Chapter 2
How Does the Hippocampus Support the Spatial and Temporal Attributes of Memory?

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In 1987, Kesner and DiMattia proposed that progress toward our understanding of memory could be improved by fragmenting memory into attributes that characterize the structural organization of memory, including space, sensory-perception, time, response, and affect. They assigned to the hippocampus a key role in the organization of memories in both space and time, and later, Kesner (1990) proposed that "the interaction between spatial and temporal attributes can provide an external context for situations." In support of this proposal, Kesner cited existing models of the hippocampus as involved in a spatial mapping of contexts (O'Keefe and Nadel 1978) and as forming a representation of temporal context (Rawlins 1985; see also Olton 1986). At that time there was compelling evidence of hippocampal neuronal activity that signaled spatial representations—place cells—and many studies, including key experiments by Kesner and his colleagues, had demonstrated critical hippocampal involvement in spatial memory. Furthermore, Kesner argued that the hippocampus is essential in supporting the temporal attributes of memory, showing that hippocampal lesions impair memory for the order of arms visited in a radial arm maze (Kesner and Novak 1982).

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One could argue that memory within the radial maze task has essential spatial as well as temporal attributes, thus confounding a demand for spatial memory with that of temporal organization. But, many additional experimental studies by Kesner and his colleagues have shown that the hippocampus is also required in a variety of tasks that contain a memory delay and in memory for the order of nonspatial stimuli (reviewed in Kesner and Hunsaker 2010). Perhaps most compelling were experiments that examined whether rats could remember unique sequences of odors, and compared their ability to remember temporal order with that for odor discrimination (Kesner et al. 2002) and for recognition of the odor stimuli that had appeared within the list (Fortin et al. 2002). In the tests of memory for order, rats initially were rewarded for sampling each of a list of five odors. A few minutes later, on the order test, they were presented two nonadjacent odors from the list and were required to choose the less recently experienced odor to obtain another reward. Rats performed well above chance on temporal order memory, and better when the lag between previously presented items was larger. Rats with selective hippocampal damage were impaired in memory for temporal order at all lags, and performance was above chance only for the largest lag. By contrast, on tests of odor discrimination and on the recognition tests, rats with hippocampal damage performed as well as normal rats; and the selective impairment in order memory compared to intact item memory was striking even when overall accuracy in normal animals was matched between tasks.

These findings indicate that the hippocampus is essential in processing the temporal organization per se, independent of the memories for the items themselves, which was intact following hippocampal damage. There is a large literature on the ability of rats to time intervals, some of which indicate a role for the hippocampus in the perception of time and memory for duration (e.g., Meck et al. 1984; Jacobs et al. 2013; reviewed in MacDonald 2014). In addition, several other brain areas have been implicated in the capacity to time intervals, so it is likely that the hippocampus utilizes temporal information from many sources in supporting its role in the temporal organization of memories (Mauk and Buonomano 2004; Buhusi and Meck 2005; Yin and Troger 2011; MacDonald 2013).

Here we consider why and how the hippocampus is involved in both the spatial and temporal attributes of memory organization. One possibility is that these attributes are supported separately by anatomically distinct subfields within the hippocampus. Some of Kesner's work supports this idea. For example, in one particularly important study, Kesner et al. (2005) tested rats with selective CA1, CA3, or control lesions on a task in which animals were taught associations between an object and an odor that were separated by a 10 s delay; they called this the object–trace–odor association task. The animals learned that if object A was presented before the delay, then a cup of sand would contain a food reward if it was scented with odor 1 (but not with odor 2). Conversely, if object B was presented first, then a cup of sand would contain a food reward if it was scented with odor 2 (but not odor 1). Memory was measured by a briefer latency to approach the scented cup on rewarded pairings (A-1 and B-2) than on non-rewarded pairings (A-2 and B-1). In control rats, the latency to approach rewarded cups gradually decreased over daily training sessions

of 12 trials each. Rats with selective CA1 lesions showed no sign of acquiring the associations, even after extensive training, whereas rats with CA3 lesions acquired the task just as rapidly as normal control animals.

The results of this study were surprising not only because a difference between the lesion groups was observed but also because the difference was so stark. The CA1 group did not learn at all and the CA3 group performed entirely normally. These findings stand in striking contrast to the findings of another by Gilbert and Kesner (2003), where rats learned associations between a particular object or odor and their locations in specific places in an open field. Normal rats learned the object–place and odor–place problems at about the same rate as in the object–trace–odor association task. However, in contrast to those findings, selective lesions of CA3 impaired acquisition of object–place and odor–place associations, whereas CA1 lesions did not. Indeed, in the case of odor–place associations, CA3 lesioned animals showed no learning, whereas animals with CA1 lesions performed normally, a pattern of results opposite to the pattern found in the authors' more recent study. Thus, CA1 and CA3 each appeared to make unique contributions, respectively, to temporal and spatial attributes of memory. These findings are difficult to reconcile with the close serial anatomical connections between CA3 and CA1, but are consistent with other evidence of differential effects of selective lesions to these subfields (reviewed in Manns and Eichenbaum 2005). Yet, other studies have continued to provide compelling evidence that CA1 may play an especially important role when associations demand bridging a substantial temporal gap (Farovik et al. 2010).

