The honeycomb maze provides a novel test to study hippocampal-dependent spatial navigation

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Here we describe the honeycomb maze, a behavioural paradigm for the study of spatial navigation in rats. The maze consists of 37 platforms that can be raised or lowered independently. Place navigation requires an animal to go to a goal platform from any of several start platforms via a series of sequential choices. For each, the animal is confined to a raised platform and allowed to choose between two of the six adjacent platforms, the correct one being the platform with the smallest angle to the goal heading-direction. Rats learn rapidly and their choices are influenced by three factors: the angle between the two choice platforms, the distance from the goal, and the angle between the correct platform and the direction of the goal. Rats with hippocampal damage are impaired in learning and their performance is affected by all three factors. The honeycomb maze represents a marked improvement over current spatial navigation tests, such as the Morris water maze[1–3], because it controls the choices of the animal at each point in the maze, provides the ability to assess knowledge of the goal direction from any location, enables the identification of factors influencing task performance and provides the possibility for concomitant single-cell recording.

The hippocampal formation generates a cognitive map of a familiar environment that supports the ability of an animal to identify its location, respond to changes in the environment and navigate to desirable locations or avoid undesirable ones[4]. These functions are supported by cells coding for location (place cells)[5], heading direction (head direction cells)[6,7], distance in a particular direction (grid cells)[8] and distance from the boundaries of an environment (boundary cells)[9,10]; these functions have previously been reviewed[11,12].

Several tasks are routinely used to test spatial navigational learning. These include the T maze and Y maze[13], Olton radial arm maze[14,15], Barnes maze[16] and Morris water maze[1–3]. All have disadvantages as tests of spatial navigation and memory, and for concomitant recording of spatial cell activity. The first four do not force animals to use a single identifiable spatial navigational strategy, because they can all be learned using directional- or object-heading strategies as well as place learning. The Morris water maze overcomes this indeterminacy because it requires the animal to approach a hidden goal from a variety of directions; the animal must therefore head in different directions and approach different cues on each individual trial. However, the unlimited number of choices at each location, the lack of independence between successive choices and the inhomogeneity of behaviour across the environment once the animal has learned the task (see Supplementary Discussion 1, 2) all present problems for studies that use the Morris water maze the honeycomb maze described here has been designed to overcome these difficulties.

Here we describe the honeycomb maze, the performance of control rats during the navigational task, the factors that affect their performance as well as the performance of rats with hippocampal lesions; and, as proof of principle, an example of a place cell recorded on the maze is included in Extended Data Fig. 1.

The honeycomb maze consists of 37 tessellated hexagonal platforms each fixed atop a pneumatic tube, which enables it to be raised independently of the others. Figure 1 shows the maze in a variety of configurations: all platforms raised (Fig. 1a), a single platform raised (Fig. 1b) or three adjacent platforms raised (Fig. 1c). The objective is to reach a specific goal platform from eight or nine starting locations (Fig. 1d, bottom right, Extended Data Fig. 2) by making a series of binary choices between two platforms adjacent to the currently occupied platform (Fig. 1d, top left and top right, Supplementary Video 1). The 'correct' choice is the platform with the smallest angle to the goal direction—in vector terminology, the one with the smallest dot or inner product with respect to the goal-direction vector. The sequence continues until the goal platform is reached, where after a short delay the animal is rewarded with a single Cheerio.

First, three groups of male Lister hooded rats were tested on a spatial navigation task using the honeycomb maze (Methods): a control group that had not been operated on and two control groups that had been operated on; one group received sham surgical procedures in the medial entorhinal cortex and the other sham hippocampal lesions. These three groups were used as controls for a further experiment with rats that had ibotenic-acid-induced lesions in

the hippocampus, described below. All three control groups rapidly learned the spatial navigation task, and after 28 trials all rats achieved a mean score of greater than 90% correct choices over four consecutive trials. There was no difference in performance between the three groups, which suggests that performance on the maze is consistent and reliable (Fig. 2).

