spatial task. Thus, *Experiment 2* provided sensory, rather than neural, deprivation to achieve a similar effect: facilitation of passive spatial learning. The manipulation of being 'boxed in' all along the different trajectories towards the goal perhaps inhibited an attempt to construct a spatial map based upon those trajectories, paving the way for a larger contribution to mapping from the end-phase of acquisition trials: the goal location itself. Thus, we

considered that by getting the rats to 'ignore' the passive transport phase, by blocking vision, any goal location learning deficits could be ameliorated. The results suggest this might indeed have occurred. It is possible that the hippocampal lesions in *Experiment 1* acted in a broadly similar fashion to the black box in *Experiment 2* by de-emphasising strategies based on 'getting there' and, in so doing, facilitating 'knowing where' based on visual cues observed from the goal location.

It must be pointed out that a number of recent studies have provided evidence of passive learning in the MWM in intact rats [28-31]. However, learning was based either on the geometric or visual properties of the pool walls and, crucially, rats in these studies were placed on the escape platform from the same start location across all trials and close to a curtain that occluded other visual cues, somewhat similar to the light tight box condition in the current *Experiment 2*, avoiding interference from different en-route-to-goal trajectories. Clearly, the kind of passive placement task used in the present study is rather difficult. Our results offer some indications of why this is so, consistent with the view that the hippocampus relies on self-motion cues for spatial mapping, and that passive transport disrupts this.

Experiment 3, like *Experiments 1 and 2*, attempted to simplify the 'getting there' component of the task to further examine hippocampal function during navigation. Rats were guided to a hidden goal with the help of two distinctive, overhead landmarks, one of which acted as a beacon. When the landmarks were removed and the rats could only search for the hidden platform using room cues ('place solution') there was no impairment observed in rats with hippocampal lesions. This result replicates findings from [7] who also used a beacon to help signal the location of the hidden goal in the MWM. The authors interpreted their findings as evidence that the hippocampus is not responsible for learning a 'place response', per se, but is critically involved in the online integration of movements pertaining to spatial mapping. Our results also lend support to this conclusion.

A further probe trial in *Experiment 3,* with extra-maze room cues hidden from view with a curtain, revealed that hippocampal lesions facilitated learning based solely on overhead landmarks. Or, put another way, the removal of room cues impacted more heavily on the performance of control than hippocampus-deprived animals. Again, and as mentioned

previously, this supports the view that in intact animals, separate memory systems can compete for control over the navigating animal's behaviour [53-55]. In line with the current results, previous work has shown that hippocampal lesions facilitate 'response' strategies, such as our landmark discrimination, when the use of extra-maze cues provide a parallel solution [42, 56-58].

The results of *Experiment 3*, demonstrate a lack of impairment during both a place and landmark test when hippocampal rats were a) released from a novel location and b) exposed to very different conditions to those during training, especially during the landmark test when all the extra-maze cues were occluded. As such, they are not obviously consistent with theories emphasising that hippocampal rats are unable to use their spatial representations in a flexible manner [38, 39, 59]. The results of the current study do, however, support the argument that rats with hippocampal damage are more than capable of solving a place solution task when certain task demands, such as the integration of non-spatial information, are made easier [7, 9, 32, 33].

Despite almost complete destruction to the CA1-CA4 pyramidal cell fields and the dentate gyrus, substantial damage to the dorsal subiculum, and partial damage to pre and para subiculum, rats with bilateral lesions of the hippocampus in Experiments 1 and 3 were still able to learn the precise location of a hidden goal, which raises the question of how these animals were accurately locating space. Of the brain structures still intact, there are several candidates capable of computing a spatial code. Perhaps the most obvious candidate is the medial entorhinal cortex (MEC), serving as a conduit for visuospatial information entering the hippocampus from regions such as the postrhinal cortex (see [60] for a review). Indeed, it has been shown that an intact dorsolateral band of the entorhinal cortex is necessary for the acquisition and retention of a hidden platform MWM task [61]. More recently, [5] showed that almost complete lesions of the MEC produced memory deficits in the MWM that were equally as severe as hippocampal lesions. Interestingly, however, it is worth noting that the platform location was eventually learned by both of these lesion groups after additional training, broadly mirroring the performance observed by rats with hippocampal damage in the current experiment (Experiment 1: Active Swim condition) and in [4]. Other candidate brain regions spared in the current experiments and identified as playing a key role in the MWM task include the anterior thalamic nuclei (for acquisition

deficits see e.g. [62, 63]; see also [64] for a review), the subicular complex [4, 65], presubiculum and parasubiculum [66, 67], and some neocortical regions [e.g. 68, 69].