On the other hand, in contrast to a clear separation of temporal from spatial coding within CA1, a major line of evidence suggesting that CA1 also processes spatial information is the prominent observation of spatial coding by place cells in area CA1. This prominent finding raises the question: Do hippocampal neurons also encode temporal attributes of memory? Temporal coding by CA1 neurons is much less studied than their role in spatial information processing, but recently, several experiments have reported temporal coding by neurons in area CA1. Here we present evidence that CA1 neurons encode both the spatial and temporal attributes of memories. Supporting Kesner's intuition that spatial and temporal attributes are organizing features of the context of memories, we will argue that spatial and temporal organization are prominent attributes of hippocampal neural networks that support memory.

How Memories are Represented in Space

Following on earlier studies of spatial and nonspatial firing properties of hippocampal neurons (e.g., Wood et al. 1999; reviewed in Eichenbaum et al. 1999; Eichenbaum 2004), in recent studies aimed at examining the mechanisms by which hippocampal networks represent memories in spatial contexts, we recorded the activity of CA1 principal neurons in rats performing a task that requires them to remember

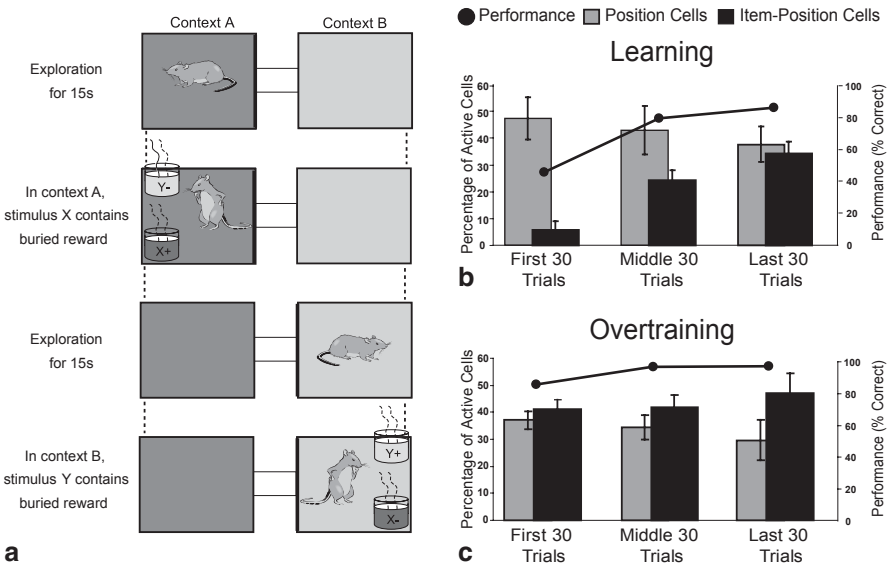

Fig. 2.1 Hippocampal neurons develop item–place representations in parallel with learning what happens where. **a** Object–context association task. The two contexts (represented by different shadings) differed in their flooring and wallpaper. The stimulus items (X or Y) differed in odor and in the medium that filled the pots. Items with a plus contained reward, whereas those with a minus did not, each depending upon the spatial context. **b** Changes in proportions of *Item-Position* and *Position cells* in learning vs. **c** overtraining sessions. (Data from Komorowski et al. 2009)

the differential reward associations of objects when they are presented in different places (Komorowski et al. 2009, 2013). In these experiments rats moved between environmental contexts that differed in visual, textural, and olfactory cues. On each trial, rats were initially allowed time to orient to the environment; then they were presented with two cups that were distinguished by both their odors and their digging media. In one environmental context (A), one of the stimuli (X) had a buried reward and the other stimulus (Y) did not, whereas in the other environmental context, the contingency was reversed (Y was baited and X was not; Fig. 2.1a). Therefore, the rat had to learn which of the two stimuli had been rewarded within each environment. We found that rats required several training sessions to acquire an initial problem of this type, but a subsequent second problem with new stimuli and new environmental contexts was typically acquired in the middle of a single 100-trial training session. This rapid learning allowed us to track the firing patterns of single neuron during the course of training on the second problem. We could therefore examine how neuronal firing patterns in the hippocampus might encode the relevant object–context associations.

We focused on the firing rates of hippocampal principal cells in areas CA1 and CA3 for a 1-s period surrounding when the rats sampled the stimuli during each trial. Earlier in training, we found that a large percentage of neurons fired when animals sampled either stimulus in a particular location in one of the two environments

(Fig. 2.1b; first 30 trials). These likely correspond to so-called place cells which fire when rats occupy a location in their environment. Some of these cells maintained the same place-specific firing patterns throughout training. At this stage, the firing patterns of virtually none of the cells distinguished the stimuli. However, as the animals acquired the context guided object association task, some neurons began to fire selectively during the sampling of one of the objects in one of the contexts and these cells continued to exhibit conjunctive object and place specificity after learning (Fig. 2.1b; middle 30 trials). The magnitude of item–context representation was robust in that, by the end of the training session, the proportion of hippocampal neurons that fired selectively during the sampling of one of the objects in a particular place or context equaled that of place cells (Fig. 2.1b; last 30 trials). This conjunctive object and place representation remained strong throughout recording sessions in which animals were highly overtrained on the task (Fig. 2.1c). Thus, a large percentage of hippocampal neurons developed representations of task-relevant object and place associations, and their evolution was closely correlated with learning those associations. Furthermore, subsequent analyses showed that the conjunctive representations developed from preexisting spatial representations into enhanced activations when particular objects were sampled in specific locations. Conversely, the representation of the objects alone was minimal throughout learning and the representation of places where any object was sampled, although strong, remained unchanged throughout training. These and other (Moita et al. 2003; Manns and Eichenbaum 2009) findings strongly suggest that the development of conjunctive object and location representations within the hippocampus underlies memories for items in the places where they occur.