Several parameters determined performance in the unoperated control rats: (i) the angular difference between the direction to the correct platform and the goal (angle α); (ii) the smallest angular separation between the two choice platforms, independent of the direction of the goal (angle β); and (iii) the distance of the choice from the goal (Supplementary Discussion 3). The testing schedule minimized the interaction between these variables so that the effect of each could be investigated (Methods, Extended Data Fig. 2). The rats performed best when choice platforms were separated by two others (angle $\beta = 180°$; $92.8 \pm 1.1\%$ (mean $\pm$ s.e.m.) correct choices overall) and deteriorated when only one (angle $\beta = 120°$) or no (angle $\beta = 60°$) platform separated them ($86.9 \pm 1.2\%$ and $85.8 \pm 1.4\%$ respectively). There was a significant effect of both angle β and day of testing on performance, but no interaction between these two variables (Fig. 3a). The differences between two-platform separations (angle $\beta = 180°$) and the rest (angle $\beta = 120°$ or $60°$) were significant (Fig. 3a, inset).

Performance decreased with increasing distance from the goal (Fig. 3b, Supplementary Discussion 3); the percentage of correct choices ranged from $88.4 \pm 1.0\%$, when platforms were adjacent to the goal, to $71.8 \pm 6.1\%$, when platforms were five platforms away from the goal. Performance also decreased as an inverse function of the difference between the direction to the goal and the correct platform (angle α); the percentage of correct choices ranged from $86.2 \pm 1.3\%$ for angles of $0°–29°$, to $61.1 \pm 3.1\%$ for angles greater than or equal to $90°$ (Fig. 3c). Rats performed significantly better when the direction of the correct platform was less than $90°$ from that of the goal (Fig. 3c, inset). Notably, even when the correct choice was $90°$ or greater from the goal direction, all rats performed with rates of success above those that would be achieved by chance ($t_8 = 3.156$, $P = 0.013$, one sample t-test).

A multiple regression analysis indicated that angle α, angle β and distance were all significant predictors of performance (Extended Data Table 1). Between them, these variables predicted 5.5% of the variance ($R^2 = 0.055$, $F_{3,391} = 7.608$, $P < 0.001$); a large proportion of the remaining variance in performance is accounted for by experience (Fig. 2).

Finally, there was evidence of an interaction between the effects of angle α and the distance from the goal, at least when choice platforms were adjacent to one another. The angle-to-goal effect decreases as a function of the distance from the goal, such that performance improved with larger angles at greater distances (Fig. 3d).

In a further experiment, learning on the honeycomb maze was compared between rats with ibotenic-acid-induced lesions of the hippocampus and sham-operated control rats. Hippocampal damage ranged from 48% to 94%; preserved tissue was observed primarily in the ventral hippocampus and small amounts of incidental damage to the caudate nucleus and putamen were found in all rats. Minor additional damage to the dorsal subiculum, medial geniculate nucleus and pre- and parasubiculum was found in a subset of rats (Methods, Extended Data Fig. 3).

Rats with hippocampal lesions were significantly deficient in learning the task, relative to controls (Fig. 4a). As in the unoperated control group, performance in rats with hippocampal lesions and in the sham-operated controls was related to three variables (Fig. 4b–d): separation between choice platforms (angle β), distance from the goal and angle of the correct choices to the goal direction (angle α). There was a significant interaction between all three variables and lesion status (angle $\beta \times$ lesion status, $F_{2,28} = 6.981$, $P = 0.003$; distance $\times$ lesion status, $F_{4,56} = 4.999$, $P = 0.002$; angle $\alpha \times$ lesion status, $F_{2.3,32.8} = 8.431$, $P = 0.001$; two-way mixed ANOVAs). Behavioural performance decreased as hippocampal damage increased, although this was not significant, possibly owing to a floor effect as half of the rats with lesions scored at chance level (Extended Data Fig. 4). Rats with hippocampal lesions were also slower to make choices ($F_{1,14} = 11.103$, $P = 0.005$; Extended Data Fig. 5). Latencies were significantly longer for incorrect choices than correct choices across all rats ($F_{1,14} = 23.839$, $P < 0.001$), which suggests that longer latencies reflect the uncertainty of the rats; this effect was larger for rats with lesions ($F_{1,14} = 4.956$, $P = 0.043$, two-way mixed ANOVA).

In summary, the performance of rats across all three control groups was comparable (Fig. 2) even when the rats were tested by different experimenters on different occasions, which shows the consistency and reproducibility of the task. Performance was affected by three variables. Success was reduced with increased distance of the choice to the goal and by increased deviation of the best-choice platform from the direction vector to the goal (angle α), and was improved by an increase in the angle between the two choice platforms (angle β).

