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Hippocampal CA1 place cells encode intended destination on a maze with multiple choice points

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Abstract

The hippocampus encodes both spatial and non-spatial aspects of a rat's on-going behavior at the single cell level. In this study, we examined the encoding of intended destination by hippocampal (CA1) place cells during performance of a serial reversal task on a double Y-maze. On the maze, rats had to make two choices to access one of four possible goal locations, two of which contained reward. Reward locations were kept constant within blocks of 10 trials, but changed between blocks, and each day's session was comprised of three or more trial blocks. A disproportionate number of place fields were observed in the start box and beginning stem of the maze, relative to other locations on the maze. 46% of these place fields had different firing rates on journeys to different goal boxes. Another group of cells had place fields before the second choice point, and of these 44% differentiated between journeys to specific goal boxes. In a second experiment, we observed that rats with hippocampal damage made significantly more errors than control rats on the Y-maze when reward locations were reversed. Together, these results suggest that, at the start of the maze, the hippocampus encodes both current location and the intended destination of the rat, and this encoding is necessary for the flexible response to changes in reinforcement contingencies.

One of the dominant views of the hippocampus is that it contains a neural representation of space – a cognitive map – that encodes locations via the spatial receptive fields of place cells (O'Keefe and Nadel, 1978; O'Keefe, 1999). Individual place cells are active, to a first approximation, whenever the animal's head is in a portion of the environment to which the cell is responsive. However, these neurons are also responsive to ongoing dimensions of the rats' purposive behavior (Markus et al., 1995; Wood et al.,1999; 2000; Frank et al., 2000; Ferbinteanu & Shapiro, 2003; Bower et al., 2005; Smith & Mizumori, 2006; Griffin et al., 2007; Ainge et al., in press). These, and additional empirical clarifications (Huxter et al., 2003; Leutgeb et al., 2005), indicate that the hippocampus likely processes spatial and episodic dimensions of the animal's experience.

Although place cells encode current location, it is not clear how they give rise to a representation of the rat's intended destination (Morris, 1990). One possibility is that a downstream set of "goal" neurons fire maximally near the desired location of the animal (Burgess & O'Keefe, 1996). Evidence for such a representation within the hippocampus has been mixed (Breese et al., 1989; Speakman & O'Keefe, 1990; Lenck-Santini et al., 2001), although representations of arrival at a goal have been observed in the water maze (Hollup et al., 2001; Fyhn et al., 2002), and in an open field task (Hok et al., 2007). Place fields have been also observed to move towards an intended goal as rats run a series of turns during a continuous T-maze alternation task (Lee et al., 2006).

One way of examining the encoding of intended destination is to look for changes in place cell activity at critical choice points on a maze. Early studies had suggested that rats, in deciding upon a destination, use an overall representation of the maze environment to guide performance (Tolman, 1948). If such a representation is

based in the hippocampus, one might expect that as a rat learns that a given choice will lead to reward, a sub-set of its place fields will begin to reflect both the animal's current location and its intended destination. Such a representation of current location and learned association has been observed with a conditioned auditory stimulus in a fear conditioning task (Moita et al., 2003).

The expectation that place cells may fire differentially at choice points on a maze is a logical extension of several recent observations of prospective, retrospective, and contextual encoding (Wood et al., 2000; Frank et al., 2000; Ferbinteanu & Shapiro, 2003; Holscher et al., 2004; Bower et al., 2005; Frank et al., 2006; Smith & Mizumori, 2006; Ainge et al., in press; see Shapiro et al., 2006 for review). If prospective encoding is critical for choice behavior, one would expect that, when faced with a number of alternative destinations, place cells would develop selective firing for specific choices. Our results provide clear evidence for conditional firing, and suggest that the hippocampus encodes the intended destination of the rat at the beginning of the maze.

Methods

Apparatus The maze was built of wood and painted black. It consisted of a start box area, three choice points, connecting alleyways, and four goal boxes. These were arranged in a double Y-maze configuration (see Figure 1A). The start box, choice points and goal boxes were all octagons with 25 cm between opposing edges. The octagons had 30 cm high walls. The interconnecting alleyways were 25 cm long and 8 cm wide with 10 cm high walls. Each goal box contained a round ceramic food bowl of 5 cm depth. To help distinguish the four goal boxes from one another, we equipped each box with a different object (unopened bottle of salad dressing, metal plate, unopened liquid soap, rock) approximately 15 cm high x 8 cm wide x 5 cm deep, and a different Perspex figure. The figure was attached to the wall opposite the object, and the shape and colour of each figure was different in each box. The maze was mounted on circular stools so that it rested 64 cm above the floor, and it was situated in a square curtained enclosure (1.85 m square) with no deliberate extramaze cues.

Behavioral training The goal of the task was for the rat to find out which of four goal boxes contained a food reward (Weetos chocolate cereal loops (Weetabix, Kettering, UK); broken in to halves or quarters) and to return to a "correct" (reinforced) box over blocks of 10 consecutive trials (Figure 1A). Two of the four goal boxes were baited on every trial, and on different days different pairs of goal boxes were baited such that all combinations of boxes were presented. To increase the likelihood that the rats would return to the rewarded goal boxes, each baited food bowl contained more cereal loop pieces than could be consumed on an individual trial. It was reasoned that rats would be more likely to return to a location where they perceived that food remained, as opposed to returning to a location where they've just

consumed all the reward. After 10 trials, the food reward was shifted to other combinations of boxes, and the rat's task was to switch to a different rewarded goal box. Within a day the order of baited boxes was pseudo-randomised to ensure the rat visited all boxes.

Prior to their training on the task, rats were habituated to the maze for two 10 min sessions with no food or food bowls present. Thereafter, food bowls were added to all four goal boxes. Two food bowls were baited, and the other two were un-baited (though they contained cereal dust, to help control for odor cues). Each rat was run for 20 minutes each day or until 20 trials had been completed, whichever came first.

On each trial the rat was placed in the start box at the base of the Y, and kept in this area briefly by a wooden barrier that blocked the start box door. The experimenter stood in a constant location directly behind the start box. The experimenter then removed the barrier and allowed the rat to explore the maze and choose a goal box. A choice was recorded as an entry into one of the four goal boxes. If the rat chose a goal box that was baited with food, it was allowed to eat for approximately 5s. If the box was not baited, a wooden barrier was placed behind the rat and the rat was kept in the goal box for approximately 5s. The rat was then picked up by the experimenter and replaced in the start box, with the wooden barrier again blocking the start box exit. The maze was then wiped with a weak solution of detergent, and the interval between the end of one trial and the beginning of the next was approximately 10s. Trials were run in blocks of 10. A correct response was a visit of either of the baited goal boxes, although in practice rats would almost always return to the same baited box on consecutive trials. Rats were trained until they reached a criterion of 75% correct (rewarded goal box choices) over 20 trials (within a day) for four days.

Experiment 1: Place fields on a Y-maze

Subjects Subjects were five male Lister hooded rats, with a weight range of 330-368g. They were housed individually, and kept on a 12 hour light/dark cycle. Behavioral testing was carried out during the light phase. During testing, rats were kept on a food deprivation schedule to maintain approximately 90% (and not less than 85%) of their free-feeding weight. Testing was carried out five days per week. In this and the subsequent experiments, compliance was ensured with national (Animals [Scientific Procedures] Act, 1986) and international (European Communities Council Directive of 24 November 1986 [86/609/EEC]) legislation governing the maintenance of laboratory animals and their use in scientific experiments.

Electrode and Microdrive Preparation The electrode arrays consisted of two groups of four tetrodes. Each tetrode consisted of four, formvar coated, 25µm nichrome wires (California Fine Wire, Grover City, CA) twisted together and reinforced by coating with superglue. Each set of four tetrodes was threaded through a 27-gauge thin-wall stainless steel cannula (Small Parts Inc., Miami Lakes, FL) and the individual wires were wrapped around 16 separate pins of an 18 pin socket (Millmax, Oyster Bay, NY). The wires were covered with silver paint to improve the connection to the socket. The remaining two pins of the socket were connected to the cannula and a copper wire respectively (both of which acted as animal grounds). All of the connections were then secured in place using dental acrylic. Three drive screws (80 threads per inch, Small Parts Inc.) were attached to each electrode array, creating a moveable microdrive similar to that described previously (Kubie, 1984). This allowed the tetrodes to be advanced into the brain following surgery. Immediately before the electrode arrays were implanted, the tetrodes were cut to approximately 2mm from

the end of the cannula. The electrodes were bubble tested, and their impedance measured. The impedance of the electrodes before implanting was typically 150 KOhm. The electrode tips were coated in a small drop of carbowax as described previously (Wood et al., 2000), and the cannula was coated in sterile petroleum jelly.

Surgery Rats were anaesthetised with isoflourane and positioned in a Kopf stereotaxic frame (Tujunga, CA). To maintain hydration, rats were given 5 mls of Hartman's solution intraperitoneally. For analgesia, rats were given a subcutaneous injection of Carprofen and buprenorphine prior to the incision. Isoflourane anaesthesia was maintained for the surgery.

Under sterile conditions, the skull was exposed and lambda and bregma made level. Two small holes were made 3.5mm posterior to bregma and ± 2.5 mm lateral of the midline, and dura was exposed. The dura was pierced and the two electrode arrays were lowered to 1.5 - 1.7 mm below dura, one in each hemisphere. Each electrode array was comprised of four tetrodes. The ground wire from each electrode array was attached to two different skull screws and coated in silver paint. The electrode arrays were then secured in place by using dental acrylic and five small screws affixed to the skull. Rats were then injected with an additional 5 mls of Hartman's solution. The rats were monitored until they awoke, and then placed in a modified home cage designed to prevent the microdrive from getting caught on the cage sides. On the day following surgery an additional dose of Caprofen was given. All animals were allowed one week to recover before screening for cells began.

Screening, testing, and data acquisition Following recovery, rats were screened daily for complex spike activity in a circular arena (68 cm diameter with 49 cm high

walls) within the curtained recording environment. Screening for place cells was done by connecting the rat to the recording system (Axona, Herts, UK) via a lightweight cable and connectors (Millmax) that fit into the sockets on the electrode arrays. The signal from each electrode was passed through an AC-coupled unity gain operational amplifier mounted at the base of the cable, proximal to the rat's head. They were then passed, via the recording cable, through a 36-channel commutator (Dragonfly Research and Development Inc., Ridgeley, WV), to the recording system. The signals on each wire within a tetrode were recorded differentially with respect to those recorded on a wire in another tetrode in that electrode array. The signal was amplified (5,000 to 20,000 times) and bandpass filtered (600-6000Hz). One channel in each array was dedicated to recording EEG. The position of the rat was monitored during the recording session through a black and white camera mounted on the ceiling above the maze. Two groups of ultra-bright LEDs were attached to the amplifier on the rat's head. These were tracked using the recording system which detected the position of the two groups of LEDs at a sampling rate of 50 Hz. To record neuronal activity, each channel was monitored every 20 μs, and 50 samples per channel collected whenever the signal on any one of the four channels of a tetrode exceeded a predetermined threshold (set based on signal-to-noise ratio). These digitized spike waveforms were stored on the hard drive of a PC, together with the LED coordinates and the time since the start of the recording session. This permitted offline analysis of correlations between cell activity and the position, head direction, and movement of the animal.

If complex spike activity was observed during the screening session, an 8 min session was recorded in the circular arena with the rat foraging for randomly distributed pieces of cereal. The arena was then removed from the recording enclosure

and replaced with the Y-maze. Rats ran at least three blocks of 10 trials on the double Y-maze while complex spike activity was recorded. At the end of the trials, this activity was recorded as the rat explored the Y-maze for 8 mins with no food present. Finally, another 8 min session in the circular arena was recorded. If no complex spike activity was seen, the electrodes were advanced by 40-80 µm and allowed to stabilise overnight.

