

# The global record of memory in hippocampal neuronal activity

Emma R. Wood, Paul A. Dudchenko & Howard Eichenbaum

Laboratory of Cognitive Neurobiology, Department of Psychology, Boston University, 64 Cummington Street, Boston, Massachusetts 02215, USA

In humans the hippocampal region of the brain is crucial for declarative<sup>1</sup> or episodic<sup>2</sup> memory for a broad range of materials. In contrast, there has been controversy over whether the hippocampus mediates a similarly general memory function in other species, or whether it is dedicated to spatial memory processing<sup>3–6</sup>. Evidence for the spatial view is derived principally from the observations of ‘place cells’—hippocampal neurons that fire whenever the animal is in a particular location in its environment<sup>7,8</sup>, or when it perceives a specific stimulus or performs a specific behaviour in a particular place<sup>3,4</sup>. We trained rats to perform the same recognition memory task in several distinct locations in a rich spatial environment and found that the activity of many hippocampal neurons was related consistently to perceptual, behavioural or cognitive events, regardless of the location where these events occurred. These results indicate that non-spatial events are fundamental elements of hippocampal representation, and support the view that, across species, the hippocampus has a broad role in information processing associated with memory.

Rats were trained to perform an odour-guided, continuous, non-matching-to-sample task at nine different locations on an open platform (Fig. 1). On each trial a cup containing sand scented with one of nine spices (for example, thyme, paprika) was placed on the platform. On half of the trials the scent was different from that of the previous trial (a ‘non-match’) and a cereal reward was buried at the bottom of the cup. On the remaining trials the scent was repeated (a ‘match’) and the cup was not baited. Rats approached each cup, sniffed the sand, and then either dug for the reward or turned away. After either response, the cup was removed and there was a 10-s interval before the next trial, during which the rat remained on the platform. The location of the cup changed on each trial and did not predict whether the trial was a non-match or a match. The cup

Table 1 Cells with significant task-related correlates

Nonspatial variables		Spatial variables	
Approach	26	Position	14
Odour	10	Position and Odour	4
M/NM*	13	Position and M/NM*	18
Odour and M/NM*	2	Position and Odour and M/NM*	4

The activity of 91 cells (out of 127) was statistically associated with the variables tested. \* M/NM, match/non-match trial type.

location, sequence of odours and match/non-match contingencies followed a pseudo-random order, such that each session comprised 108 trials, with 12 presentations of each odour and each cup location. This design enabled us to dissociate hippocampal neural activity associated with approaching the stimulus cups, the different odours, and the match and non-match trial types from the locations where these events occurred.

After being trained to dig in sand, rats learned within three testing sessions to perform the differential response that involved either digging through the sand to find the buried reward on non-match trials, or turning away without digging on the match trials (a range of 100–280 trials was required to perform correct responses on 90% of trials over 20 consecutive trials). They continued to perform the task consistently during the recording sessions (mean 96.8% correct (range 88–100%) for each 108-trial session). On all of several non-match probe trials in which the reward was omitted, each rat dug appropriately, confirming that their accurate performance was not based on detecting the food directly.

The activity of 127 single units identified as complex spike cells<sup>9</sup> was recorded from the CA1 and CA3 pyramidal cell fields of four rats performing the non-matching-to-sample task. The activity of 91 cells was statistically associated with one or more of the variables tested, and over half of these were associated only with non-spatial variables (Table 1). We identified ten cells that fired differentially across the different odour stimuli, but not across the match/non-match trial types or across the different locations where the trials were performed. For example, the cell depicted in Fig. 2a responded to odour 5 more than to any other odour, and was not influenced by trial type or cup location. To examine the robustness of the discrimination among odours for all cells of this category, we normalized their firing rates and compared average responses to the best two odours (those associated with the highest firing rates)