Memories in Space and Time

Kesner and colleagues suggested that the entire hippocampus is engaged when a task demands both spatial and temporal attributes of memory (Hunsaker et al. 2006). In recent years, recordings of hippocampal neurons in animals performing tasks that require memory for spatial sequences have provided insights into how spatial and temporal attributes are integrated by hippocampal neuronal activity.

In addition to representation of elapsed time as a regularity of experiences, there is substantial evidence that hippocampal neuronal ensembles encode the order of events in sequence memories as revealed in studies showing that hippocampal neural ensembles “replay” sequences of place cell activations that occurred during previous experiences. The earliest studies on sequence replay by hippocampal neural ensembles focused on the tendency of place cells that fired in order during behavior to also fire in the same order when animals subsequently slept (Wilson and McNaughton 1994). Since then, numerous studies have reported forward and reverse replay of place cell sequences, both when animals are asleep and during periods of quiet wakefulness (see Karlsson and Frank 2009). Furthermore, when rats are engaged in vicarious trial and error of maze choices, hippocampal neurons

replay firing sequences that reflect possible paths of response choices (Johnson and Redish 2007). And place cell sequences anticipate paths to be taken even in open fields (Pfeiffer and Foster 2013). Conversely, interfering with hippocampal replays retards learning of critical choices in spatial memories, but not the general skills of performance in the maze (Jadhav et al. 2012). In addition, hippocampal replays are synchronized with cortical replays, consistent with the view that sequence replays reflect a temporal organization involved in remembering and memory consolidation (Ji and Wilson 2007).

In a particularly striking recent study linking place cell replay with learning, Singer et al. (2013) recorded from CA1 and CA3 principal cells in rats performing a spatial alternation task in a "W" shaped maze. They examined neuronal activity during local field potential events known as sharp wave ripples (SWR), in which several earlier reports have shown a speeded "replay" of neuronal firing sequences that had occurred in earlier experiences. Specifically, their analyses focused on SWRs when the rat was relatively still while outbound on the center arm, heading toward the critical choice between the left or right arm as having the next reward. During these SWR events, they identified replays as co-activations of place cell activity that typically occurred during actual runs toward the left or right goals. They found that more replays occurred preceding subsequent correct choices than incorrect choices, and in the latter, the likelihood of replay was at chance level. In addition, there were usually multiple replays at these times, corresponding to both the correct and incorrect choice paths. Also, replays were common early in learning but no longer appeared when rats had mastered the task. Thus, associated with the course of learning, the hippocampus replays alternative paths just before a critical choice between those paths is made, and the occurrence of replay increases the accuracy of the subsequent choice.

The findings by Singer et al. (2013) showing that the hippocampus replays multiple alternative memories build on many earlier observations about hippocampal replay, including, in particular, that hippocampal neural ensembles replay both recent paths and paths not recently taken (Gupta et al. 2010). Also, the occurrence of replays is greater after novel experiences and correlates with memory performance (Dupret et al. 2010). And replays of alternative paths have also been observed when rats investigate possible choices during vicarious trial and error at a critical decision point (Johnson and Redish 2007). Here the trial-by-trial prediction of accuracy by the proportion of replays of alternative paths suggests that hippocampal replay reflects the retrieval of multiple relevant memories that can be evaluated to guide the correct subsequent choice, and this is of particular value early in learning.

The findings on hippocampal replay and its association with memory are paralleled by several observations on trajectory dependent activity of place cells (reviewed in Shapiro et al. 2006). In these studies, rats traverse overlapping routes through a maze and a typical observation is distinct place cell firing sequences for each route, including different firing patterns when the rat is traversing the overlapping part of different routes. In our first study of this phenomenon, rats were trained on the classic spatial T-maze alternation task in which successful performance depends on distinguishing left- and right-turn episodes to guide each subsequent

choice (Wood et al. 2000). We reasoned that, if hippocampal neurons encode each sequential behavioral event within one type of episode, then neuronal activity at locations that overlap in left-to-right and right-to-left turn trials should vary according to the route currently under way. Indeed, virtually all cells that were active as the rat traversed these common locations were differentially active on left-to-right versus right-to-left trials. Although most cells exhibited similar quantitative differentiation of trial types, other cells fired exclusively on one type of trial. Similar results have subsequently been observed in several versions of this task (Bower et al. 2005; Ferbinteanu and Shapiro 2003; Frank et al. 2000; Griffin et al. 2007; Lee et al. 2006; Ainge et al. 2007; Pastalkova et al. 2008; for review, see Shapiro et al. 2006; but not all versions of the task Lenck-Santini et al. 2001; Bower et al. 2005). Furthermore, these observations are consistent with recent results in animals and humans showing that hippocampal neuronal activity captures sequential events that compose distinct memories (Ginther et al. 2011; Paz et al. 2010). These findings suggest a reconciliation of the current controversy about spatial navigation and episodic memory views of hippocampal function: Place cells represent the series of places where events occur in sequences that compose distinct memories.