Both direction and distance factors were noted in early research on maze learning (Supplementary Discussion 3), and it has been proposed that the direction factor is generated by the hippocampal cognitive map. The ability of control rats to identify the better of two directions even when neither is aligned with the goal suggests that the brain is capable of vector computations. Rats with hippocampal damage performed significantly worse for all three factors, which suggests that these vectors are computed in the hippocampus itself. An alternative explanation is that the computation is performed in another brain region—such as the parietal cortex—that requires input from hippocampal place cells that encode the current location of the animal, the location of the goal and the choice platform locations. A simple vector schema can account for these data (Extended Data Fig. 6), as has previously been proposed [17,18].

Finally, the honeycomb maze makes an ideal environment for correlating the activity of place cells with spatial navigation performance (Extended Data Fig. 1), which is not easily accomplished in the water maze. The honeycomb maze therefore represents an improvement on the Morris water maze, which is the current gold standard for testing hippocampal-dependent spatial navigation (Supplementary Discussion 2).

<jrn>1. Morris, R. G. M. Spatial localization does not require the presence of local cues. *Learn. Motiv.* **12,** 239–260 (1981). </jrn>

<jrn>2. Morris, R. G., Garrud, P., Rawlins, J. N. & O'Keefe, J. Place navigation impaired in rats with hippocampal lesions. *Nature* **297,** 681–683 (1982). </jrn>

<jrn>3. Morris, R. Developments of a water-maze procedure for studying spatial learning in the rat. *J. Neurosci. Methods* **11,** 47–60 (1984). </jrn>

<bok>4. O'Keefe, J. & Nadel, L. *The Hippocampus as a Cognitive Map* (Oxford Univ. Press, 1978).</bok>

<jrn>5. O'Keefe, J. & Dostrovsky, J. The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Res.* **34,** 171–175 (1971). </jrn>

<jrn>6. Ranck, J. B. Head-direction cells in the deep cell layers of the dorsal presubiculum in freely moving rats. *Abstr. Soc. Neurosci.* **10,** 599 (1984).</jrn>

<jrn>7. Taube, J. S., Muller, R. U. & Ranck, J. B. Jr. Head-direction cells recorded from the postsubiculum in freely moving rats. I. Description and quantitative analysis. *J. Neurosci.* **10,** 420–435 (1990).</jrn>

<jrn>8. Hafting, T., Fyhn, M., Molden, S., Moser, M. B. & Moser, E. I. Microstructure of a spatial map in the entorhinal cortex. *Nature* **436,** 801–806 (2005). </jrn>

<jrn>9. Lever, C., Burton, S., Jeewajee, A., O'Keefe, J. & Burgess, N. Boundary vector cells in the subiculum of the hippocampal formation. *J. Neurosci.* **29,** 9771–9777 (2009). </jrn>

<jrn>10. Solstad, T., Boccara, C. N., Kropff, E., Moser, M. B. & Moser, E. I. Representation of geometric borders in the entorhinal cortex. *Science* **322,** 1865–1868 (2008). </jrn>

<edb>11. O'Keefe, J. in *The Hippocampus Book* (eds Morris, R. *et al.*) Ch. 8, 471–544 (Oxford Univ. Press, 2007).</edb>

<jrn>12. Moser, M. B., Rowland, D. C. & Moser, E. I. Place cells, grid cells, and memory. *Cold Spring Harb. Perspect. Biol.* **7,** a021808 (2015). </jrn>

<jrn>13. Dudchenko, P. A. How do animals actually solve the T maze? *Behav. Neurosci.* **115,** 850–860 (2001). </jrn>

<jrn>14. Olton, D. S., Walker, J. A. & Gage, F. H. Hippocampal connections and spatial discrimination. *Brain Res.* **139,** 295–308 (1978). </jrn>

<jrn>15. Olton, D. S. & Samuelson, R. J. Remembrance of places passed: spatial memory in rats. *J. Exp. Psychol. Anim. Behav. Process.* **2,** 97–116 (1976). </jrn>

<jrn>16. Barnes, C. A. Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. *J. Comp. Physiol. Psychol.* **93,** 74–104 (1979). </jrn>

<edb>17. O'Keefe, J. in *Brain and Space* (ed. Paillard, J.) 273–295 (Oxford Univ. Press, 1991).</edb>