Experiment 4 tested the rats used in *Experiments 1* and *3* with the aim of identifying a previously reported hippocampal-induced deficit in learning based on environmental shape [20-22]. Though the deficit was modest, the results replicated previous studies and revealed that hippocampal lesions impair learning based on shape. The results confirm that the lesions produced their intended destructive function, and ensure that the unique pattern of results from *Experiments 1* and *3* did not reflect any failure to damage hippocampal integrity.

5. Conclusions

Our rats appeared to be incapable of passively learning the location of a hidden goal based on distal room cues in the water maze. Furthermore, hippocampal lesions: a) facilitated passive spatial learning, with behavioural evidence pointing to degraded self-motion cues during passive transportation to the hidden goal as a potential reason; b) facilitated landmark learning in a dual solution task in which both distal room cues and two proximal landmarks signalled the location of the goal during training; c) impaired learning based on the geometry of environmental boundaries, showing that the lesions were effective. In conclusion, we suggest the following interpretation of our results; that self-generated motion cues are crucial to spatial mapping strategies subserved by the hippocampus, and that hippocampal output routinely competes with output from other systems to control spatial behaviour. When hippocampus output is neurally inhibited (lesions, *Experiments 1* & *3*) or, we suggest, 'ignored' (box, *Experiment 2*), spatial strategies subserved by other neural systems (e.g. the striatum) gain more control over behaviour. Thus, when self-motion inputs to the hippocampus are so degraded as to render hippocampal output erroneous, the net result of inhibiting/ignoring such output can be to actually enhance spatial learning and behaviour controlled by other systems.

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Figure Legends

Figure 1. ***Hippocampal lesions facilitated passive spatial learning***
Experiment 1 procedure and results. (A) Training procedure for *Group Active Swim*. Rats were released from various start locations at the edge of the pool and had to swim to the location of the escape platform (dashed circle), with extra-maze cues informative of the platform's location. (B) Training procedure for *Group Passive Placement*. Rats were placed directly onto the escape platform (dashed circle) by the experimenter from different start locations at the edge of the pool, with extra-maze cues informative of the platform's location. (C) Probe trial procedure. All rats searched for the now absent escape platform. Time spent searching at the correct location (denoted by a tick) and opposite location (denoted by a cross) was recorded. (D) Probe trial data for *Group Active Swim*. The mean exploration times of the correct (white bars) and opposite (grey bars) locations for groups *Sham* and *HPC*. (E) Probe trial data for *Group Passive Placement*. The mean exploration times of the correct (white bars) and opposite (grey bars) locations for groups *Sham* and *HPC*. Asterisks denote significance: * $p < .05$; ** $p < .01$.

Figure 2. ***Degrading sensory cues en route to the hidden goal facilitated passive spatial learning***
Experiment 2 procedure and results. (A) Training procedure for *Group Passive Placement*. Rats were placed directly onto the escape platform (dashed circle) by the experimenter from different start locations at the edge of the pool, with extra-maze cues informative of the platform's location. (B) Training procedure for *Group Passive Box*. Rats were placed directly onto the escape platform (dashed circle) by the experimenter from different start locations at the edge of the pool but were kept in a box en route. Extra-maze cues were informative of the platform's location. (C) Probe trial procedure. Searching for the now absent escape platform was recorded in the correct location (denoted by a tick) and opposite location (denoted by a cross). (D) Probe trial data. The mean exploration times of the correct (white bars) and opposite (grey bars) locations for groups *Passive Placement*, *Passive Swim*, and *Passive Box*. Asterisks denote significance: * $p < .05$.

Figure 3. Hippocampal lesions did not impair place learning, but did facilitate landmark learning
Experiment 3 procedure and results. (A) Training procedure. Extra-maze cues and landmarks were informative in signalling the location of the escape platform (dashed circle). (Bi) Landmark Probe Trial procedure. Curtains were drawn around the pool so that only the overhead landmark cues

(now rotated relative to the laboratory) were available. Searching for the now absent escape platform was recorded underneath the correct landmark (denoted by a tick) and opposite landmark (denoted by a cross). (Bii) Landmark Probe Trial results. The mean exploration times of the correct (white bars) and opposite (grey bars) landmarks for groups *Sham* and *HPC*. (Ci) Extra-Maze Probe Trial procedure. The overhead landmarks and escape platform (present during training) were removed so that search strategy could only rely on extra-maze room cues. Search times were recorded in the correct zone (denoted by a tick) and opposite zone (denoted by a cross). (Cii) Extra-Maze Probe Trial results. The mean exploration times of the correct (white bars) and opposite (grey bars) zones for groups *Sham* and *HPC*. Asterisks denote significance: * $p < .05$; *** $p < .001$.