Perfusion and histology At the end of the experiment rats were given an overdose of sodium pentobarbitol and the electrode position was marked by passing current through the tetrodes making small electrolytic lesions. Rats were perfused transcardially with saline, followed by 4% formalin, and the brains removed. Brains were kept in 4% formalin mixed with 4% potassium ferrocyanide for at least 48 hours to elicit a Prussian blue reaction at the tetrode tips. Brains were sectioned on a freezing microtome, with 30 µm sections taken from area of the electrode track.

Place cell identification and analysis Initial data analysis was performed using TINT analysis software (Axona, Herts, UK) on the data from the circular arena and the free exploration of the Y-maze. Spikes were sorted into clusters using comparisons of peak amplitude, trough, and time to peak and trough on each channel. Autocorrelograms were generated for each cluster to ensure that no spikes fired within 1.5 ms of any other spikes from the same cluster. Clusters were then processed by a program (F-rate; Axona, Herts, UK) to measure mean spike duration, amplitude and firing rate over the whole session. Only clusters with mean amplitude greater than 95 µV on at least one channel, mean spike duration of over 250 ms, and mean firing rate of less than 2.5Hz over the whole session were accepted for analysis. Firing rate maps

were generated by dividing the maze into a grid of 60 x 60 pixels (each pixel being 3cm x 3cm). The firing rate for each pixel was calculated by dividing the number of spikes fired in that pixel by the number of seconds that the rat spent there. Cells were deemed to have place fields on the maze if there were at least six adjacent pixels with a firing rate of at least three times the session mean firing rate. Only cells with well defined place fields on the Y-maze were used for further analysis. Recordings from consecutive days were closely examined and cells with similar cluster boundaries across days were only counted once. Cluster quality was examined using a custom-written Matlab program (Steven Huang, Edinburgh). For each tetrode, energy and first principle component of the waveforms for each session were calculated. Using these, the L_{ratio} and isolation distance, as described by Schmitzer-Torbert et al. (2005), were derived.

Influence of intended destination For cell isolation, the cluster parameters from the 8 min session on the Y-maze were applied to the preceding test trials. In some cases very few spikes were seen in the test trials using these cluster parameters. If less than 50 spikes were recorded across trials within a day's recording session, the cell was excluded from further analysis.

To examine firing rates during the test trials the maze was divided up into areas of interest (see Figure 1B), and assessed using a Matlab script. For each trial the number of spikes fired and time spent in each area were extracted and these data were used to calculate an average firing rate in each area for every trial. To avoid double-counting fields that occupied more than one adjacent area, such fields were assigned to one of the two areas on an alternating basis. The trials were then separated by the goal box the rat selected. As is evident from Figure 1B, the start box (area 1) and

common stem (area 2) of the maze were common to all four goal box trajectories. To see whether place cell firing in the start box or common stem differed as a function of the rat's destination, we compared the firing rate of cells using a univariate ANOVA with destination (goal box) as the independent factor. Further analyses examined firing rates in the alleyways after the first choice point (areas 3 and 4). In both of these locations there are two possible subsequent destinations that the rat may choose. To examine this, the firing rates of cells with place fields in areas 3 and 4 were analysed using independent sample t-tests with goal destination as the independent factor.

Experiment 2: Y-maze performance following a hippocampal lesion

Subjects 10 male hooded Lister rats (weight range: 542-618g) served as subjects for this experiment. These rats had participated previously in a behavioral study on maze learning, but had not been exposed to the Y-maze or undergone invasive procedures. Rats were housed and fed as in experiment 1.

Y-maze training Prior to surgery, rats were trained on the Y-maze task as in Experiment 1. Rats were first habituated to the maze in a single 10 minute session, and then given daily sessions comprised of 10 trials. Reward was available in two of the four goal locations, and the rewarded locations were changed daily. Thus, on the initial trial of a day, the rats had a 50% chance of making a correct response. Once a rat completed 10 trials in less than 6 mins for two consecutive days, and did so with six or more correct responses on each day, they were moved to the final phase of pre-surgery testing. Here, rats were given 20 trials per day, and the two initial reward locations were changed to the previously non-rewarded goal locations after the first

10 trials. Rats were trained in this version of the task until they made an average of seven or more correct responses in their first 10 trials over three consecutive days. In addition, rats had to get five out of their last six responses correct within their first 10 trials for two consecutive days. Two rats did not achieve this last criterion on consecutive days, but did so on non-consecutive days.

Surgery Following pre-training, rats were assigned to one of two groups: a HIPPOCAMPAL LESION group, which received ibotenic acid lesions of the hippocampus (dentate gyrus and CA fields) (n = 6); or a surgical sham lesion, CONTROL group (n = 4). The general anaesthesia and surgical procedures were as in Experiment 1. Following exposure of the skull, a craniotomy was made bilaterally, exposing the dura above the hippocampus on each side. For rats in the HIPPOCAMPAL LESION group, 13 injections of ibotenic acid (Sigma, UK; dissolved in phosphate-buffered saline at 10mg/ml) were made in each hemisphere using techniques adapted from Jarrard (1989) and previously described (Ainge et al., 2006; Ainge et al *in press*). The injections were made via a 1µl syringe (SGE) securely attached to a Kopf stereotaxic arm. Ibotenic acid was injected at a rate of 0.1µl/minute, beginning 30 seconds after the needle was lowered. The needle was removed slowly 1 minute and 30 seconds after the injection. A total of 0.91µl per hemisphere was used for each lesion (see Ainge et al. *in press* for coordinates and volumes). For rats in the CONTROL group, no injections were made. Instead, the dura above the hippocampus was punctured with a needle nine times on each side, at sites corresponding to those at which injections were made in the lesion group. For rats in both groups, the skin was sutured together over the skull. The animals were then placed on a warmed blanket for a short time after surgery. All rats were

permitted free access to food and water following surgery, and allowed 10 days of recovery. The food restriction schedule (see Subjects section) was then resumed and behavioral testing began.

Post-lesion testing Following surgery, rats were tested on the double Y-maze for 15 20-trial sessions, using the same protocol as in pretraining. In each trial, two of the four goal boxes contained reward on each trial, and the specific boxes that were rewarded at the start of each day varied. After completing the first 10 trials within a session, the reward locations were reversed: the previously unrewarded boxes now contained reward, while the previously rewarded boxes did not. To examine the possibility that rats with hippocampal damage may have difficulty extinguishing or inhibiting a previously correct response, a further three sessions were run with the number of trials in each block being increased to 20. Our prediction was that increasing the number of trials in each block would exacerbate any tendencies to choose previously rewarded locations following a reversal.

The rats were then tested on a standard, within-trial alternation task using only the right side (goals 3 and 4 in Fig. 1A) of the Y-maze. The left side of the double Y-maze was made inaccessible by removing the alleyway leading to it after the first choice point. Alternation tasks on a T-maze are particularly sensitive to disruption of the hippocampus if there is a delay between arm choices (e.g., Ainge et al., in press). These tasks also require a reversal, in that the rat must choose a location that was not reinforced during the immediately proceeding sample run. This might be a particular challenge for the animals trained on the Y-maze task, as the rats were reinforced for going back to the same locations for 10 or 20 trials ("win-stay"), and then, following the change in reward location, for shifting to the new location ("lose-shift").

On the sample run, only one of the two goal boxes was open. After entering the box, the rat was picked up by the experimenter and replaced in the start box at the base of the Y-maze. The rat was then permitted to run up the maze. Both the previously visited, "sample" goal box, and the goal box that had been blocked off were open, and reward was only available in the box that had not been visited in the sample run. Rats were given 10 trials per day on the task, and each of the two goal boxes served equally as the sample and goal box.

Perfusion and histology At the conclusion of the behavioral testing rats were given an overdose of sodium pentobarbitol and perfused transcardially with saline, followed by 4% formalin. The brains were removed and fixed in egg-yolk, and kept in a 4% formalin solution. Subsequently, the brains were sectioned at 50 um using a freezing microtome. Sections were mounted on gelatin-coated slides, and processed for Nissl stain. The extent of hippocampal tissue loss in the lesion group was quantified using an image analysis program, Leica QWin. The tissue volume through the anterior-posterior extent of the hippocampus was compared to the average hippocampal volume of two sham-lesioned rats.

Results

Experiment 1

Behavioral analysis: Rats reached a criterion level of performance (75 % correct for four consecutive days) on the Y-maze in an average of 13 days. During the test trials they made 77.3 % correct choices. It should be noted that, due to the serial reversal nature of the task, a number of errors were inevitable. If a rat learned the correct strategy of returning to a reward goal box, it should make an error on the first trial of

every new block as it would not know that the reward location has changed. If these errors are removed from the analysis rats performed at 84.5 % correct during the test trials. Figure 1C illustrates paths taken by a rat on 20 consecutive trials of a session. These paths suggest that the rat's movements were ballistic; once the rat was released from the start box it ran quickly to the goal without deviating or hesitating. The paths also demonstrate the rat's ability to learn, in one trial, that once no reward was found in a goal box it should search elsewhere.

Histology Inspection of the brain histology confirmed that the electrodes were situated in the hippocampus (Figure 2; Supplementary Figure 1). For 4 of the 5 subjects, the Prussian blue reaction was consistent with an electrode placement in the CA1 cell layer. In the 5th subject (not shown), the track of the electrode array was somewhat deeper, and the location of this animal's recordings could not be specified. The data from this animal (6 cells) were excluded from the analysis.

Place field identification and distribution A total of 139 cells with place fields fitting the specified criteria were recorded on the Y-maze. A number of these cells had two or more fields and so a total of 205 place fields were available for analysis. Table 1 presents the cluster quality measures for the cells, using the L_{ratio} and Isolation Distance measures (see Schmitzer-Torbert et al. (2005) for a comparison). Based on these measures, 110 of the 139 clusters were classified as good (i.d. > 30; $L_{ratio} < 0.1$), 26 were classified as intermediate (i.d. > 20; $L_{ratio} < 0.15$), and 3 were classified as poor (i.d. < 20; $L_{ratio} > 0.1$). Goal-sensitive cells (discussed below) were observed in nearly equal proportions in both the good and intermediate clusters; none of the 3 poor clusters were goal-sensitive.

Our initial analysis examined the position of the place field on the maze (Figure 1E) and found the majority of the place fields were clustered around the start box area. A total of 70 place fields were identified in the start box area, with 52 fields in the start box and 18 fields in the common stem of the maze. A Chi-square test comparing the distribution of fields in the start box, choice points, and goal boxes (all of which were the same size) indicated that the observed distribution of field differed significantly from one in which these locations were represented equally (χ^2 (7, n = 122) = 108.5, $p < .005$). A further 39 fields were found after the first choice point (20 fields on the left alleyway, and 19 fields on the right). All of these were assessed for encoding of intended destination.

Influence of goal destination on place fields in the start box area Firing rates in the start box and common stem of the maze were calculated for each of the test trials. The test trials were next grouped in terms of the final goal destination (i.e., goal box 1, 2, 3 and 4) and differences in firing rate as a function of goal destination were examined with univariate ANOVA for each cell. Of the 70 cells with place fields in the start box and common stem of the maze, 32 (46%) showed significant effects of intended goal destination, indicating that firing rates for these cells differed between journeys to different goal boxes. Examples of cells that fired at high rates on journeys to a specific box are shown in Figure 3A. In these 4 examples, differential place cell activity was manifest in a relatively high rate of firing when the rats made the journey to one goal box, and substantially fewer spikes when the rats ran to the other three goal boxes. 25 of 32 place fields with significant differential activity exhibited this pattern of firing (see Supplementary Table 1). 6 of 32 significant fields showed a more graded difference in firing rates between the highest- and next highest-rate

journeys, and only 1 field showed a high firing rate for journeys to 3 goals, with a low firing rate to the remaining goal (Supplementary Table 1; Supplementary Figure 2). Clear examples of differential activity were seen in all rats. In addition, in cells showing differential activity, the firing to "non-preferred" goal boxes journeys, when present, appeared in the same location as the firing to the high rate journey, consistent with a rate-remapping perspective (Leutgeb et al., 2005).