Similar to the findings of Singer et al. (2013) on replays, trajectory-dependent activity of place cells is also strongly linked to memory performance, as its occurrence both prior to a memory delay and during memory retrieval predicts subsequent trial-by-trial memory accuracy (Robitsek et al. 2013). In that study, we first trained rats on the continuous spatial alternation task used in the Wood et al. (2000) study then, on subsequent recording sessions, recorded CA1 principal neurons as rats performed separate blocks of trials on the continuous alternation and on a delayed alternation version where they were constrained at the start of the common segment of the maze. Performance during delayed alternation was approximately 70% correct, allowing a comparison of firing properties during accurate trials and errors when the animal ran on trajectories from left-to-left or right-to-right (Fig. 2.2). We found hippocampal place cells that fired when the rat traversed locations throughout the maze and their activity predicted accuracy of subsequent choices. In particular, we found that many place cells that fired at locations just before the delay were strongly activated in advance of subsequent correct choices, whereas the same cells fired much less or not at all in advance of errors. For example, the cell in Fig. 2.2a fires robustly as the rat approaches the end of the left return arm on correct but not error trials and the cells in Fig. 2.2b and c fire strongly as the rat is in the midst of the right return arm on correct trials, and much less on errors. Also, many of the cells that fired selectively associated with retrieval of left-to-right or right-to-left trials as the rat traversed the common segment of the maze also fired strongly in advance of correct choices but less so or not at all in advance of errors. For example, the cell in Fig. 2.2d fired robustly as the animal traverses the stem on correct left-to-right trials, much less so on right-to-left trials, and hardly fired on errors. Figures 2.2e and f show cells that fired at different locations on the common maze segment most strongly on correct left-to-right trials and slightly less on correct right-to-left trials, and did not fire on either type of error. The combined evidence on replay and trajectory-dependent firing strongly suggest that the activity of place cells in spatial

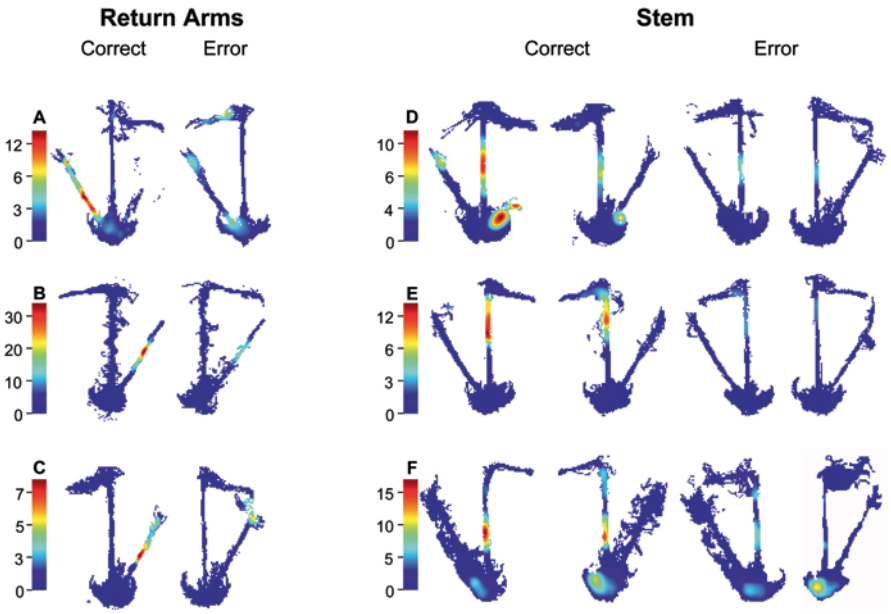

Fig. 2.2 CA1 neurons signal subsequent accurate memory on a spatial alternation task. **a**–**c** Cells that fired differentially as rats traversed different parts of the maze arm just prior to the memory delay. **d**–**f** Cells that fired differentially as rats traversed different parts of the maze common to both routes through the maze. See text for description. (Data from Robitsek et al. 2013)

memory tasks reflects the encoding and retrieval of sequences of places traversed that compose the memories of routes taken.

Do Hippocampal Neurons Represent the Temporal Attributes of Experience, Independent of Spatial Coding?

While there is an extensive literature on the spatial firing properties of hippocampal neurons, much less attention has been paid to how time itself is represented in the hippocampus, despite substantial evidence of hippocampal involvement in the temporal organization of memory (reviewed in Eichenbaum 2013). Recently, evidence has emerged showing that hippocampal neuronal networks compose a gradually changing representation of the flow of time, independent of explicitly identifiable locations or specific events that might directly drive sequential neural activations. Furthermore, the temporal signal has been dissociated from potential confounds of moving through space as well as self-generated movement cues (path integration) that could underlie an apparent temporal modulation of neural activity, as discussed in the interpretation of several experiments below.

The initial evidence of gradually changing temporal context representations in the hippocampus came in a study in which ensembles of CA1 neurons were recorded as rats performed the above-described task wherein rats encode and remember unique sequences of odors (Kesner et al. 2002; Fortin et al. 2002). The firing patterns of CA1 ensembles gradually evolved over entire recording sessions. Moreover, within those sessions, CA1 ensemble representations gradually changed even over a few minutes in which individual sequences were encoded, and the extent of ensemble change during the sequence of odor sampling events predicted subsequent success in remembering the order of odors experienced on each trial (Manns et al. 2007). Consistent with this observation, Naya and Suzuki (2011) observed that, when monkeys perform a task where they bridge a delay between two visual stimuli, hippocampal neural ensembles represent the evolving temporal context between the stimulus events.