Figure 4. Hippocampal lesions robustly impaired learning based on the shape of the environment
Experiments 4A and *4B* procedure and results. (A) Training procedure. Curtains were fully drawn around the pool (note the curtains are part opened in the schematic for illustrative purposes only) so that only the shape of the pool's walls signalled the location of the escape platform (dashed circle), which resided in one corner of the rectangular pool. (B) Test procedure. Searching for the now absent escape platform was recorded in the correct corner (denoted by a tick) and incorrect corner (denoted by a cross). (C) Probe trial data for *Experiment 4A*. The mean exploration times of the correct (white bars) and incorrect (grey bars) corners for groups *Sham* and *HPC*. (D) Probe trial data for *Experiment 4B*. The mean exploration times of the correct (white bars) and incorrect (grey bars) corners for groups *Sham* and *HPC*. Asterisks denote significance: * $p < .05$; *** $p < .001$; **** $p < .0001$.

Figure 5. Histology for *Experiments 1* & *4A* (left panel) and *2* & *4B* (right panel).
A and C show the minimum (black shading) and maximum (grey shading) extent of hippocampal damage on a series of coronal sections. B and D show photomicrographs of representative hippocampal lesions. Numbers indicate distance (mm) posterior to bregma.

FIGURES

FIGURE 1

EXPERIMENT 1

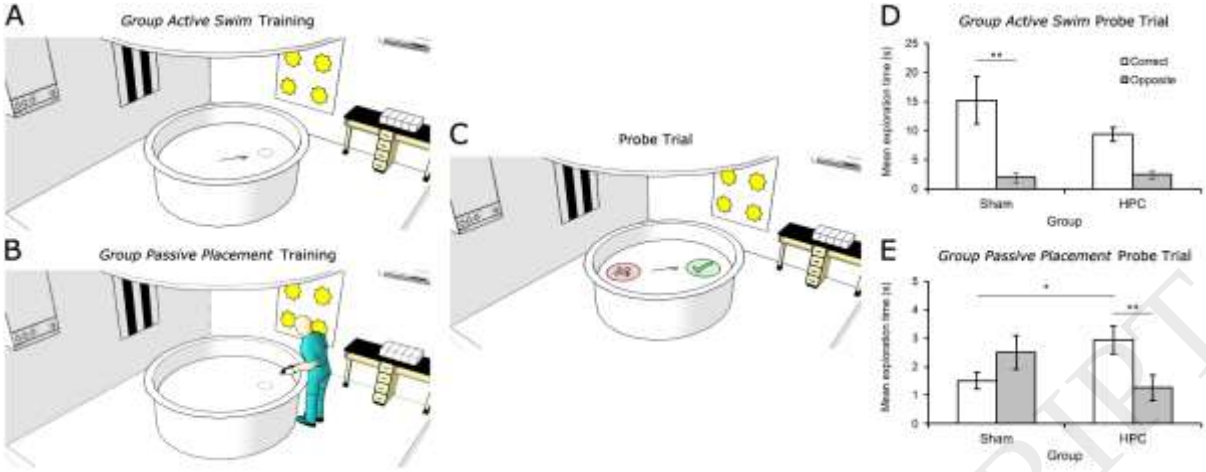


FIGURE 2

EXPERIMENT 2

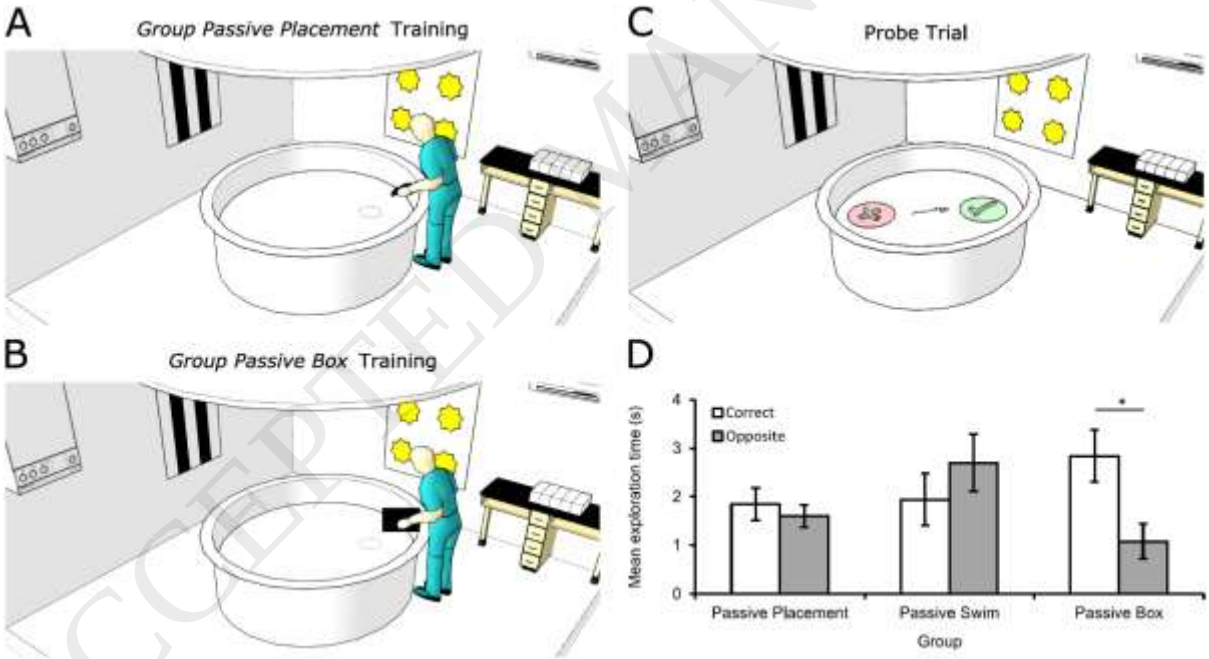
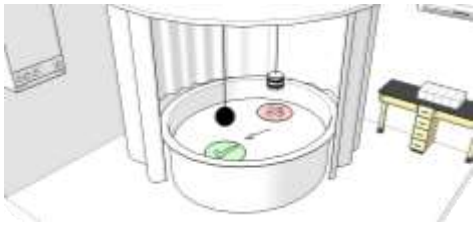