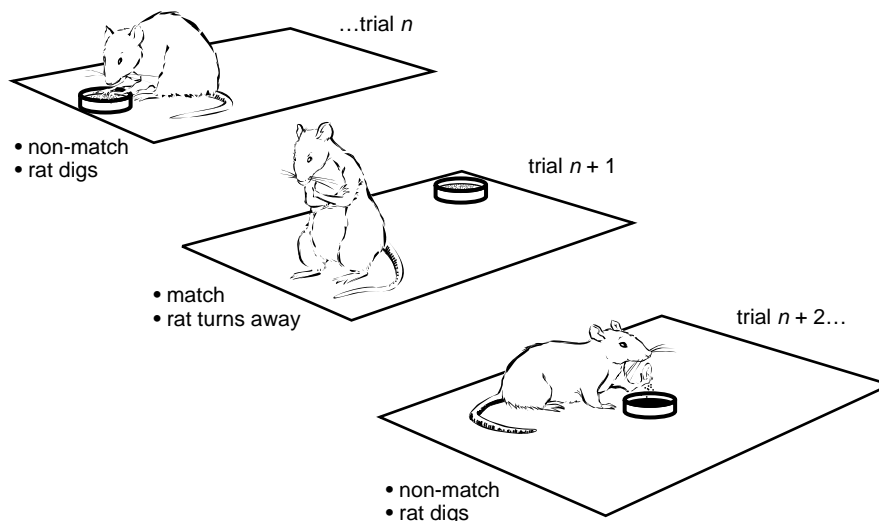


Figure 1 Continuous non-matching to sample task. Trial  $n$  represents a non-match trial where the odour differs from that presented on the previous trial, and the rat digs to find a buried reward. On the next trial ( $n + 1$ ), the same scent is repeated, although in a different location. As no reward is available, animals learn

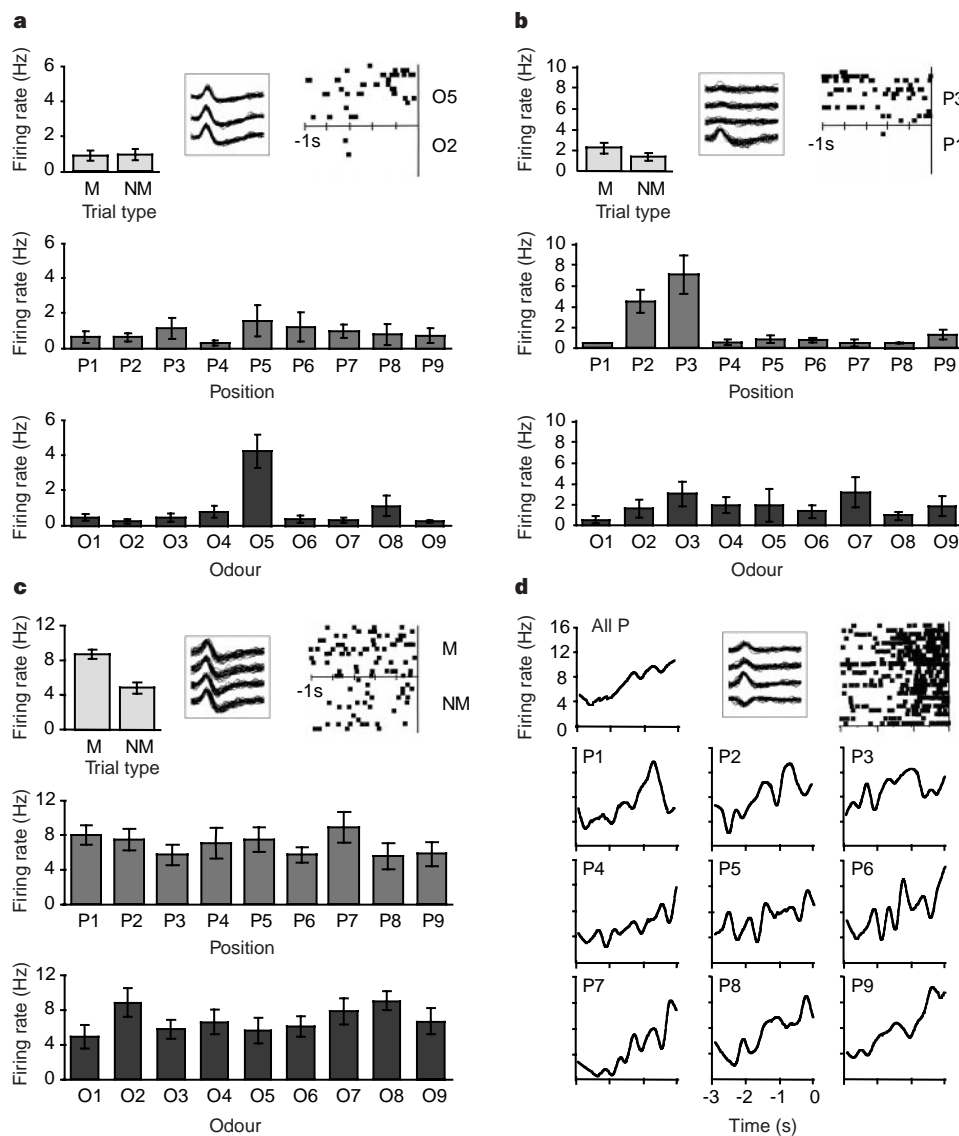
not to dig on these match trials and to turn away from the cup. On the subsequent trial ( $n + 2$ ), the odour again differs from that of the previous trial and the animal digs for a buried reward. Note that the position of the cup is independent of the match/non-match contingency.