Figure 3B is an example of a cell whose firing did not differ as a function of the rat's goal box choice. 38 of the 70 fields found in the start box and first common stem did not fire differentially. The auto-correlograms for the cell in Fig. 3B and those of Figure 3A show theta-modulation of the cell firing, consistent with the activity in these fields occurring during theta. (The theta-modulation of the 3rd example in Figure 3A was somewhat less robust, but this may be due in part to the spikes at the arm ends, where the rat stopped to consume its reward.) It's possible that the number of non-differential fields may be an overestimate of the true non-differential firing, as firing on multiple reference frames (see Redish, 1999) will occasionally occur in the same location.

Influence of goal destination on place fields after the first choice point Differential firing was also observed beyond the first choice point. Firing rates in the alleyways to the left and right of the first choice point (areas 3 and 4) were calculated for each trial. Trials were then grouped by goal destination, and a comparison of firing rates for a given place field as a function of the rat's intended destination was performed using an independent samples T-test. Of the 39 cells with fields in the alleyways immediately beyond the first choice point, 17 (44%) showed a significant difference in firing rate depending on the rat's destination. Examples of cells that have higher

firing rates on journeys to one goal box relative to the other are shown in Figure 4A. As in the start area, place fields that did not differentiate between intended destinations were observed, and an example is shown in Figure 4B.

As is evident in the second example in Figure 4A, some cells had multiple place fields. To see if these fields encoded common elements of the maze, we reviewed the place field plots of all 45 cells with multiple fields (Supplementary Table 2). The most common pattern, seen in 12 cells, was the presence of a field in the start area and in one of the goal arms. However, only one of these cells fired differentially in the start box to the goal encoded by the second field. 8 cells had a field in the left or right arm after the first choice point, and a second field in one of the goal boxes. Only one of these cells fired differentially for the same goal encoded by the second field. 6 cells had fields in both the start area and the left or right arms after the first choice point, but only one of these cells showed differential firing in both fields to the same goal. This cell's firing could be construed as encoding a specific path, but if so it represents the only example we observed. Only one cell appeared to encode second choices – e.g., right turns at both second choice points (Figure 3A, 4th example). However, this cell also fired in the start box when the rat travelled to one of the other arms. Overall, although different combinations of fields were observed (see Supplementary Table 2), a clear hierarchy in the conditional activity of the fields was not evident. Rather, multiple fields from the same cells appeared to be relatively independent of one another.

The proportions of place fields showing goal sensitivity are shown in Figure 5A. The top plot shows the proportions of place fields found in the start box area (areas 1 and 2) relative to those found before the final choice point (areas 3 and 4). For place fields in the start box area, 32 fields were goal-sensitive and 38 showed no

significant influence of intended destination. For the place fields before the second choice point, 17 fields were goal-sensitive, while 21 were goal-non-sensitive. Thus, in both regions there were somewhat more non-differential cells (e.g., Figure 3B and 4B) than goal-sensitive cells. Nonetheless, the percentages of fields showing significant differences in firing rate as a function of intended goal, 46% in the start box area and 44% after the first choice point, were considerably higher than the 5% one would expect based solely on type I error.

To assess further the possibility that place fields with differential activity simply reflect the extremes of a distribution of non-differential fields, we plotted the test statistic for all 70 place fields from areas 1 and 2 (Figure 5B). The test statistic, the F-ratio from the ANOVA, is the ratio of firing rate variability between goals relative to the variability within trials to the same goals. Higher F-ratios indicate greater differences in firing rates as a function of intended destination. (Ratios for the differential fields after the first choice point weren't included in this analysis as their test statistics were based on different degrees of freedom.) The distribution of the lower, non-significant F-ratios in Figure 5B resembles a typical F distribution, with a peak near 1. However, the number of fields with statistically significant F-ratios (> 3) suggests that there is also a separate distribution of goal-sensitive fields.

Dynamic changes in place cell firing within a session Although the majority of the cells recorded on the maze showed consistent firing within a place field across trials, a number of place cells showed clear changes in firing rates within a test session. From the total 205 place fields recorded, 46 showed intra-session changes in firing. This was characterised as a period of complete inactivity (at least 1 trial with 0 spikes in

the place field) followed by a period of sustained firing (at least 3 trials with firing rate in the place field greater than 3 times the average firing rate for the session). Nine examples of these changes are shown in Figure 6. The changes fell into three categories. The most prevalent were cells that were silent at the beginning of the sessions and then developed robust place fields after a number of trials (Figure 6A). 37 of the 46 fields showed this pattern of place field change. A second category had robust place fields at the beginning of the session which disappeared after a number of trials (Figure 6B). 5 fields showed this pattern. A third category of cells had fields that moved within a session from one area of the maze to another, for example from a goal box to the start box (Figure 6C). 4 place fields exhibited this pattern of change.

In Figure 6 there is some evidence for out-of-field spikes. Although we've restricted our analysis to place fields, it is possible that such activity may occur at critical junctures on the maze (Johnson and Redish, 2006).

Experiment 2

Lesion extent Infusions of ibotenic acid produced significant, although not complete removal of the neuropil within the hippocampus. Damage was greater in the anterior-dorsal hippocampus; substantial neuronal sparing was observed in the more posterior-ventral hippocampus. As can be seen in Table 1, the percentage of dorsal hippocampus removed ranged from 46.2 - 64.6%, with an average tissue loss of 57.4%. For the entire hippocampus, the average amount of tissue loss was 45.8%, with a range of 37.5-54.7%.

Post-lesion behavior Figure 7A shows the performance of the hippocampal lesion and control groups on the first 10 trials of each day over 15 post-surgery testing sessions. For clarity, the data are presented in three-session blocks. As is evident in

the left plot, there was no difference between the groups in the number of correct choices made in the first 10 trials in each block of sessions ($F(1,8) = 0.241$, $p = 0.64$). There was also no change in performance across blocks ($F(4,32) = 0.454$, $p = 0.77$), or difference between groups as a function session block ($F(4,32) = 1.1$, $p = 0.37$).

Following the first 10 trials each day, the reward locations were reversed. In the second 10 trials of each testing session (Figure 7B), the hippocampal lesion group were significantly impaired relative to the control group ($F(1,8) = 9.08$, $p < 0.017$). Again, there was no difference in overall performance across blocks of sessions ($F(4,32) = 1.18$, $p = 0.34$), or difference between groups as a function of session block ($F(4,32) = 0.067$, $p = 0.98$). A regression analysis revealed that the degree of impairment following reversal was not predicted by the overall size of the lesion ($r = .12$; $F(1,5) = .06$, $p = 0.82$) or by the amount of damage to the dorsal hippocampus ($r = .52$; $F(1,5) = 1.48$, $p = .29$). However, the power of this analysis may be constrained by the limited variability in the lesioned animals' performance (range: 5.53 – 6.47 correct).

To examine the possibility that rats with hippocampal damage may have difficulty inhibiting a previously correct response, both groups were given three sessions of two blocks of trials where the number of trials in each block was increased to 20. Reward locations were constant within a block. As in the previous condition, there were no differences between the groups in the number of correct choices in the first set of (now 20) trials ($F(1,8) = 0.203$, $p = 0.65$). There was also no overall difference in performance across the three sessions ($F(2,16) = 1.129$, $p = 0.35$) or difference between groups as a function of session ($F(2,16) = 0.02$, $p = 0.98$). Following reversal of the reward locations, the hippocampal lesion group appeared to exhibit lower average scores than the control group in each session (Figure 7C),

although this difference was not statistically significant ($F(1,8) = 1.99$, $p = 0.2$). No overall difference across sessions was found ($F(2,16) = 0.99$, $p = 0.39$), and the performance of the two groups did not differ in different sessions ($F(2,16) = 0.36$, $p = 0.96$).

As is evident in Figure 7B (and to a lesser extend in 7D), the hippocampal lesion group performed worse than the control group when the reward locations were reversed. To see whether this impairment was due to perseverative responding by the lesioned animals when the reward locations were reversed, we examined the performance of both groups on each of the 20 trials for the 15 testing sessions shown in figures 7A and B. In Figure 7E, the performance of the control and hippocampal lesioned groups across each of the 20 trials is plotted. In the first 10 trials of each daily session, performance for both groups began at near chance (50%), and improved. Statistically, this was revealed by a significant effect of trials ($F(9,72) = 14.1$, $p < .001$), but no overall difference between groups ($F(1,8) = 0.34$, $p = .58$) or interaction between groups as a function of trials ($F(9,72) = 1.18$, $p = .32$). When the reward locations were reversed, both groups' performance dropped below 50% on the next (the 11^{th}) trial. Following this reversal, performance improved for both groups, although the hippocampal lesion group was consistently worse than the control group. Statistically, this was supported by a main effect of trials ($F(9,72) = 27.4$, $p < .001$), a significant difference between groups ($F(1,8) = 10.3$, $p < .012$), but no interaction between groups as a function for trials ($F(9,72) = 0.67$, $p = .74$).

Figure 8 shows the performance of the hippocampus lesion and control groups in learning the spatial alternation task. Both groups were tested for 21 sessions, and for clarity of presentation, the data has been grouped into seven three-session blocks. As is evident from the graph, the control animals learned this task more readily than

the animals with hippocampal damage. Both groups improved with training, and by the final training block the animals with hippocampus damage were at nearly the same performance levels as the control animals. Statistically, the overall improvement across training blocks was significant ($F(6,48) = 21.92$, $p < 0.001$). The performance of the hippocampal damage group was significantly worse than the control group ($F(1,8) = 29.34$, $p < 0.001$), and there was a significant interaction between groups and testing session ($F(6,48) = 4.65$, $p < 0.001$). Post-hoc, independent samples T-tests revealed that the source of the this interaction was a non-significant difference between groups in the 1st and 7th testing block (p's $\geq$ 0.26), and significant differences between groups in all other testing blocks (blocks 2-6, all p's < 0.05).

Discussion

In a double Y-maze task where reward is found repeatedly at the end of some arms, but not others, a subset of hippocampal place cells exhibited differential firing at the start of the maze and before the final choice point that predicted the rat's ultimate destination. Partial removal of the hippocampus did not impair learning of the initial reward locations, but did impair performance when reward locations changed.

The encoding of intended destination

46% of the place fields at the beginning of the maze – in the start box and the first alleyway - exhibited significant differences in firing rates as a function of the rat's intended destination. This trajectory-specific encoding was also observed in 44% of the place fields beyond the first choice point, but before the final choice. This

result reinforces previous demonstrations of conditional or contextual place field activity (Wood et al., 2000; Frank et al., 2000; Ferbinteanu & Shapiro, 2003; Holscher et al., 2004; Bower et al., 2005; Smith & Mizumori, 2006; Bahar & Shapiro, 2006) but provides an important extension of these by showing that such encoding is not simply dichotomous, but instead reflects the encoding of a specific trajectory among several alternatives. Such encoding may reflect a trajectory-specific intention, present from the beginning of the trial.

Goal-sensitive place fields may reflect rate-remapping between trajectories (Leutgeb et al., 2005). As suggested by Redish (1999), multiple maps or reference frames may allow both current location and intended destination to be represented in situations where reward locations change. A test of the multiple-map view would be to see whether place fields initially fire in a single reference frame when a rat is first exposed to a maze, but later develop differential firing and multiple reference frames when the rat learns that reward is in different locations in different trial blocks.

Distribution of place fields on the maze

A disproportionate number of place fields were observed at the start of the maze. It's possible that this over-representation was due to the greater amount of time the rats spend in the start box (where they were placed between trials) relative to the rest of the maze, but rats also spent longer periods of time in the goal boxes at the maze end, and these locations were not over-represented. Alternatively, the over-representation may reflect a greater significance of this maze area for the rats, similar to the over-representation of the area near the goal platform in an annular water maze (Hollup et al., 2001). Another possibility is that, if each of the goal-box trajectories is represented independently, the locations common to all four paths will of necessity

have greater numbers of place fields. Conceptually, this would be akin to the independent (but spatially overlaid) representations that are seen when rats move in opposite directions on a maze (e.g., McNaughton et al., 1983; Gothard et al., 1996).