<edb>18. O'Keefe, J. in *Language and Space* (eds Bloom, P. *et al.*) 277–316 (MIT Press, 1996).</edb>

<jrn>20.   Hollup, S. A., Molden, S., Donnett, J. G., Moser, M. B. & Moser, E. I. Accumulation of hippocampal place fields at the goal location in an annular watermaze task. *J. Neurosci.* **21,** 1635–1644 (2001).</jrn>

<jrn>21.   Hull, C. L. The goal gradient hypothesis and maze learning. *Psychol. Rev.* **39,** 25–43 (1932). </jrn>

<bok>22 Dashiell, J. F. *Direction Orientation in Maze Running by the White Rat. (Comparative Psychology Monographs 7)* (Johns Hopkins Press, 1930).

<jrn>23.   Spence, K. W. The order of eliminating blinds in maze learning by the rat. *J. Comp. Psychol.* **14,** 9–27 (1932). </jrn>

<jrn>24.   O'Keefe, J. & Speakman, A. Single unit activity in the rat hippocampus during a spatial memory task. *Exp. Brain Res.* **68,** 1–27 (1987). </jrn>

<jrn>25.   Sarel, A., Finkelstein, A., Las, L. & Ulanovsky, N. Vectorial representation of spatial goals in the hippocampus of bats. *Science* **355,** 176–180 (2017). </jrn>

<jrn>26.   O'Keefe, J. A computational theory of the hippocampal cognitive map. *Prog. Brain Res.* **83,** 301–312 (1990). </jrn>

Supplementary Information is available in the online version of the paper.

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Figure 1 | The honeycomb maze. a–c, Honeycomb maze with all (**a**), one (**b**) and three (**c**) platforms raised. **d,** Schematic navigation paradigm. Top left, at any given starting location (blue) two choices are offered: the correct choice (orange) possesses a smaller angle with respect to the heading direction towards the goal (black) than does the incorrect choice (grey). Top right, the previously chosen platform becomes the new 'occupied' platform (blue), and two further platforms are presented as choices. Bottom left, each choice is described by two angles: angle α between the correct choice and goal-heading direction, and angle β between the correct and incorrect choices. Bottom right, illustration of eight potential starting platforms (blue).

Figure 2 | Learning to navigate the honeycomb maze. Performance of three groups of rats; data are mean ± s.e.m. and each data point is representative of four trials. $n = 9$ unoperated controls (blue), 8 controls with sham hippocampal lesions (red) and 8 controls with sham medial entorhinal lesions (green). All rats were completed between 68 and 72 trials, over 13–17 days; data are shown for the first 68 trials. Unoperated controls did six trials per day, sham-operated controls did four trials per day. Performances were similar for all groups ($F_{2,22} < 0.001$, $P > 0.999$, two-way mixed ANOVA).

Figure 3 | Factors that affect maze performance in controls. Three factors influence performance in unoperated control rats ($n = 9$ rats). **a,** Performance improved with increasing platform separation (performance versus angle β: $F_{2,16} = 8.850$, $P = 0.003$) and with experience (performance versus testing day: $F_{11,88} = 11.361$, $P < 0.001$), but there was no significant interaction between angle β and experience ($F_{22,176} = 1.438$, $P = 0.102$). All two-way repeated measures ANOVA. Inset, mean performance for different values of angle β across all days, with pairwise comparisons: 60° versus 180°, mean difference = 7.0%, $P = 0.034$; 120° versus 180°, mean difference = 5.9%, $P = 0.044$; 60° versus 120°, mean difference = 11.0%, $P = 1.000$; post hoc Bonferroni test. Six trials per day. **b,** Performance decreased with increasing distance of choice from goal, measured as in the inset ($F_{3,24} = 3.707$, $P = 0.025$, one-way repeated measures ANOVA). **c,** Performance decreased with increasing angle to goal (angle α, $F_{4,32} = 20.670$, $P < 0.001$, one-way repeated measures ANOVA). Inset, mean scores were higher for choices that were 'ahead' (angle $\alpha < 90°$, orange) than they were for choices that were 'behind' (angle $\alpha > 90°$, grey) ($t_8 = 6.620$,

$P < 0.001$, two-sided paired t-test). **d**, There was an interaction between distance from goal and angle to goal (angle α) for adjacent platform choices ($F_{3,24} = 9.133$, $P < 0.001$, two-way repeated measures ANOVA). The y axis shows the difference in mean performance for 'ahead' and 'behind' choices. For **a–d**, error bars indicate s.e.m., *$P < 0.05$, ***$P < 0.001$.