with responses to the worst two odours (lowest firing rates). Odour cells strongly differentiated best and worst odours (Fig. 3a, left panel). To examine whether odour-selective activity was present across the different locations, we compared the normalized responses among positions rank-ordered for each cell according to the magnitude of the overall firing rate associated with each position (see Methods). Although the magnitude of the odour specificity declined across the rank-ordered positions, odour cells discriminated best and worst odours at all positions (Fig. 3a, right panel).

We also identified 14 cells that fired differentially across the different positions of the trial, but not across the different odours or match/non-match trial types. For example, the cell depicted in Fig. 2b fired at higher rates when the rat performed trials at positions P2 and P3 than at other positions, but was not influenced

by the trial type or odour. As a group, location cells strongly discriminated their two best positions (those associated with their highest firing rates) from their two worst positions (Fig. 3b, left panel). Furthermore, although the magnitude of location specificity declined across the rank-ordered odour series, the ability of location cells to discriminate positions was found across the whole odour set (Fig. 3b, right). Notably, the magnitude of the normalized firing rates and of the discrimination between best and worst conditions was similar for odour cells and location cells (compare panels in Fig. 3a, b), indicating that the robustness of odour responses is as great as that for location responses within these analyses.

In addition, 13 cells fired differentially between match and non-match trials, but not across the different odours or locations. For example, the cell depicted in Fig. 2c had a greater firing rate on match trials than on non-match trials, regardless of the odour or