A final possibility is that the over-representation of the start area of the Y-maze participates in, or is the product of, changes in the representation of the task as it becomes habit-based. Jog et al. (1999) observed that on a conditional T-maze task, 88% of task-related neurons recorded in the sensorimotor striatum responded at the start of the maze. Barnes et al. (2005) confirmed and extended this finding by showing that increases in activity at the beginning of the T-maze develop with over-training. The striking similarities between these findings and the over-representation of the start area we observed may suggest that some aspects of a habit-based task are represented in the hippocampus, even when the task becomes dependent on the caudate (Packard & McGaugh, 1996).

Determinants of behavior at a choice point

The current results complement the intriguing results of Johnson and Redish (2006), who recorded from CA3 neuronal ensembles on a T-maze apparatus with return arms. They observed that at the critical choice point on the maze, the ensemble appeared to "look ahead" down the potential arm choices before the rat made its choice. These results imply a difference in the way the rats behave on their task relative to the current double Y-maze task, as, presumably, the rats paused briefly at the choice point on their T-maze, but showed little evidence of doing so on the Y-maze. However, it is possible that at the start box, a part of the over-representation we observed was based on the type of anticipatory activity observed by Johnson and Redish (2006).

Changes in place fields within a series of trials

Nearly 23% of the place fields recording on the Y-maze exhibited changes across trials within a session, the most common of which was the appearance of a place field in an initially quiet cell. Frank et al. (2004) also showed this on a novel arm of a radial maze, where some place fields only became evident after 1-2 minutes experience. In a subsequent study, 20% of CA1 place fields were observed to develop rapidly, whereas very few entorhinal cortex cells did so (Frank et al., 2006).

A striking difference between the current results and those of Frank et al. (2004; 2006) is that in this study the entire Y-maze apparatus was, presumably, highly familiar to the rats. All rats were trained on the Y-maze task before surgery, and once a place cell was encountered, the rats received repeated testing using all maze arms. The changes observed by Frank et al. occurred primarily within the first two days on the new maze arm; by day three, the representation appeared stable. One possibility is that our Y-maze task has elements (e.g., the change in reward locations) that are perceived as novel in each session. However, even if this is true, it's unclear how the appearance or disappearance of a field after several maze trials contributes to essentially stable maze behavior.

Additional types of place field changes have been described by Mehta et al. (1997) and Lee et al. (2006). Our data were based on fewer repeated journeys than Mehta et al., but some beginnings of backward place field expansion (e.g., the 1st and 4th examples in Fig 6A) may be evident. Backward expansion presumably occurs whenever a rat runs repeatedly in the same direction through a place field, and it's possible that the synaptic potentiation underlying this effect also contributes to the emergence of place fields in previously quiescent place cells. It is unclear, however,

how backward field expansion could account for goal-sensitive activity we observed, as the goal encoding seemed to be in the form of large differences of firing rate in the same location (i.e., rate-remapping), and not a shift in field locations between trajectories. The forward shifts in place fields observed by Lee et al. (2006) were not evident in our results, although this may be due to 1) the smaller number of repeated trials we ran, 2) the separation of trials by a brief delay, and 3) the reward of the same location within blocks of trials, as opposed the reward of alternate locations.

Reversal and alternation on the Y maze

The impairment in reversal performance following the partial lesions of the hippocampus is consistent with contemporary views of the hippocampus as a component of a memory system necessary for the flexible use of relations between stimuli to guide behavior (Eichenbaum & Cohen, 2001). This deficit also agrees with earlier data on reversal learning (e.g., Kimble & Kimble, 1965; but see Murray & Ridley, 1999) and earlier views of hippocampus function provided by Kimble (1968), Hirsch (1974), and Gray (1982). Kimble argued that impairments in reversal learning, amongst other tasks, indicate that the hippocampus is essential for the ability to inhibit responses which the animal has a predisposition to make. Hirsch's view was that the hippocampus is necessary for the use of contextual information to guide behavior, and in the absence of a hippocampus, animals rely on stimulus-response, habit based strategies. Thus, animals with hippocampus damage can learn initial discriminations, but are impaired in reversals, as they require segregation of the previously rewarded responses and the new responses. The related perspective of Gray is that the septo-hippocampal system is essential to act on mismatches between the animal's expectations and its actual experience. In the current study such a mismatch may

occur on the first trial of the reversal when the rat discovers that a previously reinforced goal box no longer contains food. In Gray's view, the septo-hippocampal system would then inhibit an ongoing motor programme, such as the rats' ballistic return to the same goal box, and initiate exploration of other potential reward sites.

The deficits in Y-maze alternation in the current experiment were consistent with a number of studies using delayed alternation tasks, where 50-90% damage to the hippocampus produced significant impairments in performance (e.g., Racine & Kimble, 1965; Hock & Bunsey, 1998). They are also consistent with a study using delayed-non-matching to sample task on a Y-maze (Higgs et al., 2001) where the hippocampus-lesioned animals were significantly impaired in choosing the non-matching box relative to the control animals, but still performed at a 75% correct level. This agrees with the current findings that lesioned animals were impaired in acquiring the alternation task, but ultimately learned the alternation rule.

Implications

The finding that place cells encode intended destination may complement recent findings implicating the human hippocampus in the ability to imagine future events. People with hippocampus damage exhibit impoverished descriptions of imagined future situations (Hassabis et al., 2007). Further, when recalling past event or imagining future events, fMRI scans show that the right hippocampus exhibits a significant activation, and the left hippocampus is significantly more active during future event elaboration (Addis et al., 2007). Addis et al. suggest that episodic memory's function is not simply to retrieve past events, but also to envision future ones. The encoding of both current location and intended destination by hippocampal place neurons shown here may reflect the single-unit instantiation of this capacity.

References

Addis DR, Wong AT, Schacter DL (2007) Remembering the past and imagining the future: common and distinct neural substrates during event construction and elaboration. Neuropsychologia 45:1363-1377.

Ainge JA, Heron-Maxwell C, Theofilas P, Wright P, de Hoz L, Wood ER (2006) The role of the hippocampus in object recognition in rats: examination of the influence of task parameters and lesion size. Behav Brain Res 167:183-195.

Ainge JA, van der Meer M, Langston RF, Wood ER (in press*)* Exploring the role of context dependent hippocampal activity in spatial alternation behaviour. Hippocampus

Bahar AS, Shapiro ML (2006) Reorganised prospective and retrospective hippocampal memory coding after switching the start and goal of a journey. Soc Neurosci Abstr 68.21.

Barnes TD, Kubota Y, Hu D, Jin DZ, Graybiel AM (2005) Activity of striatal neurons reflects dynamic encoding and recoding of procedural memories. Nature 437: 1158-1161.

Bower MR, Euston DR, McNaughton BL (2005) Sequential-context-dependent hippocampal activity is not necessary to learn sequences with repeated elements. J Neurosci 25(6):1313-1323.

Breese CR, Hampson RE, Deadwyler SA (1989) Hippocampal place cells: stereotypy and plasticity. J Neurosci 9(4):1097-111.

Burgess N, O'Keefe J (1996) Neuronal computations underlying the firing of place cells and their role in navigation. Hippocampus 6(6):749-62.

Eichenbaum H, Cohen, NJ (2001) From conditioning to conscious recollection. New York, Oxford University Press.

Ferbinteanu J, Shapiro ML (2003) Prospective and retrospective memory coding in the hippocampus. Neuron 40(6):1227-1239.

Frank LM, Brown EN, Stanley GB (2006) Hippocampal and cortical place cell plasticity: implications for episodic memory. Hippocampus 16:775-784.

Frank LM, Brown EN, Wilson ML (2000) Trajectory encoding in the hippocampus and entorhinal cortex. Neuron 27:169-178.

Frank LM, Stanley GB, Brown EN (2004) Hippocampal plasticity across multiple days of exposure to novel environments. J Neurosci 24(35):7681-7689.

Fyhn M, Molden S, Hollup S, Moser M-B, Moser E (2002) Hippocampal neurons responding to first-time dislocation of a target object. Neuron 35(3):555-566.

Gothard KM, Skaggs WE, McNaughton BL (1996) Dynamics of mismatch correction in the hippocampal ensemble code for space: interaction between path integration and environmental cues. J Neurosci 16(24):8027-40.

Gray JA (1982) Precis of The neuropsychology of anxiety: An inquiry into the functions of the septo-hippocampal system. Behav Brain Sci 5(3): 469-484.

Griffin AL, Eichenbaum H, Hasselmo ME (2007) Spatial representations of hippocampal CA1 neurons are modulated by behavioral context in hippocampus-dependent memory task. J Neurosci 27(9):2416-2423.

Hassabis D, Kumaran D, Vann SD, Maguire EA (2007) Patients with hippocampal amnesia cannot imagine new experiences. Proc National Acad Sci USA 104(5):1726-1731.

Higgs S, Bannerman DM, Rawlins JN (2001) The effect of cytotoxic lesions of the hippocampus on recognition memory in the rat: effects of stimulus size. Behav Neurosci 115(6):1193-203.

Hirsch R (1974) The hippocampus and contextual retrieval of information from memory: a theory. Behav Biol 12: 421-444.

Hock BJJ, Bunsey MD (1998) Differential effects of dorsal and ventral hippocampal lesions. J Neurosci 18(17):7027-7032.

Hok V, Lenck-Santini P-P, Roux S, Save E, Muller RU, Poucet B (2007) Goal-related activity in hippocampal place cells. J Neurosci 27(3):472-482.

Hollup SA, Molden S, Donnet JG, Moser M-B, Moser, EI (2001) Accumulation of hippocampal place fields at the goal location in an annular watermaze task. J Neurosci 21(5):1635-44.

Holscher C, Jacob W, Mallot HA (2004) Learned association of allocentric and egocentric information in the hippocampus. Exper Brain Res158:233-240.

Huxter J, Burgess N, O'Keefe J (2003) Independent rate and temporal coding in hippocampal pyramidal cells. Nature 425:828-832.

Jarrard LE (1989) On the use of ibotenic acid to lesion selectively different components of the hippocampal formation. J Neurosci Methds 29(3): 251-259.

Jog MS, Kubota Y, Connolly CI, Hillegaart V, Graybiel AM (1999) Building neural representations of habits. Science 286(5445): 1745-9.

Johnson A, Redish AD (2006). Neural ensembles in CA3 transiently encode paths forward of the animal at a decision point: a possible mechanism for the consideration of alternatives. Soc Neurosci Abstr 574.2.

Kimble DP, Kimble RJ (1965) Hippocampectomy and response perseveration in the rat. J Comp Phys Psych 60(3):474-476.

Kimble DP (1968) Hippocampus and internal inhibition. Psych Bull 70(5): 285-295.

Kubie JL (1984) A driveable bundle of microwires for collecting single-unit data from freely-moving rats. Physiol Behav 32:115-118.

Lee I, Griffin AL, Zilli EA, Eichenbaum H, Hasselmo ME (2006) Gradual translocation of spatial correlates of neuronal firing in the hippocampus toward prospective reward locations. Neuron 51:639-650.

Lenck-Santini PP, Save E, Poucet B (2001) Place-cell firing does not depend on the direction of turn in a Y-maze alternation task. Eur J Neurosci 13(5):1055-8.

Leutgeb S, Leutgeb JK, Barnes CA, Moser EI, McNaughton BL, Moser M-B (2005) Independent codes for spatial and episodic memory in hippocampal neuronal ensembles. Science 309:619-623.