Figure 4 | Performance of rats with hippocampal lesions on the honeycomb maze. a, Rats with hippocampal damage (red, $n = 8$) are significantly impaired when learning the task compared to controls with sham hippocampal lesions (blue, $n = 8$) ($F_{1,14} = 10.240$, $P = 0.006$, two-way mixed ANOVA), four trials per day. **b–d**, Rats with hippocampal damage (red, $n = 8$) are significantly more influenced than controls (blue, $n = 8$) by separation between choice platforms (**b**, angle β; $F_{2,28} = 40.024$, $P < 0.001$, two-way mixed ANOVA), distance from the goal (**c**, $F_{4,56} = 34.740$, $P < 0.001$, two-way mixed ANOVA) and angle to goal (**d**, angle α; $F_{2.3,32.8} = 28.812$, $P < 0.001$, two-way mixed ANOVA). For **a–d**, error bars indicate s.e.m., ** $P < 0.01$.

METHODS

Maze description

The honeycomb maze consists of 37 tessellated hexagonal platforms (11.5 cm each side) in an overall hexagonal configuration (total maze diameter 145.5 cm, five platforms across with 1-cm gaps between each platform). Each platform is fixed atop a pneumatic tube that enables it to be raised independently of the others. Each raised platform is 81.5 cm above the base (49 cm when lowered). Each platform consists of three layers: the movable top is made of 3-mm thick acrylonitrile butadiene styrene (ABS) with a pinseal finish, and is connected to the bottom layer by six microswitches that register the presence of an animal on the platform; the middle layer is made of aluminium, which is grounded to reduce electrical artefacts. Custom-made software that was written in Labview is used to monitor the platform switches, which enables the raising and lowering of platforms on the basis of the animal's location. Only unoccupied platforms are moved. Plastic skirts around the top of the pneumatic tubes protect the wiring and tubing under the platform. The maze sits within a standard laboratory, which gives the animal access to abundant extra-maze cues for location and direction (Fig. 1a–c).

Animals and surgical procedures

Four groups of rats weighing between 310 and 374 g were trained: three control groups and a group with hippocampal lesions. All rats were male Lister hooded rats purchased from Charles River Laboratories and aged between 12 and 16 weeks at the start of behavioural

testing. Rats were all housed in open caging along with their littermates, in groups of three to four. All animal experiments were carried out in accordance with British Home Office Regulations (UK Animals Scientific Procedures Act 1986; Project License PPL 70/8202 to J.O.). Study protocols were in accordance with the terms of the Project License, which was reviewed by the Animal Welfare and Ethical Review Board at University College London.

The first group consisted of nine unoperated control rats. The second group comprised eight control rats with sham hippocampal lesions, and the third group comprised eight rats with sham lesions in the medial entorhinal cortex. Group sizes were determined on the basis of extensive prior experience with maze studies. In the operated controls, sham lesions that caused no neural damage were made by insertion of a borosilicate glass micropipette (World Precision Instruments) without injection into fourteen sites per hemisphere for the sham hippocampal lesions (Extended Data Table 2), and eight sites per hemisphere for the sham medial entorhinal cortex lesions (Extended Data Table 3). In the final group of eight rats, pressure injections of between 50–80 nl of ibotenic acid ($10 \ \mu g \ \mu l^{-1}$) were made via a glass micropipette at fourteen injection sites in each hemisphere to lesion the hippocampus (Extended Data Table 2). Surgeries were performed under sterile conditions under isoflurane anaesthesia and the rats were given analgesics (Metacam) for three days after the operation.

For the lesion study, rats were randomised into an intervention group, which received hippocampal lesions, or an operated control group, which received sham hippocampal lesions. Randomisation was stratified to ensure that littermates were equally distributed between the two groups.

Behavioural training

After one week of recovery, all rats were food-restricted and reduced to 90% of their free-feeding body weight over a two-week period, before training. During this time, they were handled daily, and during the final three days they were placed on a holding platform in the testing room where they were habituated to the sounds made by the maze platforms being raised and lowered.