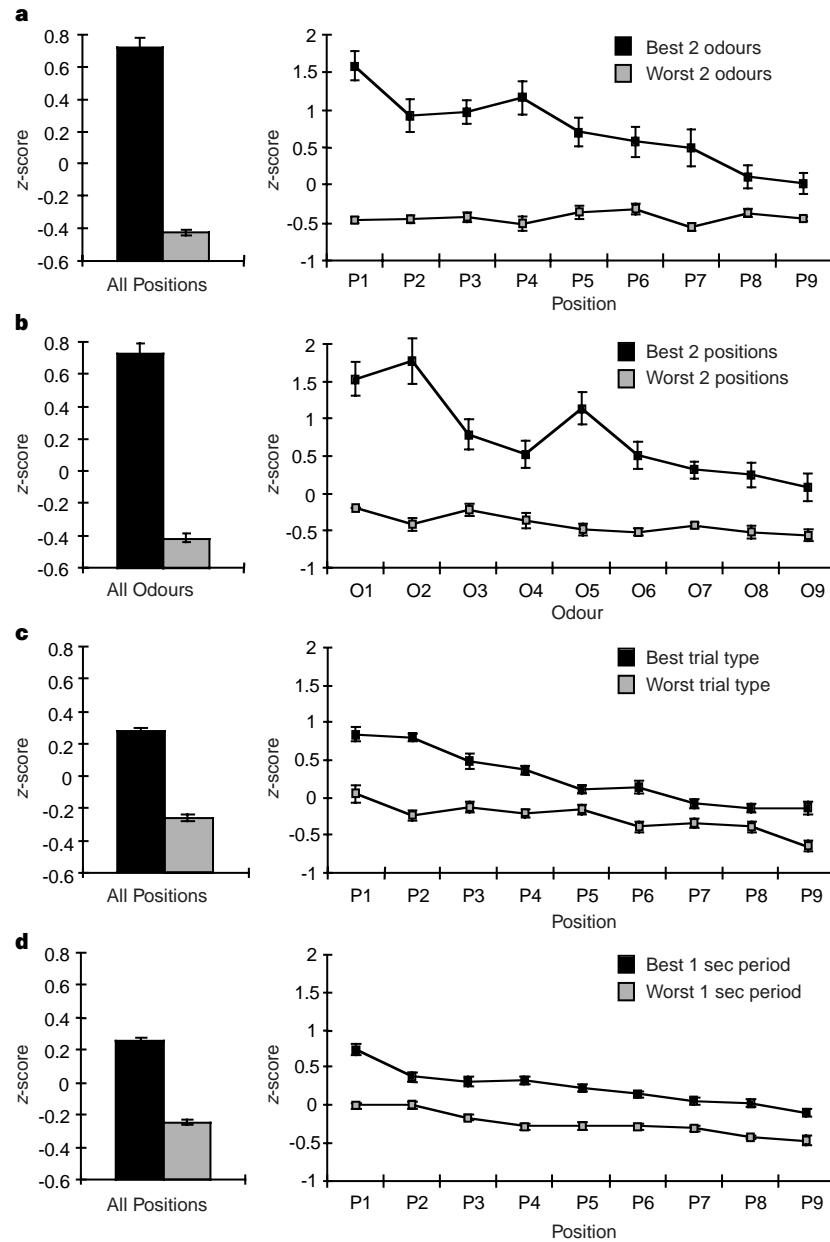
**Figure 2** Examples of task-related firing patterns of hippocampal neurons. **a-c**, Firing rate in 1-s analysis period for each trial type (M, match; NM, non-match), cup location (P1-P9) and odour (O1-O9) for: **a**, an odour cell (odour,  $F_{(8,74)} = 8.59$ ,  $P < 0.0001$ ; trial type,  $F_{(1,74)} = 0.04$ , not significant (NS); cup location  $F_{(8,74)} = 1.03$ , NS; odour  $\times$  trial type,  $F_{(8,74)} = 0.75$ , NS; location  $\times$  trial type,  $F_{(8,74)} = 1.74$ , NS); **b**, a location cell (cup location,  $F_{(8,74)} = 8.60$ ;  $P < 0.0001$ ; odour,  $F_{(8,74)} = 0.84$ , NS; trial type  $F_{(1,74)} = 2.76$ , NS; odour  $\times$  trial type,  $F_{(8,74)} = 1.14$ , NS; cup location  $\times$  trial type,  $F_{(8,74)} = 1.58$ , NS); and **c**, a match cell (trial type,  $F_{(1,74)} = 22.95$ ,  $P < 0.0001$ ; odour,  $F_{(8,74)} = 1.42$ , NS; location,  $F_{(8,74)} = 1.17$ , NS; odour  $\times$  trial type,  $F_{(8,74)} = 0.68$ ,

NS; location  $\times$  trial type,  $F_{(8,74)} = 1.20$ , NS). **d**, Firing rates (200-ms bins) for 3-s period when the rat approached each cup position (P1-P9), and averaged across all positions (All P) for an approach cell (trial period,  $t_{(1,107)} = 10.77$ ,  $P < 0.001$ ; trial type,  $F_{(1,74)} = 0.06$ , NS; odour  $F_{(8,74)} = 0.47$ , NS; location  $F_{(8,74)} = 1.42$ , NS; odour  $\times$  trial type,  $F_{(8,74)} = 0.96$ , NS; location  $\times$  trial type,  $F_{(8,74)} = 1.00$ , NS). Each panel also shows the waveform of the cell recorded on each tetrode channel and a raster display of firing patterns time-locked to the end of the odour sample period.

location where the trial was performed. Match/non-match cells as a group strongly discriminated the two trial types (Fig. 3c, left panel), and this effect was observed at all rank-ordered positions (Fig. 3c, right panel).