FIGURE 3

EXPERIMENT 3

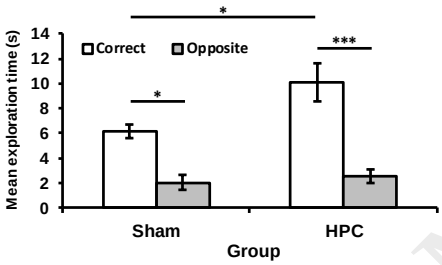
A *Extra-Maze + Landmark* Training

Bi *Landmark* Probe Trial



Ci *Extra-Maze* Probe Trial

Bii



Cii

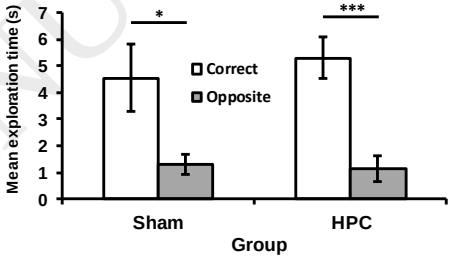


FIGURE 4

EXPERIMENT 4

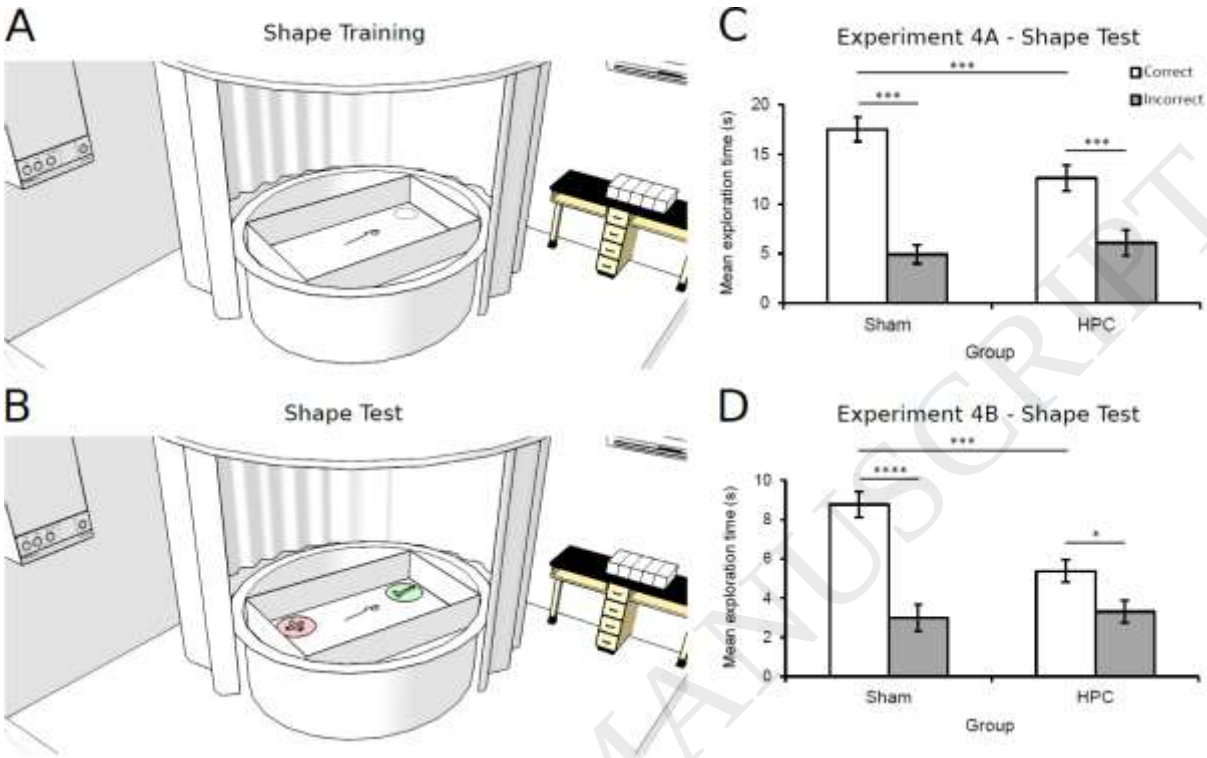
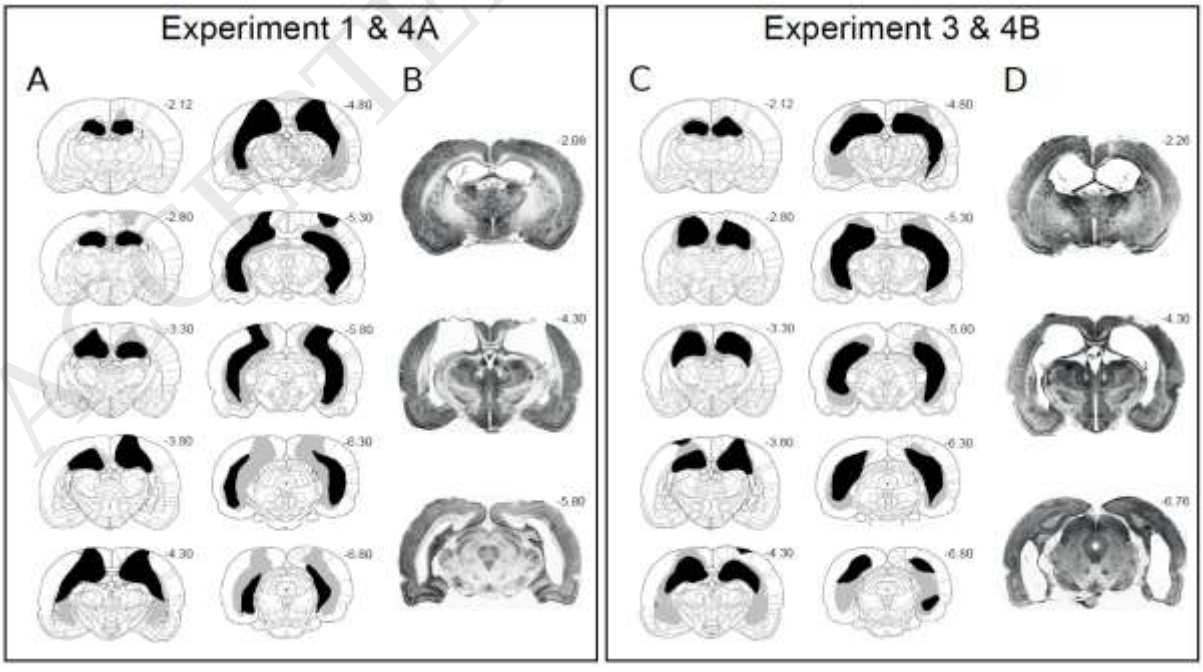


FIGURE 5



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