There was no period of maze exploration before training on the task. At the beginning of each trial, the rat was placed on one of eight or nine starting platforms and after a delay of four seconds, two of the six adjacent platforms were raised (Fig. 1d). When the rat chose one of these two platforms, the previously occupied and non-chosen platforms were lowered and

after an interval of four seconds, two new platforms were raised. Neither of these was the platform from which the rat had just come (with one exception), which ruled out the strategy of avoiding that platform as a potential solution. If a choice was not made within one minute, the rat was gently guided onto a platform, which was alternatively chosen as correct or incorrect—ruling this out as a source of information about the correct choice. The choice was then scored as incorrect, and the next choice in the sequence initiated. If the rat had not reached the goal within five minutes, the trial was terminated and the rat guided to the goal platform and given a reward. On some 'choices', the rat was presented with only a single platform and was required to move to that platform. This forced choice occasionally became necessary to eliminate the strategy of avoiding a platform that had immediately previously been occupied.

Platform choices varied along several dimensions: (i) in the angle between the best choice platform and the heading direction to the goal, which varied from 0° to 135° (angle α, Fig. 1d), (ii) in the angle between the two platforms (angle β, Fig. 1d), with choices that ranged from neighbouring platforms to platforms separated by two others and (iii) in the distance of the platform from the goal in terms of the number of platforms (1–5) to be traversed on the direct route to the goal (Fig. 3b, inset).

The trial was terminated when the rat reached the goal, or after five minutes had elapsed. Upon reaching the goal, rats were confined there and given one Cheerio after a delay of approximately five seconds. This delay of reward procedure ensured that the rat could not locate the goal platform by its odour. The maze was also cleaned between trials to eliminate odour cues.

Two trial protocols were used in this study (see Testing schedules and Extended Data Fig. 2 for further discussion of different schedules). Unoperated controls had three trials per day on the first two days and six trials per day for the next eleven days (72 trials in total). Operated controls were given four trials per day for seventeen days (68 trials in total). The number of choices per trial varied between 2 and 37 according to the rat's success rate, with a median of 5 choices per trial.

In the lesion study, the experimenter was blinded to lesion status.

Statistics

Differences in learning rates between the different control groups (unoperated versus sham hippocampal versus sham medial entorhinal cortex) were assessed using a two-way mixed

ANOVA (Fig. 2). Two-way repeated measures ANOVAs were used to compare the performance of unoperated controls on trials with different values of angle β (Fig. 3a), with post hoc Bonferroni testing for pairwise comparisons (Fig. 3a, inset), and to test for an interaction between angle α ('ahead' choices versus 'behind' choices) and distance in trials in which choices consisted of adjacent platforms (Fig. 3d). One-way repeated measures ANOVAs were used to test for a relationship between performance and distance, and performance and angle α, in the unoperated control group (Fig. 3b–c). Performance on 'ahead' choices (angle $\alpha < 90°$) versus 'behind' choices (angle $\alpha > 90°$) was compared using a paired t-test, and a one-sample t-test was used to determine whether performance on 'behind' choices was significantly better than chance. A multiple regression analysis was conducted to evaluate the contributions of the three maze factors (angle α, angle β and distance) to performance in the unoperated control group (see main text and Extended Data Table 1).

Differences in learning curves were assessed in rats with hippocampal lesions and control rats using a two-way mixed ANOVA (Fig. 4a). Two-way mixed ANOVAs were also used to ascertain the effect of each maze variable (angle α, angle β and distance) on performance, and their potential interaction with lesion status (Fig. 4b–d). A Spearman's correlation was used to correlate lesion extent with performance (Extended Data Fig. 4). Differences in latencies between rats with hippocampal lesions and control rats over time were tested using a two-way mixed ANOVA, and a two-way mixed ANOVA was used to investigate the relationship between latencies, whether a choice was correct or incorrect and lesion status.

For Figs 2, 3a–c, 4 and Extended Data Fig. 5, data points indicate the mean score of one group of rats over a specified number of trials. In all figures, error bars indicate the s.e.m. For all statistical tests, data were tested to ensure they met the necessary assumptions before proceeding to analysis.

Histology

On completion of behavioural testing, rats were euthanized under anaesthesia and the brain fixed via intracardial perfusion with saline and 4% paraformaldehyde. Horizontal sections (40 μm) of the brains of rats with hippocampal lesions and of operated control rats with sham hippocampal lesions were mounted on gelatinized slides and stained with cresyl violet acetate. Slides were imaged using a Axio Scan.Z1 (Zeiss). Lesion volume was quantified by a

blinded observer via manual tracing of the hippocampus on every fourth section, using ImageJ. The remaining volume of hippocampal tissue in rats with lesions was expressed as a percentage in relation to the measured volume of a typical operated control rat with a sham hippocampal lesion.