Markus EJ, Qin YL, Leonard B, Skaggs WE, McNaughton BL, Barnes CA (1995) Interactions between location and task affect the spatial and directional firing of hippocampal neurons. J Neurosci 15(11):7079-94.

McNaughton BL, Barnes CA, O'Keefe J (1983) The contributions of position, direction, and velocity to single unit activity in the hippocampus of freely-moving rats. Exp Br Res 52(1):41-9.

Mehta MR, Barnes CA, McNaughton, BL (1997). Experience-dependent, asymmetric expansion of hippocampal place fields. Proc Nat Acad Sci 94(16): 8918-21.

Moita MAP, Rosis S, Zhou Y, LeDoux JE, Blair HT (2003) Hippocampal place cells acquire location-specific responses to the conditioned stimulus during auditory fear conditioning. Neuron 37:485-497.

Morris RGM (1990) Does the hippocampus play a disproportionate role in spatial memory? Disc. Neuroscience VI: 39-45.

Murray TK, Ridley RM (1999) The effect of excitotoxic hippocampal lesions on simple and conditional discrimination learning in the rat. Behav Br Res 99:103-113.

O'Keefe J (1999) Do hippocampal pyramidal cells signal non-spatial as well as spatial information? Hippocampus 9(4):352-64.

O'Keefe J, Nadel L (1978) The hippocampus as a cognitive map. New York, Oxford University Press.

Packard MG, McGaugh JL (1996) Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. Neurobio Learn Mem 65: 65-72.

Racine RL, Kimble DP (1965) Hippocampal lesions and delayed alternation in the rat. Psychon Sci 3:285-286.

Redish, A. D. (1999). Beyond the cognitive map. London, MIT Press.

Schmitzer-Torbert N, Jackson J, Henze D, Harris K, Redish AD (2005) Quantitative measures of cluster quality for use in extracellular recordings. Neurosci 131:1-11.

Shapiro ML, Kennedy PJ, Febinteanu J (2006) Representing episodes in the mammalian brain. Curr Opin Neurobio 16(6):701-709.

Smith DM, Mizumori SJY (2006) Learning-related development of context-specific neuronal responses to places and events: the hippocampal role in context processing. J Neurosci 26(12):3154-3163.

Speakman A, O'Keefe J (1990) Hippocampal complex spike cells do not change their place fields if the goal is moved within a cue controlled environment. Eur J Neurosci 2(6):544-555.

Tolman EC (1948) Cognitive maps in rats and men. Psych Rev 55:189-208.

Wood ER, Dudchenko PA, Eichenbaum H (1999) The global record of memory in hippocampal neuronal activity. Nature 397(6720):613-6.

Wood ER, Dudchenko PA, Robitsek RJ, Eichenbaum H (2000) Hippocampal neurons encode information about different types of memory episodes occurring in the same location. Neuron 27(3):623-33.

Figure legends

Figure 1. (A) Schematic representation of the concatenated Y-maze. In pre-training, two of the four goal boxes contained reward in each block of 10 trials. In this example boxes 2 and 4 are rewarded in the first 10 trials and boxes 1 and 3 in the second 10 trials. (B) Representation of the binned areas used to examine the influence of goal destination on place cell firing. (C) Example of the paths taken by a rat on 20 consecutive trials. The paths are ballistic and reflect little hesitation at choice points. The rat returns to the same goal on every trial until it is not rewarded, and then immediately chooses another box. If that box is rewarded it returns to this location in subsequent trials. This illustrates the ability of the rat to learn in one trial that the reward location has changed. (D) Frequency distribution of place fields on the maze. Higher peaks and warmer colours indicate higher numbers of place fields.

Figure 2. Electrode placement. The arrow indicates the mark of the electrode tip in the dorsal hippocampus.

Figure 3. CA1 place cells in the start box encode intended destination. (A) Four examples (one on each row) of cells with place fields in the start box which fired predominantly on journeys to one of the four goal boxes. The left column shows all of the paths for a single recording session with red dots indicating the spikes from one neuron. The shaded grey box is the place field assessed for intended trajectory. The middle left column shows the data separated into journeys to each goal box. The middle right column shows the average firing rate of the cell in the start box on journeys to each of the goal boxes. The right column shows the cluster, waveforms

and autocorrelogram of the cell. The horizontal black bars on the waveforms represent 300 μs while the vertical black line represents 100 μV. (B) An example of a cell that had similar firing rates in the start box on journeys to all goal boxes.

Figure 4. CA1 place cells before the final choice point encode intended destination. (A) Two examples (one on each row) of CA1 place cells with place fields before the final choice point that fired predominantly on trials to one of the two possible goals. The left column shows all of the trials from a single session with the spikes from an individual neuron represented as red dots. The shaded grey box indicates the area of the maze with the place field of interest. The middle left column shows journeys to each goal box separately. The middle right column shows the average firing rate in the grey shaded area for journeys to the two possible goal boxes. The right column shows the cluster, waveforms and autocorrelogram of the cell. The horizontal black bars on the waveforms represent 300 μs while the vertical black line represents 100 μV. (B) An example of a cell that had similar firing rates on journeys to both goal boxes.

Figure 5. (A) Proportion of place fields in the start of the maze (areas 1 and 2), and after the start areas, but before the second choice point (top pie-chart). These are broken down into the proportions of goal-sensitive and goal-non-sensitive cells in each of these two regions (bottom pie-chart). (B) Distribution of F-ratios for goal-sensitive and goal-non-sensitive fields in the start box and first common stem of the maze (areas 1 and 2).

Figure 6. Place field changes within a session. (A) Five examples (one on each row) of cells that developed robust place fields after several trials. The left column shows the whole session with red dots indicating spikes fired by an individual neuron. The right column shows the individual trials to a specific goal box (the first one is on the left). (B) Two examples (one on each row) of cells that initially had robust place fields but ceased to fire at some point during the session. The left column shows the whole session with red dots indicating spikes fired by an individual neuron. The right column shows the individual trials to a specific goal box (the first one is on the left). (C) Two examples of cells (one on each row) whose fields changed locations over maze trials.

Figure 7. Performance on the double Y-maze task. (A) The mean (±SEM) number of correct choices made by the hippocampal lesion and control group in the first 10 trials of each session (reward locations constant) in the 15 post-surgery sessions (for clarity the data are presented in three-session blocks). The dashed line indicates the chance performance level. (B) The mean (±SEM) number of correct choices made in the second 10 trials of the same sessions following the reversal in reward location in the same session blocks. (C) The mean (±SEM) number of correct choices made in the first 20 trials of each session (reward locations constant) by the hippocampal lesion and control group in the three 40-trial testing sessions. (D) The mean (±SEM) number of correct choices made in the second 20 trials of the same sessions following the reversal in reward location. (E) Average performance of the hippocampal lesion and control groups on each trial. Each data point is the mean percentage of correct choices (±SEM) for a given trial across the 15 post-surgery testing sessions.

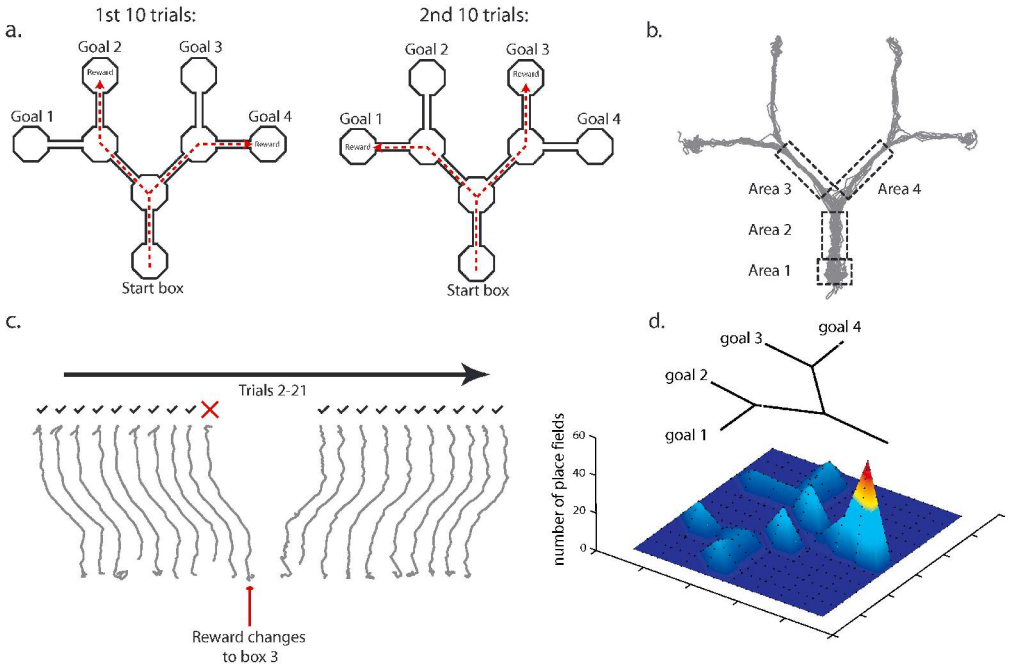
Figure 8. Performance on the alternation task. The mean (±SEM) number of correct choices (out of 10) in the 21 training sessions for both the hippocampal lesion and control groups are plotted. For clarity, the data are presented in blocks of three sessions. The dashed line is the number of correct responses expected by chance.

Table 1. Cluster quality measures

	Total	Good (id>30, l-ratio<0.1)	Intermediate (id>20, l-ratio<0.15)	Poor (id<20, l-ratio>0.1)
# Cells	139	110	26	3
Average i.d.	68.28	81.62	25.65	15.02
Average l-ratio	0.062	0.045	0.106	0.213
Goal-sensitive cells	48	39	9	0
Proportion goal sensitive	34.53	35.35	34.64	0

Table 2. Percentage of tissue loss within the hippocampus

Subject	% dorsal lesion	% total lesion
7	64.4	54.7
5	61.9	45.6
2	60.2	52.7
6	56.2	42.6
10	55.4	37.5
1	46.2	41.4
average:	57.4	45.8



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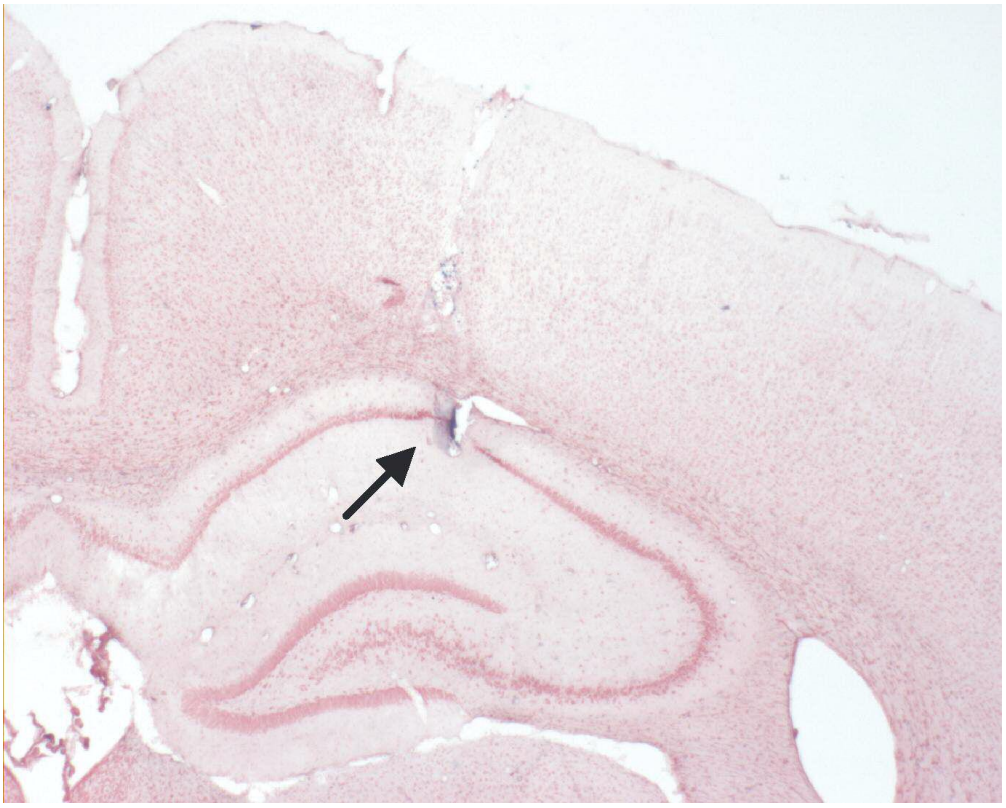