As the Manns et al. (2007) task involved unique memories on each trial, it could not be determined whether distinct evolving temporal context representations are generated for specific memories. However, Pastalkova et al. (2008) recorded the activity of hippocampal (CA1) neurons as rats ran in a running wheel in between trials in a spatial alternation task and observed that different hippocampal ensemble sequences were associated with different subsequent memory choices and, when the animals made errors, these sequences were disrupted. Although Pastalkova et al. (2008) referred to these neurons as "episode cells," we prefer to call them "time cells" because, just as place cells encode locations in a specific space, time cells encode moments in a specific period of experience. The populations of time cells observed in Pastalkova's study likely reflect the repetition of ensemble firing patterns that gradually changed in the Manns et al. (2007) study.

The phenomenon of time cells was further examined using a nonspatial task developed by Kesner et al. (2005) that identified the hippocampal CA1 region as necessary for rats to learn distinct sequences in which an object and an odor were separated by a 10 s temporal gap (Fig. 2.3a). In this version of the task, rats moved through three sections of a linear maze, each of which composed a key phase in a sequence of events. Each trial began with the presentation of one of two objects that the rat investigated for a short period. Then the rat was confined in a small area for 10 s, after which it was presented with one of two odors mixed into common playground sand. Each odor was paired with one of the objects, such that if the odor followed the correctly paired object then the rat could dig in the sand for a buried reward. Conversely, the rat obtained no reward for digging when the odor followed the object with which it was not paired. Critically, the object–delay–odor sequences were presented repeatedly during each testing session, so the rats had to remember across the delay the object that had started the trial in order to respond appropriately to the odor at the end of the trial. As described above, rats with lesions of the CA1 region show no evidence of learning these object–odor sequences (Kesner et al. 2005). Conversely, rats with CA3 lesions learn the sequences with a time-course that is comparable to control rats. Taken together, these results are consistent with a selective role for the CA1 in representing a temporally extended sequence of events to compose a distinct experience.

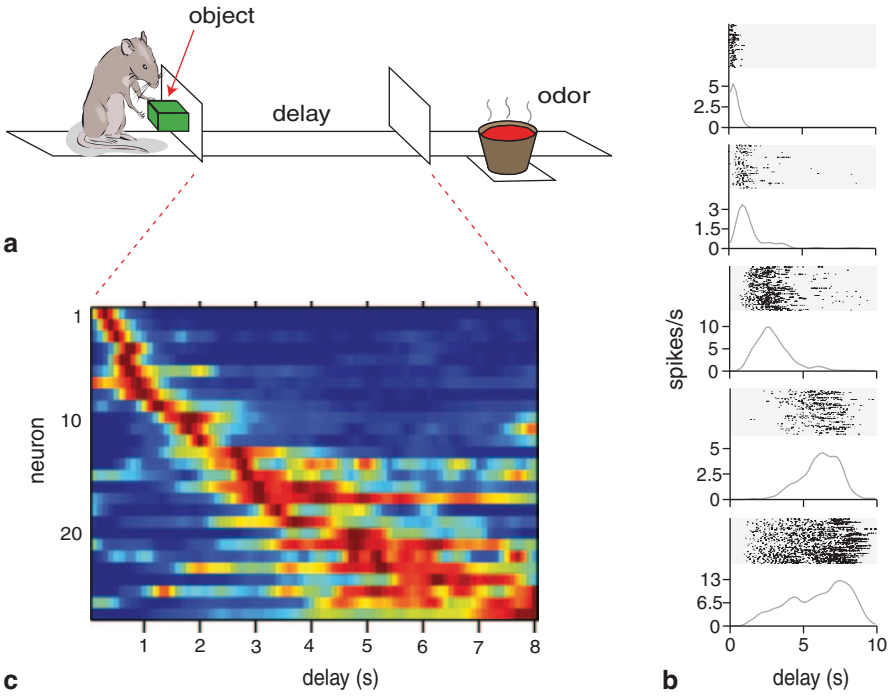

Fig. 2.3 **a** The trial structure for object–delay–odor sequences. **b** Each panel shows a raster plot and peri-event time histogram illustrating neural activity of a time cell during the delay period. **c** Normalized firing rates of 26 neurons recorded simultaneously during the delay period. Each row represents the activity pattern of a single neuron. (Data from MacDonald et al. 2011)

To explore the nature of the hippocampal representation supporting performance in this task, MacDonald et al. (2011) adapted the task and examined activity from large ensembles of hippocampal CA1 neurons monitored simultaneously. Many neurons activated during presentation of the object or odor and often fired differently depending on the object that started the trial, indicating that the hippocampus distinguished the key events composing each object–odor sequence. Most striking, nearly half of the cells that were recorded activated during the delay period, and the period of activity of each cell was typically selective for a specific moment (Fig. 2.3b). To better illustrate the temporal signature of these cells, Fig. 2.3c plots normalized firing patterns from an ensemble of cells recorded simultaneously during the delay. It is readily apparent that the cells activated in sequence, and the overlap among their firing fields bridged the delay. Importantly, time cells distinguished the object starting the trial, which is consistent with a function in integrating the object with its paired odor across the delay. These results confirmed a robust temporally organized representation for a sequence of events in the hippocampus, highlighted by cells that bridged the delay and composed the flow of time in a distinct memory.