Testing schedules

Testing schedules were designed to meet a number of criteria. First, for each choice the rat was not offered a platform it had just occupied, with the exception of one instance, to eliminate the strategy of avoiding a platform that had immediately previously been occupied. Second, to prevent the task being solved using an egocentric strategy, correct choices were selected so that there was an approximately equal number of choices that required the rat to turn left (or anticlockwise), when facing the goal, as there were right (or clockwise) turns. Third, the starting platform changed between trials and these eight or nine platforms were distributed approximately equally around the maze.

The design of the maze enables a large number of different spatial navigation schedules to be run, from those most sensitive to hippocampal function to those least so. At the most sensitive end of the spectrum are schedules in which no trial is ever repeated, which rules out the possibility that the task can be solved by non-hippocampal dependent guidance or stimulus–response strategies. The animal must learn to approach a location in space from any starting position and any direction. At the other end of the spectrum are schedules that can easily be learned using a guidance strategy, such as always approaching a distal cue or a stimulus–response routine (for example, a sequence of body turns, such as left–right alternations). A guidance-biased training protocol might involve always starting the animal from the same location and offering the same sequence of choices, one of which always leads directly towards the goal (for an example, see ref. 19). Intermediate between these two schedules are ones that restrict the types of choices available to the animal (for example, to choices between platforms at three different angles), which we have used in our first study to identify the factors contributing to successful performance.

Schedule 1

The schedule used in the unoperated control group was specifically designed to investigate the effect of the three metrics (angle α, angle β and distance) on task performance. In this experiment, the nine unoperated control rats undertook three different types of trial, which we named 'Aβ60', 'Aβ120' and 'Aβ180'. In each trial type, the smallest angle between the two

choice platforms offered (angle β) was fixed at 60° (Aβ60), 120° (Aβ120) or 180° (Aβ180) degrees. The values of angle α were selected to ensure that for any given distance from the goal there were a range of values of angle α for each choice. This maximized the number of choices with unique combinations of distance, angle α and angle β, which enabled us to collect a dataset with 50 such unique combinations. Rats were tested in groups of three. Each rat completed six trials per day, which consisted of two trials of each type. In the testing schedule, trial type was staggered to control for the effect of experience on performance. Start platforms were also rotated amongst eight different locations and the combinations of start platforms and trial type were counterbalanced; Extended Data Fig. 2a illustrates the three trial types used in testing schedule 1.

Schedule 2

When testing the rats with lesions, we designed a protocol that enabled us to examine the correlation between lesion size and task performance. In order to make a meaningful comparison between individual subjects with lesions, it was important to ensure that all rats experienced trials of the same difficulty in the same temporal order. This was particularly important given the rapid rate at which rats learn the honeycomb maze task—as an illustration of this, on the first trial, control rats did not perform significantly better than rats with hippocampal lesions, but significantly outperformed them by the end of the first day of testing. For the lesion study, we therefore designed a schedule that contained choices with all possible values of angle β, to enable direct comparisons between rats with lesions and control rats on a day-by-day basis, and amongst rats with lesions of different sizes. Nine start platforms were used; this protocol is illustrated in Extended Data Fig. 2b.

Code availability

The custom software, written in LabView, used to run the maze trials is available from the corresponding author upon request.

Data availability

All relevant data are included within the paper and its Extended Data. Additional information including trial protocols is included in the Methods and Extended Data Fig. 2. The original and analysed datasets generated during the current study and any further methodological details required are available from the corresponding author upon request. The maze is designed to be as simple as possible, so that individual laboratories with access to a machine shop can build it. All detailed methods and materials on honeycomb maze building and

control will be made available by the authors upon request, and we are currently searching for a manufacturer who can build and provide the mazes at a reasonable cost.