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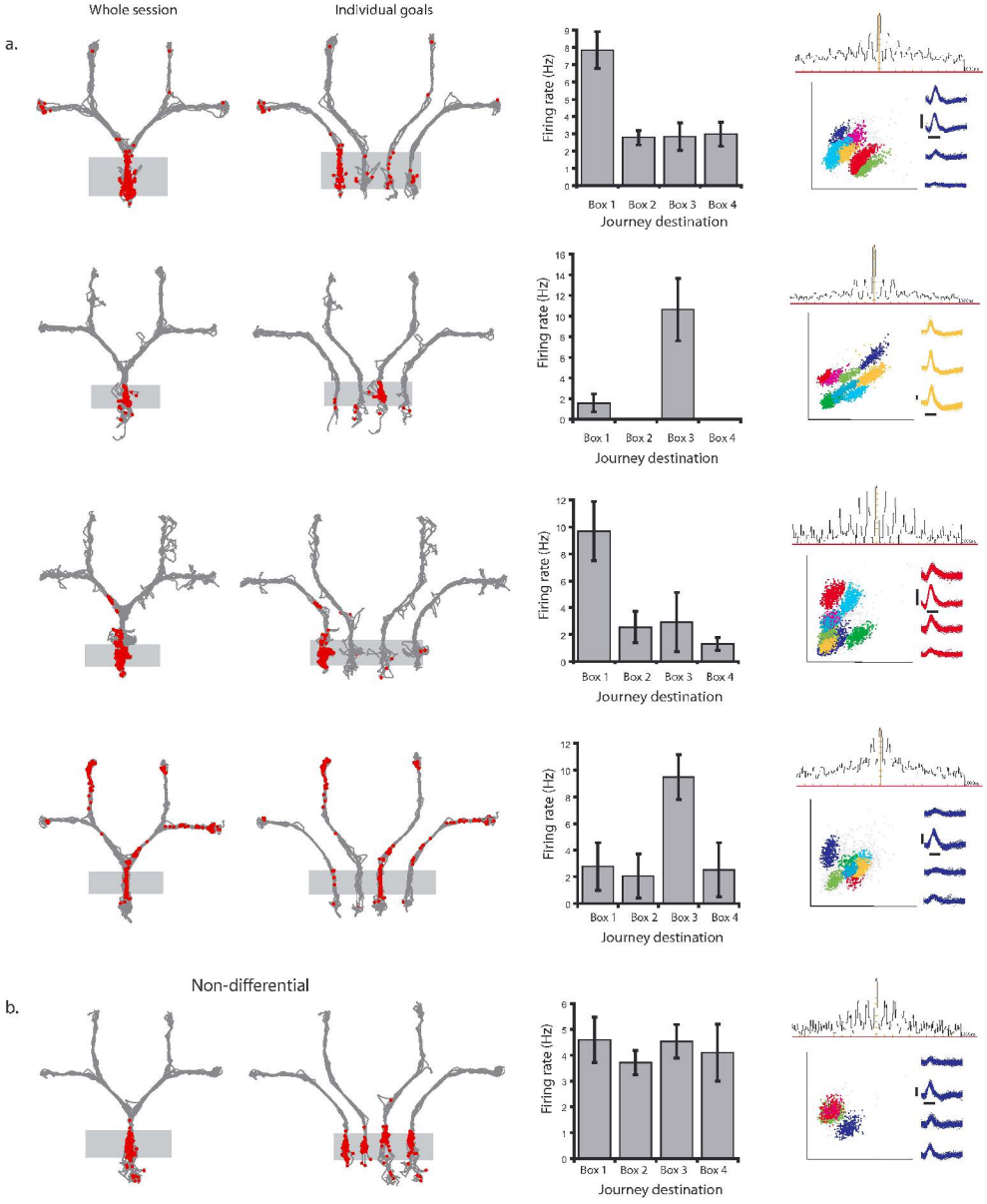

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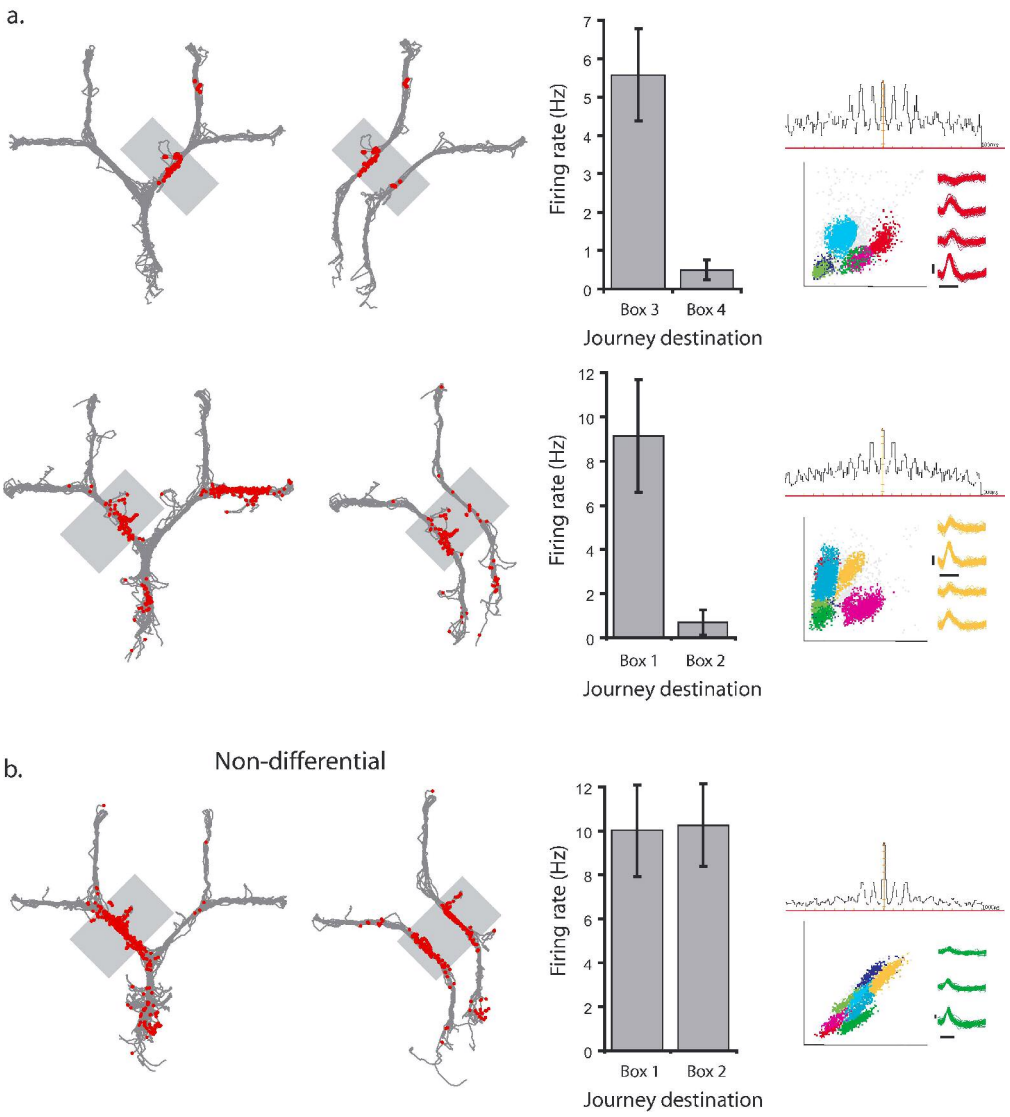
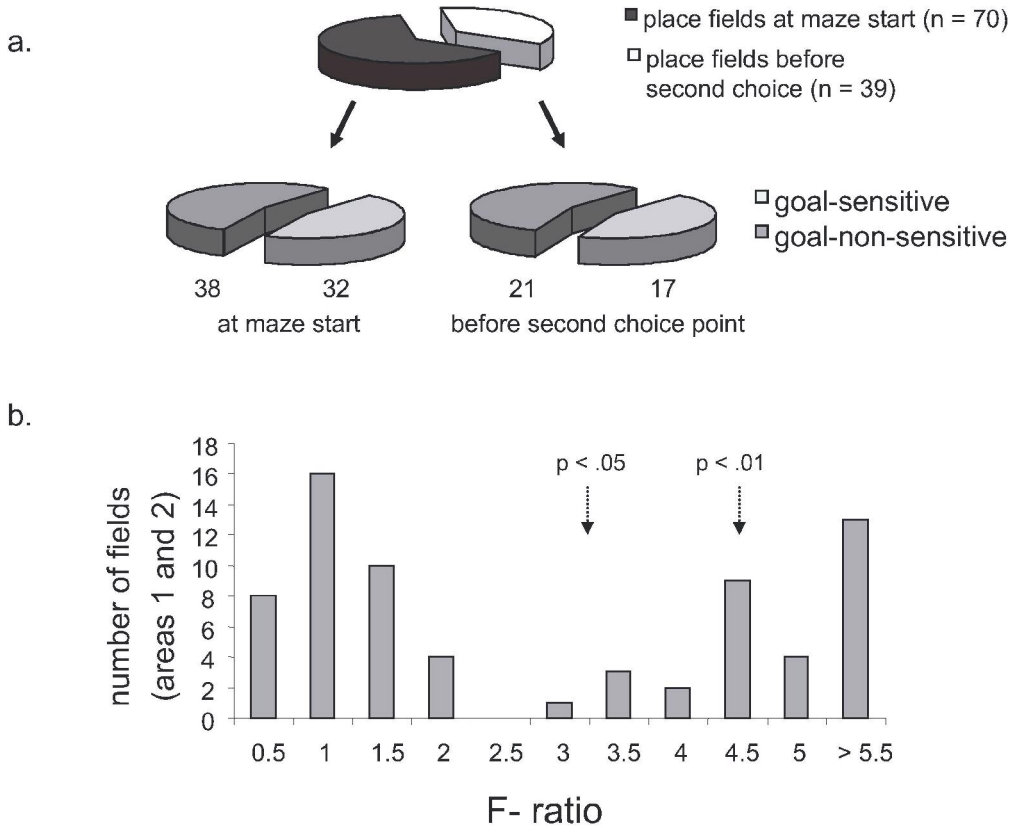


162x181mm (600 x 600 DPI)