Could temporal signals reflected in the activity of time cells be confounded with a reliable sequence of behaviors or a sequence of locations occupied during the delay? MacDonald et al. (2011) performed a detailed statistical analysis of the firing patterns of neurons and found that, while many of these cells also represented the spatial location and ongoing behavior during the delay, these factors did not account for the timing signal reflected in the activity of these cells. Thus, while many of these cells did incorporate information about spatial and behavioral events into the neural representation of the delay period, the temporal signal encoded by time cells was independent of the rat's location and movements.

Another alternative explanation of these findings is that hippocampal neurons integrated the path of movement animals took during the delay phase of the task (McNaughton et al. 1996). In the McDonald et al. (2011) and the Pastalkova et al. (2008) studies, as well as another study that observed time cells during the delay periods in a delayed spatial task (Gill et al. 2011), the rats were in motion over the entirety of the key delay periods. Therefore, the distance moved and time elapsed were entirely confounded during the periods when time cells were observed, and other studies have reported that hippocampal neurons can signal the accumulated linear distance that a rat has moved from a reference point (Gothard et al. 1996; Redish et al. 2000). Thus, it was unclear whether hippocampal neurons can signal the flow of time independent of self-generated cues that may support path integration (McNaughton et al. 2006). To address this issue, MacDonald et al. (2013) eliminated movement-related variables altogether by developing a head-fixed preparation for rats and recorded hippocampal CA1 activity while their memory was tested using an odor delayed matching to sample task. Each trial began with the presentation of a sample odor, followed by a fixed 2–5 s delay period, then presentation of a test odor. The restrained rats were rewarded with water for licking at a lick spout if the test odor matched the sample odor, but were not rewarded for licking when a nonmatching test odor was presented. This task was similar to the object–delay–odor sequence memory task in that there were a small number of highly repeated sequences that composed each combination of sample and test odors, and on each trial the rat had to remember the sample odor across the delay period to identify a target odor sequence.

Many hippocampal neurons activated at brief moments in sequence during the delay period. Therefore, even in head-fixed rats, hippocampal CA1 neurons segmented the delay period into discrete temporal units that reflected the flow of time within the trial. Moreover, many time cells were temporally modulated during the delay specifically following presentation of a particular odor that started the trial (Fig. 2.4a). Furthermore, most time cells contributed to a representation of only one odor memory while others contributed to more than one odor memory representation, though rarely to all four (Fig. 2.4a, b). In the latter case, some of these cells fired around the same time during delay following different odors, typically at different rates. Other cells had distinct temporal firing patterns after different sample odor presentations. Thus, each sample odor was represented during the delay by a largely distinct temporally organized ensemble of time cells. These data indicate that different neural ensembles activate in sequence over extended intervals to

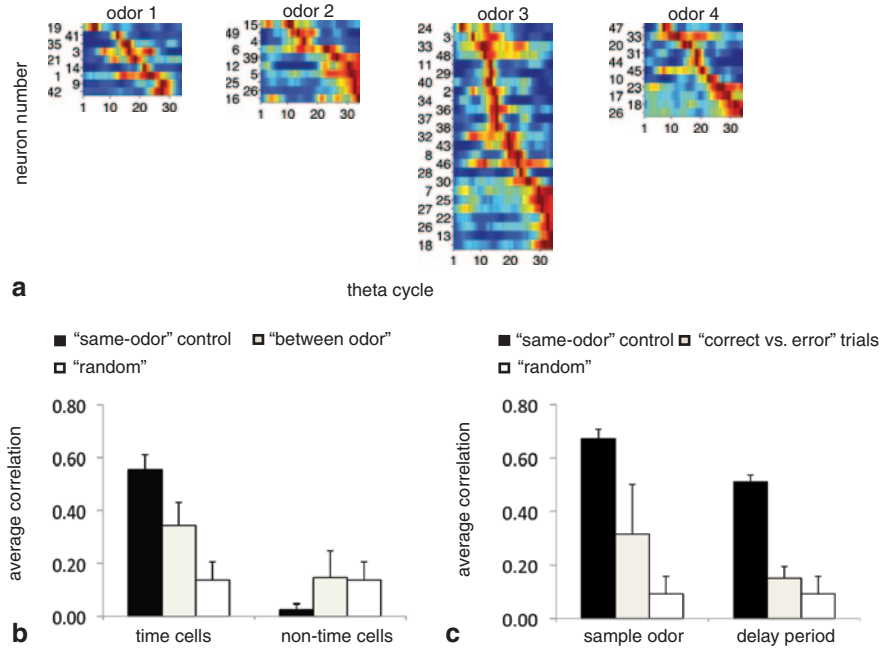

Fig. 2.4 Odor memory representations during the delay for each sample-odor defined trial type involved largely distinct, temporally organized neural ensemble activity. **a** Normalized firing rates over the delay for time cells (numerically labeled) for each of 4 sample-odor defined trial types in rat 5. **b** Average correlation coefficient between ensemble vectors for each trial type against the population vector for same set of neurons in all other trial types ("between-odor"). As one control, the average correlation coefficient between subsets of trials (even vs. odd) that began with the same odors is shown ("same-odor" control). As a second control, the average correlation coefficient between independent, randomly rearranged population vectors is shown ("random"). **c** For ensembles of cells that were temporally modulated in the sample odor or delay period, shown is the average correlation coefficient between populations vectors from correct trials that began with the same odor and error trials that began with the same odor ("correct vs. error" trials). The average correlation for the "same-odor" and "random" conditions are also shown. (From MacDonald et al. 2013)

compose the flow of time in specific odor memories. Moreover, the overlap among the different odor memories, embodied in cells that fire at the same or different rate at comparable moments during the delay, is consistent with the crucial role of the hippocampus in linking together different experiences (Eichenbaum et al. 1999; Eichenbaum 2004). Finally, these memory-specific, temporally organized representations predicted accurate memory performance, such that while ensemble representations were reliable during the sample and delay periods on successful trials, there was significantly less reliability during the sample phase and loss of the representation during the delay phase of error trials (Fig. 2.4c).