<jrn>19.   Eichenbaum, H., Stewart, C. & Morris, R. G. Hippocampal representation in place learning. *J. Neurosci.* **10,** 3531–3542 (1990).</jrn>

Extended Data Figure 1 | Place cell recorded on the honeycomb maze. A single place cell recorded during navigation on the honeycomb maze. **a**, Behaviour (black line) from a single trial (trial *i*) in which the rat was offered two consecutive choices (left, 0–73 s) to go from the start platform (second left, 0–25 s), to an intermediate platform (third left, 25–50 s) to the goal (right, 50–73 s). The program detained the rat on each platform for 20 s before the two choice platforms were raised or, in goal, the food presented. Non-chosen platforms not shown. As the rat waited on each platform, it sampled the immediate environment by circling the perimeter of the platform. **b**, The firing of a place cell during this trial (trial *i*); maximum rate in red shown top left. **c**, Rate map for the same cell on a separate trial (trial *g*) from a different start platform. **d**, Composite rate map from ten trials (trials *c–l*), each from a different starting location in which the rat took a different path to the goal. **e**, Firing rate map of the same cell when all platforms were raised and the rat foraged for food over a period of 20 min (trial *m*). In this cell, the firing fields during navigation trials (**d**) and during the foraging condition (**e**) were similar (spatial correlation = 0.77). Not all place cells displayed this profile, and others (not shown) fired in a different location(s) during the navigation trials from that seen in the foraging condition (that is, remapped).

Extended Data Figure 2 | Protocols used on the honeycomb maze. a, Schedule 1 trial protocols for Aβ60 (left), Aβ120 (middle) and Aβ180 (right) trials. **b**, Schedule 2 protocol. For **a** and **b**: goal platform, black; start platforms, blue; orange vectors, correct choices; grey vectors, incorrect choices; green vectors, 'forced' choices. See Methods for more detail.

Extended Data Figure 3 | Histology of brains from rats with hippocampal lesions. Representative sections from brains of rats with hippocampal lesions, and one operated control with a sham hippocampal lesion (R2322), alongside mean performance scores on the honeycomb maze. Subjects are arranged in order of increasing lesion size. Horizontal sections (40 μm) stained with cresyl violet.

Extended Data Figure 4 | Correlation between hippocampal volume and performance. Correlation between remaining hippocampal volume and performance on the spatial

navigation task on the honeycomb maze in eight rats with hippocampal lesions ($n = 8$ rats, $\rho_6 = 0.452$, $P = 0.260$; Spearman's correlation).

Extended Data Figure 5 | Rats with hippocampal lesions have longer latencies. Rats with hippocampal lesions (red, $n = 8$) have longer latencies than operated controls with sham hippocampal lesions (blue, $n = 8$) ($F_{1,14} = 11.103$, $P = 0.005$). Latencies also changed as a function of experience (day) ($F_{16,224} = 5.612$, $P < 0.001$) with a significant day $\times$ lesion interaction ($F_{16,224} = 2.464$, $P = 0.002$, two-way mixed ANOVA). ***$P < 0.005$. Error bars indicate s.e.m.

Extended Data Figure 6 | Vector-based navigation schema. Left, The hippocampus represents a goal-direction vector pointing from the rat to the goal (***A***), which decreases as the rat is farther from the goal (right). The navigation system computes the projection of each choice platform vector (***B***, ***C***) onto the goal-direction vector (inner product, ***B***$_{gd}$, ***C***$_{gd}$) and selects the larger of the two. This choice is easier with increased angle between choices (angle β) and consequent increased difference in the magnitudes of their projection vectors. The projection vector of the preferred platform, ***B***$_{gd,}$ is the output of the system that competes with other potential solutions to the problem (for example, choose between the leftmost or northmost platform).

Extended Data Table 1 | Summary of multiple regression analysis
A multiple regression analysis was undertaken to establish the contributions of angle α, angle β and distance to maze performance. Data were taken from nine unoperated control rats and performance assessed across all choice types. B, unstandardized regression coefficient; SE$_B$, standard error of the coefficient; β, standardized coefficient.

Extended Data Table 2 | Coordinates of the sites of hippocampal lesions, and the volume of ibotenic acid used in rats with lesions
AP, anteroposterior with respect to bregma; ML, mediolateral with respect to bregma; DV, dorsoventral with respect to the brain surface; IBO, ibotenic acid.

Extended Data Table 3 | Coordinates of the sites of the sham medial entorhinal lesions
AP, anteroposterior; ML, mediolateral with respect to lambda; DV, dorsoventral with respect to the brain surface. The glass micropipette was angled at 22° from vertical, pointing rostrally along the antero-posterior axis.