The firing rate of a further 26 cells changed significantly during the approach or arrival at the stimulus cup, but the cells did not fire differentially across the different odours, trial types, or trial locations. The cell depicted in Fig. 2d increased its firing rate as the rat arrived at each cup, regardless of any other variables. As a group, approach cells strongly differentiated the initiation of the trial and arrival at the stimulus cup (Fig. 3d, left panel), and this effect was observed at all rank-ordered positions (Fig. 3d, right panel).

Of the 127 cells analysed (Table 1), 51 (40.2%) had solely non-spatial correlates, which is reflected in a statistically significant main effect for odour, trial type or approach in the absence of a significant main effect or interaction involving cup position. Nearly all of these cells encoded just one of these non-spatial properties. A further 40 cells (31.5%) had a spatial correlate, reflected in a statistically significant main effect or interaction involving cup location. Only 14 of the spatial cells were pure location cells. The remaining 26 spatial cells, as well as two non-spatial cells, encoded combinations of multiple variables. The activity of the remaining 36 cells (28.3%) was not significantly affected by any of the variables measured. This overall pattern of results indicates that non-spatial and spatial



**Figure 3** Different groups of hippocampal neurons discriminate odours, positions, match/non-match trial types and approach period. **a**, Odour cells ( $n = 10$ ); **b**, location cells ( $n = 14$ ); **c**, Match/non-match cells ( $n = 13$ ); **d**, approach cells ( $n = 26$ ). Left: comparison of mean ( $\pm$ s.e.) normalized firing rates to **a**, the best and worst two odours ( $t_{(1,9)} = 19.75, P < 0.0001$ ); **b**, the best and worst two positions ( $t_{(1,13)} = 14.37, P < 0.0001$ ); **c**, the best and worst trial type ( $t_{(1,12)} = 14.34, P < 0.0001$ ); and **d**, the best and worst approach period ( $t_{(1,25)} = 13.89, P < 0.0001$ ) for all cells in that category. Right: normalized responses compared across the rank-ordered positions (P1–P9; **a, c, d**) or rank-ordered odours (O1–O9; **b**). **a**, odour,

$F_{(1,72)} = 527.43, P < 0.0001$ ; position,  $F_{(8,72)} = 3.92, P < 0.01$ ; odour  $\times$  position,  $F_{(8,72)} = 3.64, P < 0.05$ ; odour effect at each position,  $P < 0.05$ ; **b**, position,  $F_{(1,104)} = 231.57, P < 0.0001$ ; odour,  $F_{(8,104)} = 7.67, P < 0.0001$ ; position  $\times$  odour,  $F_{(8,104)} = 3.91, P < 0.01$ ; position effect for each odour,  $P < 0.05$ ; **c**, trial type,  $F_{(1,96)} = 205.71, P < 0.0001$ ; position,  $F_{(8,96)} = 139.44, P < 0.0001$ ; trial type  $\times$  position,  $F_{(8,96)} = 3.26, P < 0.05$ ; trial type effect at each position,  $P < 0.05$ ; **d**, trial period,  $F_{(1,200)} = 192.95, P < 0.0001$ ; position,  $F_{(8,200)} = 136.88, P < 0.0001$ ; trial period  $\times$  position,  $F_{(8,144)} = 1.54, NS$ ; trial periods effect at each position,  $P < 0.05$ .

information are both fundamental components of hippocampal representations.

Previous studies have associated hippocampal neural activity with a variety of specific non-spatial stimuli and behaviours in animals performing various tasks<sup>10–20</sup>. In most of these studies the events occurred at just one place, raising the possibility that the activity was location specific, or that the non-spatial events were defining features of the locations where they occurred<sup>10,11,13–19</sup>. Other studies presented the same stimuli in multiple locations, but these stimuli were enclosures, landmarks or walls, which might have been perceived as distinctive spatial reference frames within the overall environment<sup>12,20–23</sup>. In our study, punctate stimuli were transferred to and from the environment while the rat was present, emphasizing interactions with them as episodes occurring within a single, constant spatial reference frame. These results provide compelling evidence that hippocampal neurons represent information related to specific perceptual, cognitive and behavioural events not tied to a particular spatial location. Furthermore, the range of specificity varied from many cells that encode single, frequently experienced events, such as a place or an odour, to a few cells encoding unique conjunctions that reflect a rare event, such as a specific odour appearing as a 'match' in a particular place. Such a range of coding specificity could reflect the emergence of consistencies within a global knowledge structure from a record of specific episodes<sup>24,25</sup>. □