While the just described study revealed a temporal signal under conditions where head location was fixed and movement prevented, time cell firing patterns during movement could reflect path integration rather than elapsed time. To address this possibility, Kraus et al. (2013) recorded from multiple hippocampal neurons as rats

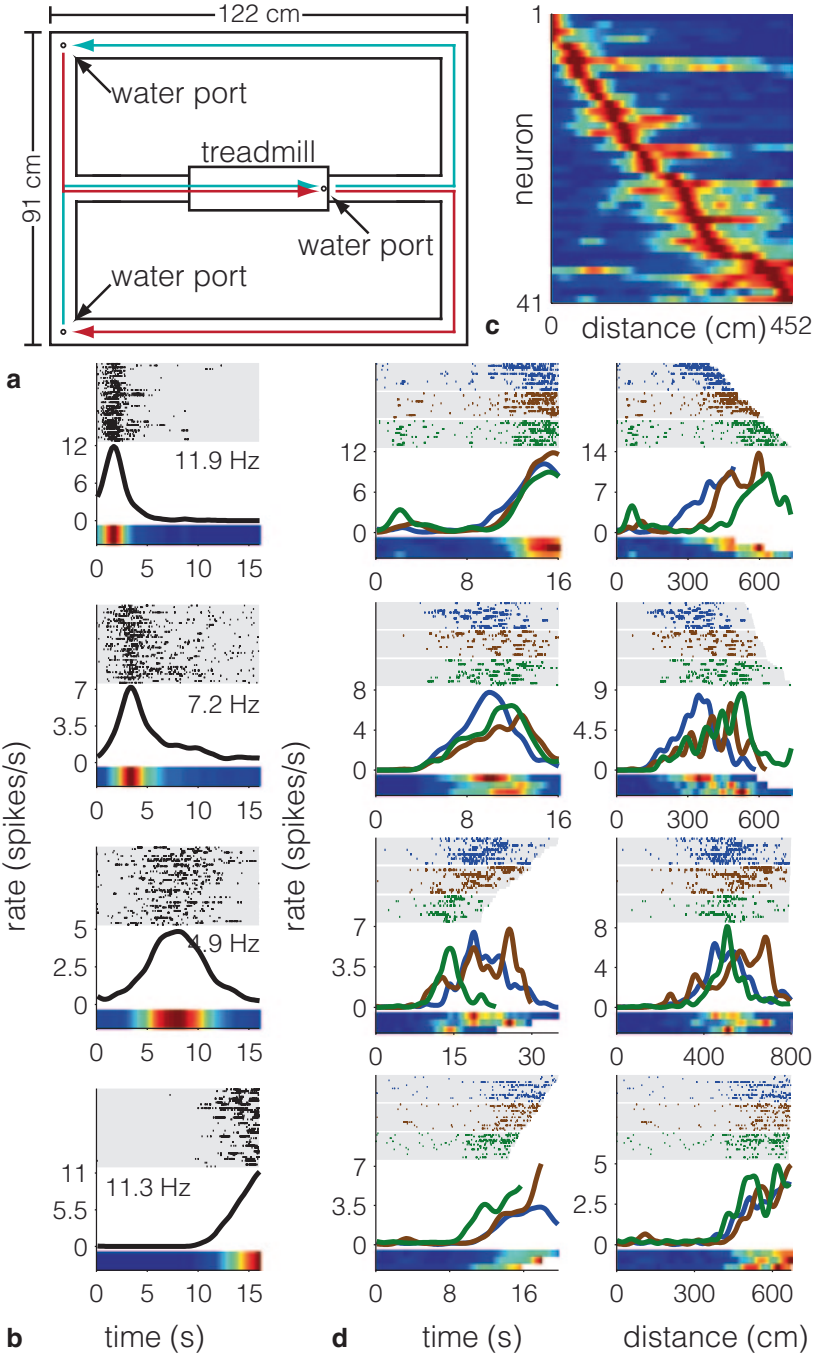

Fig. 2.5 Hippocampal activity during stationary treadmill running: temporal integration versus path integration. **a** Diagram of the figure-eight maze indicating the dimensions and location of the water ports and treadmill. *Cyan line* indicates *right-to-left* alternation; *red line* indicates *left-to-right* alternation. **b** Firing patterns of four different example neurons active during stationary

ran continuously in place at different speeds on a treadmill placed in the stem of a figure-eight maze (Fig. 2.5a). On each trial, the rats entered the central stem of the maze from one of two directions (left or right), and then walked onto the treadmill where they received a small water reward. After a short delay, the treadmill accelerated to a speed randomly chosen from within a predetermined range, and the rats ran in place until the treadmill stopped automatically and another small water reward was delivered. Subsequently, the animals finished the trial by turning in the direction opposite from their entry into the stem (spatial alternation) to arrive at a water port at the end of a goal arm. To distinguish behavior, location, time, and distance as factors influencing neuronal activity, behavior, and the location of the animal on the maze were "clamped," and the treadmill speed was varied to decouple the distance the rat traveled from its elapsed time on the treadmill.