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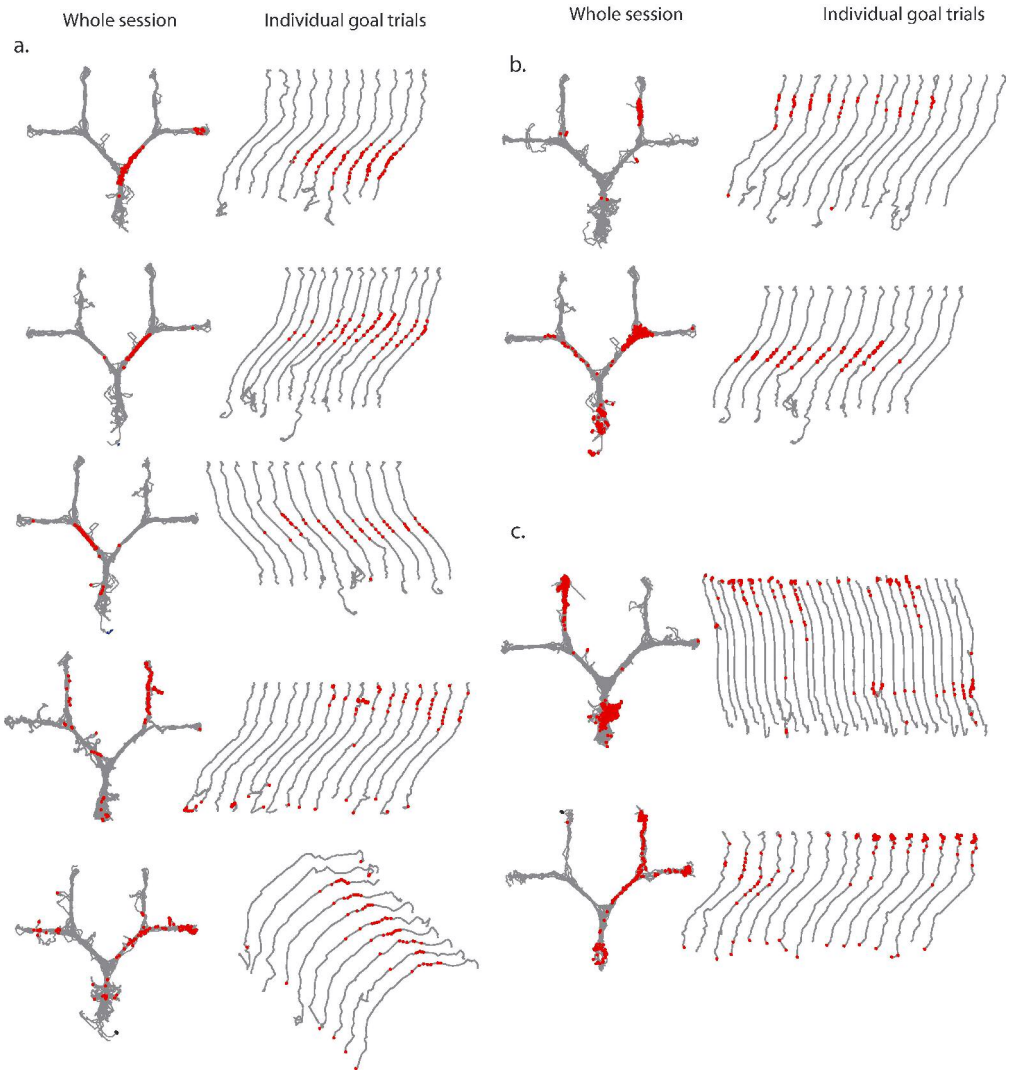
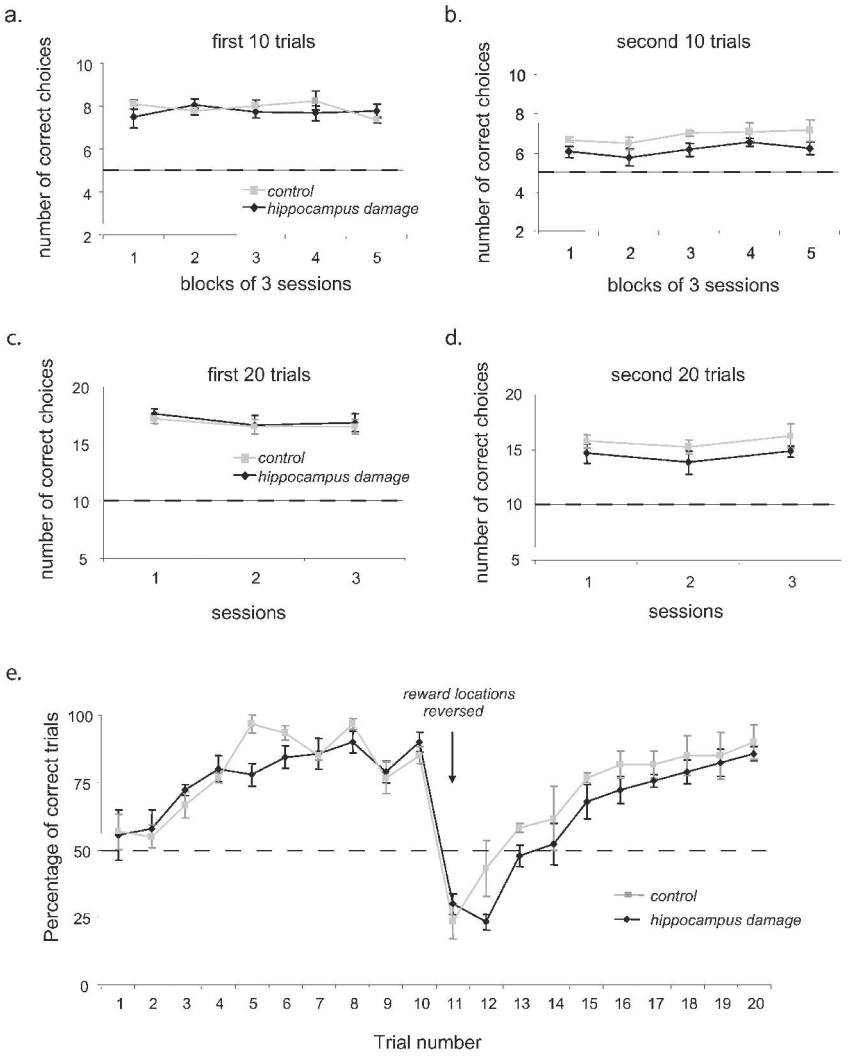
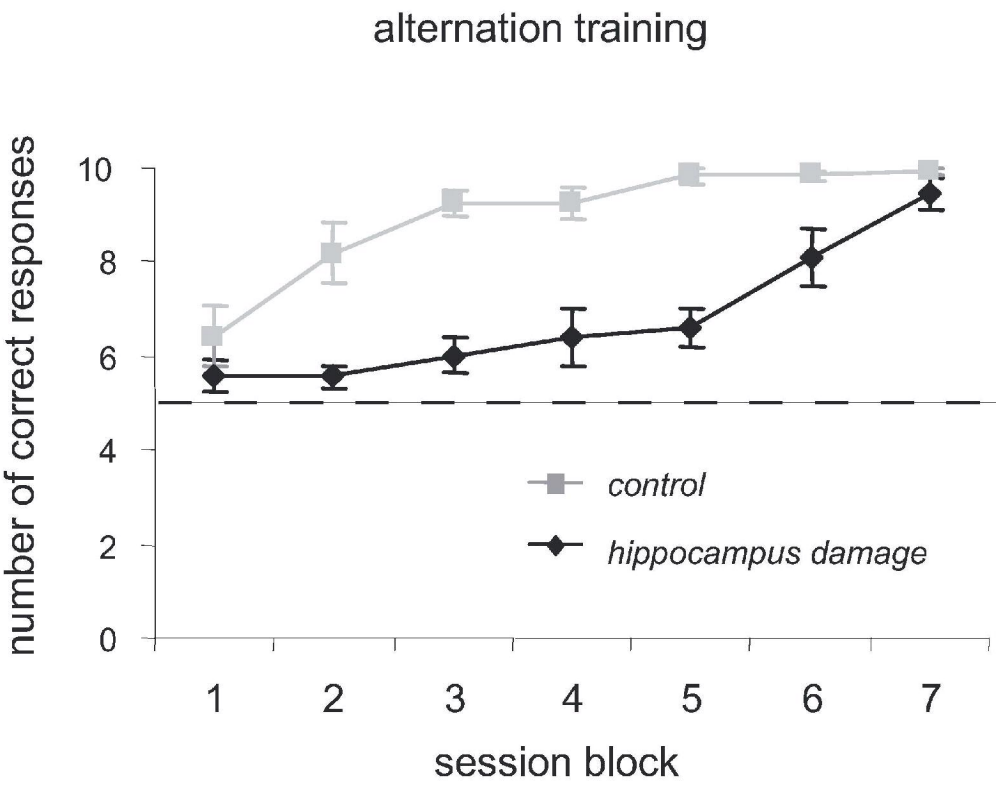


173x185mm (600 x 600 DPI)



182x192mm (600 x 600 DPI)



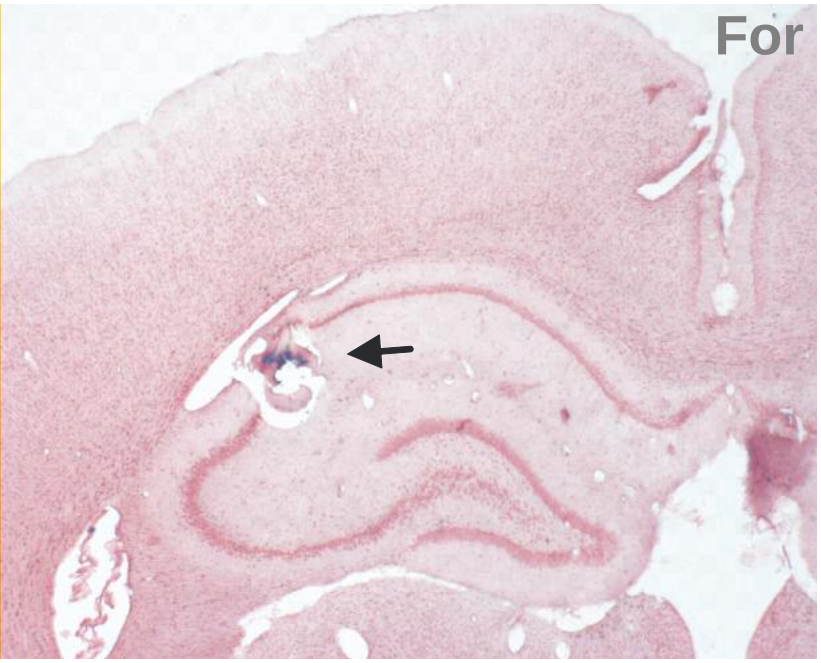
85x67mm (600 x 600 DPI)

Supplementary Figure legends

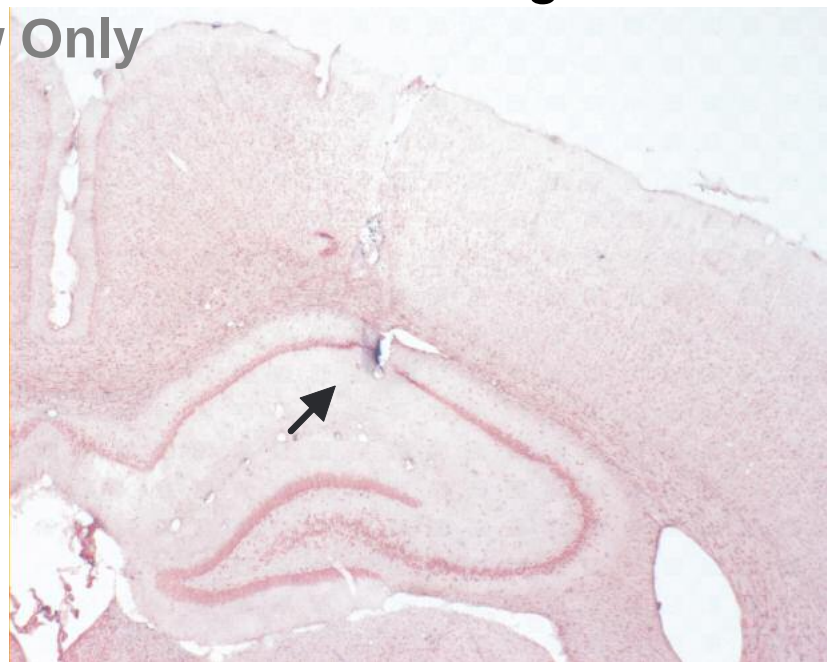
Supplementary Figure 1 Electrode placements for rats in Experiment 1. The arrow indicates the electrode tips.