## Methods

**Behavioural apparatus.** Rats were tested on a square, black, plexiglass platform (92 cm × 92 cm) with 2-cm high walls along each side, elevated 70 cm above the floor. The stimuli were plastic cups (12 cm diameter and 4.5 cm deep) containing 100 g of sand scented with 1 g of a common spice such as cinnamon or cumin. The stimuli could be placed in any one of nine locations: in the four corners, half way along each side, or in the centre of the platform. The platform was located at one end of a large testing room, amid a rich array of constant distal spatial cues. See text for behavioural testing methods.

**Electrophysiological recording.** Electrophysiological recording techniques were as described previously<sup>26</sup>. Briefly, four rats were surgically implanted with two or four moveable tetrodes just above the dorsal hippocampus (3.5 mm posterior and 2.3 mm lateral to bregma). Unit activity and the rat's location were recorded while rats were run on the odour-guided non-matching-to-sample task. Single units were isolated after the recording session by identifying clusters defined on the basis of waveform parameters (Autocut, Datawave Technologies). The recordings were considered stable if the clusters remained within the same fixed boundaries throughout the recording session (see example waveforms in Fig. 2). Cells with a signal-to-noise ratio of more than 3:1, a spike duration (peak time to valley time) of greater than 250 ms, a mean firing rate (total number of spikes divided by total session time) of less than 2.5 Hz, and a minimum spike count of 100 over the recording session were defined as complex spike cells. Electrode placements in CA1 and CA3 subfields were verified histologically.

**Analysis of cellular activity.** To assess whether each cell's activity was associated with odour, cup location or match/non-match trial type, we examined the last second of each trial when the rat arrived at the cup and sampled the scented sand, immediately before it either dug or turned away. Activity during this period was subjected to a three-way analysis of variance (ANOVA), which analysed the firing rates across the nine different odours, the nine cup locations and the two trial types (match and non-match), and the two-way interactions between odour and trial type, and between cup location and trial type. Cells with one significant main effect ( $P < 0.05$ ) in the absence of any other main effects or interactions were categorized as odour, location or match/non-match cells accordingly. To assess changes in activity associated with approach to the cup, we compared (by using paired  $t$ -tests) the firing rate during the 1-s period beginning 3 s before the behavioural response, when the animal initiates its approach to the cup, with that during the 1 s immediately before the behavioural response. Cells with a significant ( $P < 0.05$ )  $t$ -value, and with no significant main effects or interactions in the three-way ANOVA, were classified as 'approach cells'.

To assess the robustness of coding for cells in each category (odour, location,

match/non-match and approach), we first normalized their responses by converting the firing rates during the critical 1-s periods for each trial to  $z$ -scores relative to the same periods for all trials. We then compared (using paired  $t$ -tests) the responses to the best with the worst relevant condition for each category of cells (best versus worst two odours or two positions, best versus worst match/non-match trial type, or best versus worst 1-s period during the approach). In addition, to determine whether odour, trial-type and approach cells had selective responses across all positions, we first rank-ordered the positions for each cell from that associated with the highest to the lowest firing rate during the 1-s trial period (averaged across 12 trials at each position). Then, for each cell type, we used a two-way ANOVA to compare the normalized responses to the best and worst conditions (as above) across the nine positions. This strategy necessarily results in an orderly decline in firing rates across the rank-ordered position series. Nevertheless, significant discrimination at all positions by odour, trial type or approach cells could be identified as a significant main effect and significant post-hoc effects for that variable at all positions (paired one-tailed  $t$ -tests). Using the same strategy, we also compared the normalized responses to the best and worst two positions for location cells across the nine rank-ordered odours. As with the other analyses, this necessarily results in an orderly decline of firing rates across the rank-ordered odour series. Nevertheless, this analysis allowed us to compare the consistency of the location responses to the responses for the other cell types.

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Correspondence and requests for materials should be addressed to H.E. (e-mail: hbe@bu.edu).