As with previous experiments that examined hippocampal activity during task delays (Pastalkova et al. 2008; Gill et al. 2011; MacDonald et al. 2011), at each point during treadmill running a subset of hippocampal neurons fired, and the subset of neurons activated in a regular sequence that repeated during every treadmill run (Fig. 2.5b, c). In addition, running speed was systematically varied to allow post hoc analyses to separate the influences of time and distance on firing patterns, and to measure the extent to which each variable influenced firing. These analyses revealed both "distance cells," that is, cells that more reliably encoded the distance the rat has run on the treadmill, and "time cells," cells that more reliably encoded the time the rat has spent on the treadmill (Fig. 2.5d). The observation of "distance cells" in this task indicates that hippocampal neurons can integrate the length of a path even in the absence of visual flow usually associated with movement through space. Also, the presence of "distance cells" in this task indicates that these neurons are not driven entirely by network dynamics without the influence of either idiothetic or allothetic cues, as suggested by Pastalkova et al. (2008), because the neurons must be responding to the treadmill speed, or self-motion cues influenced by the speed of the treadmill, in order to encode distance. In addition, the observation of temporal modulation in addition to or without distance modulation indicates that these neurons are not exclusively driven by path integration but also by elapsed time (McNaughton et al. 1996, 2006; Etienne and Jeffery 2004). Thus, Kraus et al. (2013) showed that, when both of these dimensions are prominent, the hippocam-

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treadmill running, aligned to the time the treadmill started. *Black lines* and *color bars* represent firing rate averaged over all runs. *Number* indicates peak firing rate in spikes per second (Hz). **c** Ensemble firing rate map showing all neurons active on the treadmill during a single session. Each row represents the normalized firing rate of one neuron, sorted by the peak firing time. In each row, *blue* represents no firing (zero spikes per second) and *red* represents peak firing for that particular neuron. **d** Examples shown in each row represent the activity from one neuron plotted both as a function of time since the treadmill started (*left column*) and distance traveled on the treadmill (*right column*). *Blue, brown, and green ticks* (*and tuning curves*) represent the slowest one third of runs, middle one third of runs, and fastest one third of runs, respectively. The rows in the raster plots in panels b and d are sorted with the slowest treadmill speed on top and fastest speed on the bottom. Note better alignment of the neural activity to time in the top two examples (time cells) and better alignment of neural activity to distance in the bottom two examples (distance cells). (Data from Kraus et al. 2013)

pus represents both the distance traveled and time elapsed. Furthermore, a large fraction of hippocampal neurons combine information about these dimensions to varying extents, such that different neurons largely reflected distance or time and others equivalently reflected the combination of spatial and temporal dimensions, consistent with a unified representation of space and time attributes.

During treadmill running, when behavior and location were held relatively constant, time and distance predominated in their influence over the firing patterns of hippocampal neurons. However, other neurons, and many of the same neurons that were active on the treadmill, had place fields elsewhere on the maze, indicating that during other components of the task, where locations on the maze were important to task success, space was a strong influence over firing patterns of even the same neurons. These observations support the view that hippocampal neuronal activity reflects both the temporal and spatial regularities, along with other salient features of experience, consistent with a combined spatial-temporal organization of memories.

Conclusions

In 1987, Ray Kesner joined the then-prominent views of hippocampal function in spatial and temporal processing to propose that this brain area supported memory for the spatial–temporal context of memories. Many subsequent studies, including those of Kesner and his students, supported this idea, which we now recognize as a fundamental attribute of hippocampal dependent memory. Yet, most studies aimed to characterize the nature of information encoded by hippocampal neurons have focused solely on the spatial firing properties of hippocampal neurons and this has led to a separation between "navigation" (O'Keefe and Nadel 1978; Moser et al. 2008) and "memory" (Squire 2009) literatures on hippocampal function. However, the recent observations on temporal coding properties of hippocampal neurons, confirming Kesner's idea that the hippocampus also represents the temporal attributes of memories, offers a reconciliation of these views. The studies reviewed here show that the hippocampus is critical to memory for temporal organization independent of space, and the same neurons that are place cells when rats forage for food in open fields and traverse maze paths also fire sequentially when rats run in one location and when rats bridge gaps between remembered events independent of behavior and location. Furthermore, the hippocampus plays and replays sequences of place cell firings as a representation of spatial–temporal organization of memories. The combination of spatial and temporal organization can be considered fundamental to memory (Gallistel 1990).

These findings are examples of a growing set of studies that reveal a prominent role of the hippocampus in memory for temporal order in animals and humans, and provides a broad range of evidence for sequential activation of hippocampal neurons during memory retrieval of serial events in rats, monkeys, and humans. In particular, the existence of hippocampal "time cells" that encode moments in temporally extended memories, much as place cells encode locations in spatially extended

environments, suggests that time, not place, is the fundamental dimension of hippocampal representation that is common to navigation and memory. Furthermore, recent evidence revealed temporal organization in hippocampal ensembles that exists prior to experiences, to which learning attaches specific memories (Dragoi and Tonegawa 2011). This observation of "preplay," which anticipates subsequent replay, suggests that temporal organization is primary, and may provide the scaffolding onto which spatial and nonspatial memories are hung. Combined with the other findings on time cells described above, these observations on temporal representation by hippocampal neurons offers considerable promise for a comprehensive understanding of the network mechanisms that underlie Kesner's prescient view on the spatial and temporal attributes of memory supported by the hippocampus.

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