Supplementary Figure 2. Examples of the three patterns of firing rate changes between intended destinations yielding a statistically significant effect. The most prevalent pattern was a high firing rate on journeys to one goal box, and low rates for journeys to the remaining three goal boxes. The example shown in the left is cell 1 from Supplementary Table 1. For 25 of the 32 significant cells (cells 1-25 in the Supplementary Table 1), the firing rate on the highest rate journey (1^{st}) was more than twice the next highest (the 2^{nd}) rate. 6 of the 32 cells (cells 26-31 in the table) showed less than 2-fold differences in firing rates between the highest firing rate journey and the next highest rate journey, and cell 31 (middle plot) is perhaps the best example of a cell with relatively high firing rates on two journeys. However, even for these cells, there was a decrease in firing rates (29% on average) between the highest rate and next highest rate journey. Only 1 of the 32 significant cells appeared to have a high firing rate on 3 journeys relative and a lower rate on the remaining journey (cell 32; right plot).

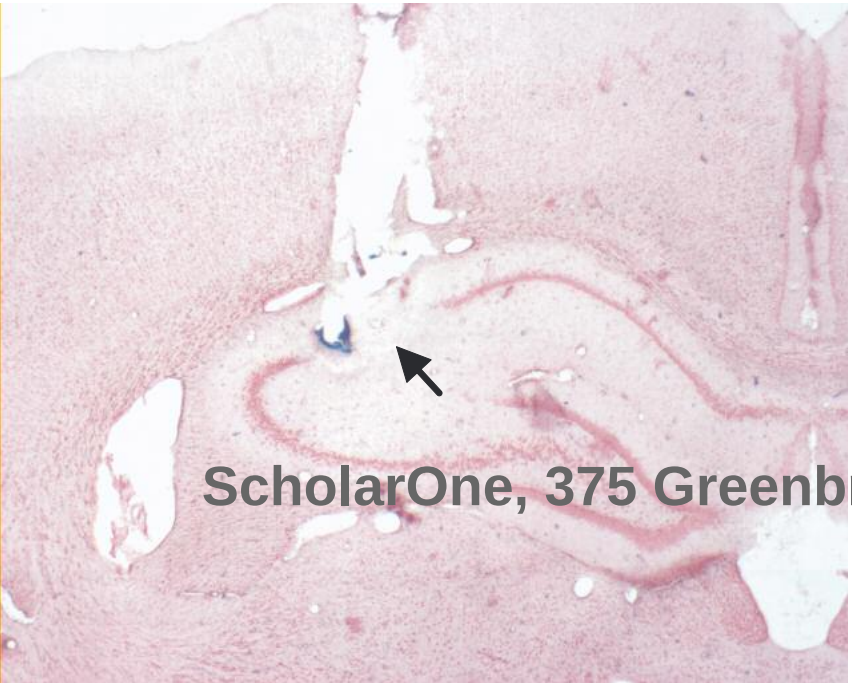
J10



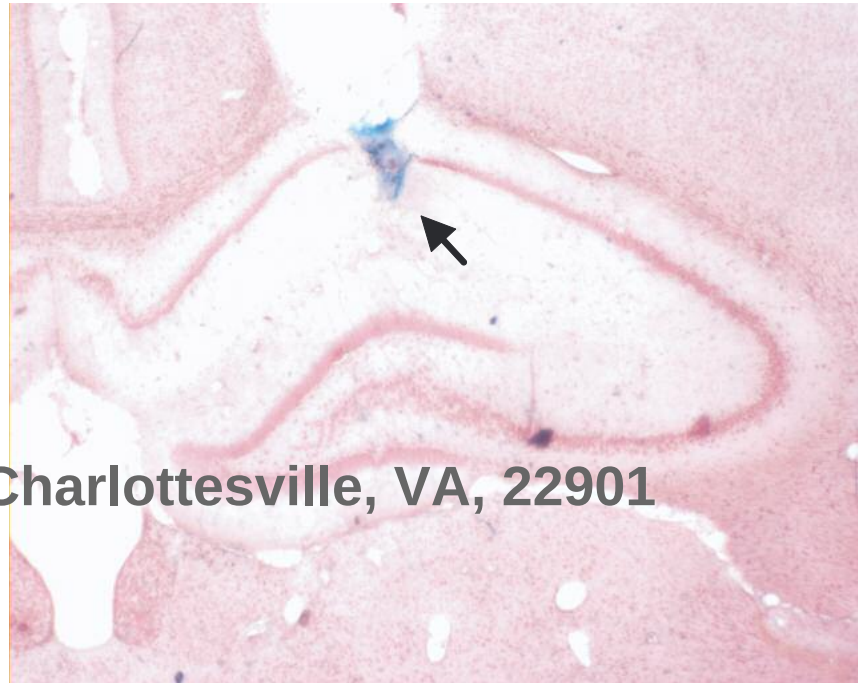
J12

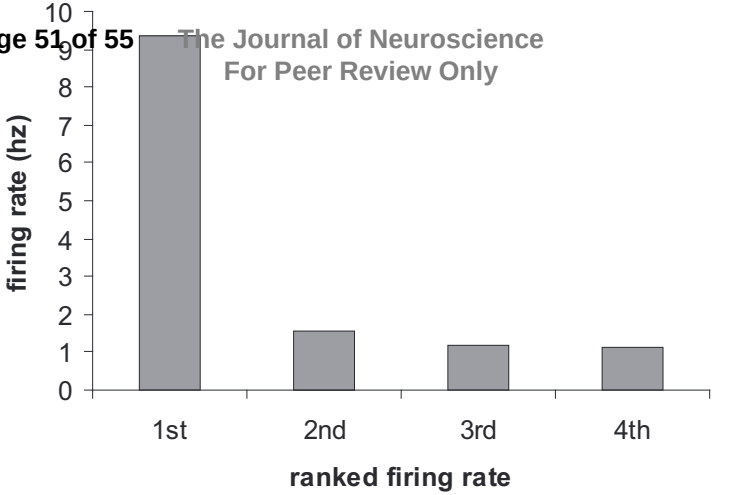


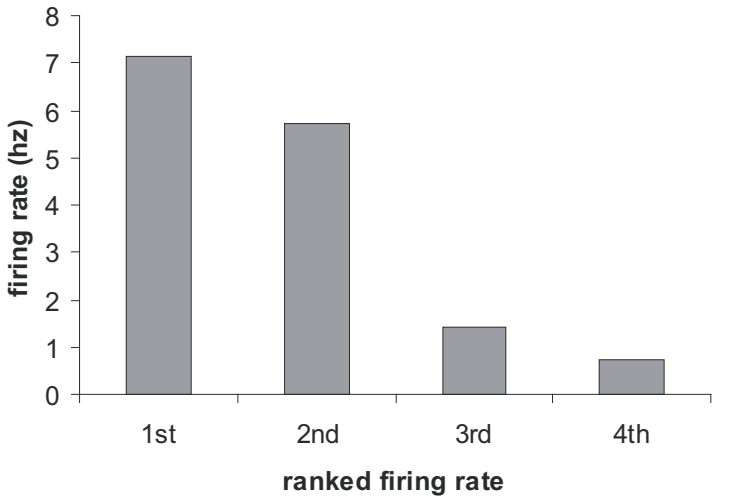
J13

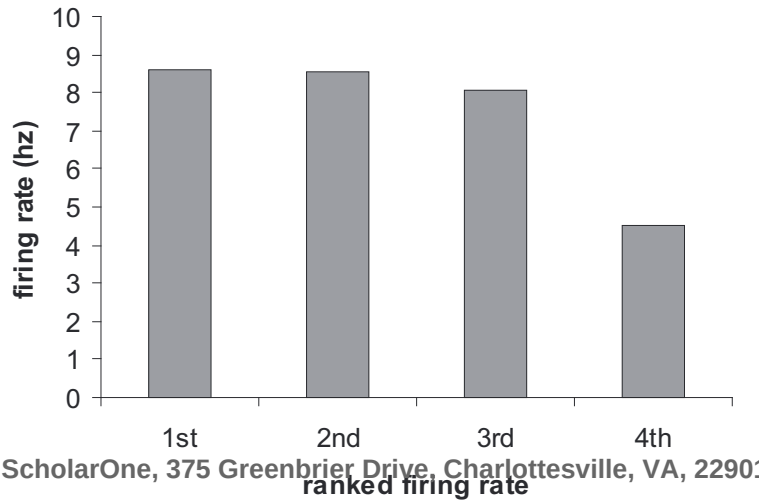


J14









Supplementary Table 1. Mean firing rates for each cell in areas 1 and 2 that had a significant difference in firing rates as a function of intended goal. For ease of comparison, the rates associated with the four goal box destinations are ranked from highest rate (1^{st}) to lowest (4^{th}). Cells are ranked by the ratio of difference between the 1^{st} and 2^{nd} highest firing rates (($1^{st} - 2^{nd}$)/($1^{st} + 2^{nd}$)). The significance of the ANOVA comparing the four destinations is shown in the "p <" column. The most common pattern of firing was a relatively high rate for one journey type, and relatively low rates for the other journeys.

cell	1st	2nd	3rd	4th	p <
1	1.31	0.07	0	0	0.0001
2	9.26	1.23	0.82	0.24	0.0001
3	6.87	1.01	0.94	0.39	0.0001
4	9.55	1.57	0	0	0.0001
5	9.34	1.57	1.16	1.11	0.008
6	7.29	1.36	0.94	0.4	0.0001
7	4.46	0.87	0.65	0.28	0.024
8	0.8	0.16	0.11	0	0.007
9	6.86	1.39	1.08	0.48	0.012
10	3.04	0.73	0.24	0.24	0.001
11	11	2.84	0.89	0.88	0.005
12	13.9	3.89	2.16	1.85	0.015
13	5.7	1.79	1.73	1.06	0.007
14	2.93	0.95	0.62	0.55	0.022
15	11.01	3.94	1.67	1.62	0.043
16	7.88	2.93	2.85	2.23	0.005
17	29	10.83	10.2	3.7	0.045
18	12.21	4.69	4.65	3.52	0.0001
19	1.34	0.53	0.28	0	0.006
20	23.85	9.54	7.26	5.1	0.031
21	11.26	4.73	3.88	3.78	0.001
22	5.49	2.33	2.04	1.52	0.005
23	8.57	3.83	1.09	0.9	0.0001
24	5.8	2.63	2.14	0.87	0.001
25	6.28	3.06	0.78	0.5	0.014
26	5.63	2.9	2.03	0.79	0.013
27	14.3	7.42	6.44	4.82	0.018
28	10.36	6.3	3.86	3.1	0.015
29	7.84	4.82	4.56	2.79	0.009

30	4.3	3.35	2.1	0.37	0.028
31	7.16	5.73	1.42	0.72	0.017
32	8.62	8.57	8.04	4.54	0.013

Supplementary Table 2. Distribution of place fields in cells with multiple fields. The presence of a place field is indicated with an "x". A boldface "x" is a goal-sensitive field.

	start area	left turn	right turn	goal 1	goal 2	goal 3	goal 4
1	**x**			x			
2	x			x			
3	x			x			
4	x			x			
5	x				x		
6	**x**				x		
7	x				x		
8	**x***				x		
9	x					x	
10	x					x	
11	x					x	
12	x						x
13	x			x			x
14	x				x	x	
15	**x**				x		x
16	x				x		x
17	x					x	x
18	x					x	x
19	x			x	x		x
20	**x**	**x**					
21	x	x					
22	x	x					
23	x	x					
24	x		x				
25	x		x				
26	x	x	x				
27		**x***		x			
28		**x**		x			
29		**x**			x		
30		x				x	
31		x					x
32		x			x		x
33			**x**	x			
34			x			x	

35			x				x
36			**x**		x	x	
37			x	x		x	
38				x			x
39						x	x
40	x		x	x	x		
41	x		x	x	x		
42	x	x				x	
43	x		x		x		
44	**x**	x			x		
45	x		x	x			x