

Trajectory Encoding in the Hippocampus and Entorhinal Cortex

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Summary

We recorded from single neurons in the hippocampus and entorhinal cortex (EC) of rats to investigate the role of these structures in navigation and memory representation. Our results revealed two novel phenomena: first, many cells in CA1 and the EC fired at significantly different rates when the animal was in the same position depending on where the animal had come from or where it was going. Second, cells in deep layers of the EC, the targets of hippocampal outputs, appeared to represent the similarities between locations on spatially distinct trajectories through the environment. Our findings suggest that the hippocampus represents the animal's position in the context of a trajectory through space and that the EC represents regularities across different trajectories that could allow for generalization across experiences.

Introduction

The superficial layers of the entorhinal cortex (EC) provide the primary source of neocortical input to the hippocampus, and the deep layers of the EC are the primary target of neocortically bound hippocampal and subicular outputs (Witter et al., 1989) (see Figure 1A). In the rat, the hippocampus and EC are necessary for the animal to perform spatial or navigational tasks (O'Keefe and Nadel, 1978; Rasmussen et al., 1989; Jarrard, 1993; Cho and Kesner, 1996), and neurons in the hippocampus, known as "place cells," are active when the animal visits restricted regions of its environment (O'Keefe and Dostrovsky, 1971; Wilson and McNaughton, 1993; Muller, 1996). The EC has received much less attention than the hippocampus, but the studies that have been done have suggested that EC neurons fire in a weakly place-specific manner (Barnes et al., 1990; Mizumori et al., 1992; Quirk et al., 1992).

While the presence of place-specific activity in the hippocampus and EC has been clearly demonstrated, the role of that activity in navigation and spatial memory in general is not yet well understood. More specifically,

hippocampal and EC neural firing appears to relate primarily to the animal's current location, but solving spatial tasks requires that the animal must know not only where it is but also where it has been and where it should go so that it can plan trajectories through the environment. To better describe the spatially related activity of EC neurons and to examine the relationship between place-specific firing and animals' ability to successfully navigate to a goal, we recorded simultaneously from cells in the CA1 subregion of the hippocampus and in the superficial and deep layers of the EC while animals performed two tasks: a spatial alternation task on a W-shaped track and a simple alternation task on a U-shaped track (Figure 1B). In the W track task, the animal ran continuously from center to left to center to right to center, and so on. To perform this task, the animal had to remember which outside arm it had most recently visited so that it could choose the next correct outside arm. This task is a continuous version of a T-maze spatial alternation task that is sensitive to hippocampal or entorhinal damage (Rawlins and Olton, 1982; Aggleton et al., 1986), and it has the advantage of having two side lengths of track that were geometrically similar to the center arm. The U track task was chosen to allow us to compare firing patterns across similar but spatially separate environments. The two environments were located next to each other in the same room.

Results and Discussion

General Characterization

An example of a brain slice showing lesions and tracks from three tetrodes is shown in Figure 1C, and examples of the "clusters" used to isolate two deep EC units and the resulting unit waveforms are shown in Figure 1D. The mean \pm SD for the amplitude of waveforms from units active on the W track (see below), measured from the baseline to the peak, was as follows: superficial EC, 127.3 ± 43.8 μ V; CA1, 195.6 ± 95.7 μ V; and deep EC, 95.3 ± 28.1 μ V. The relatively low amplitude of deep EC units may help explain why relatively few deep EC cells have been recorded from in previous studies (Mizumori et al., 1992; Quirk et al., 1992).

In both tasks the animal ran along paths from one food well to another. To allow a comparison of firing along the different paths between food wells, we converted the positions on each path into distances from the food well at the beginning of the path. That yielded four paths (center to left, center to right, left to center, and right to center) for the W track and two paths (left to right and right to left) for the U track. Each cell's firing was examined on each path. Considering first the W track and counting only those neurons with distinct place fields, 36 neurons were recorded from superficial EC, 174 neurons were recorded from deep EC, and 288 principal cells were recorded from CA1 across 48 data sets. Of those cells, 15 superficial EC, 90 deep EC, and 136 CA1 principal cells had fields on the U track as well. Plots of firing rate along the W track paths for a superficial EC neuron, a CA1 principal neuron, and a deep EC neuron are shown in Figure 2.

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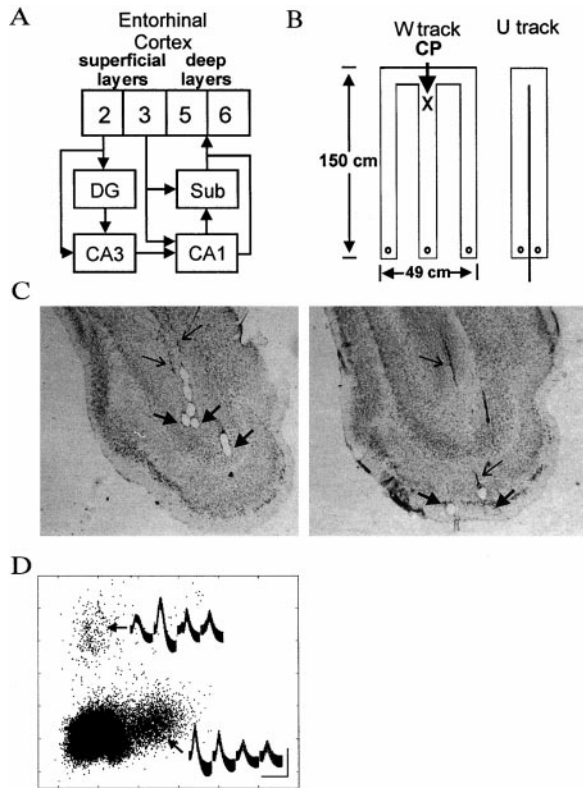


Figure 1. Tetrode Recordings from CA1 and the Entorhinal Cortex in U- and W-Shaped Environments

(A) A simplified sketch of the anatomy of the connections between the entorhinal cortex and the hippocampus. The superficial layers of the EC (2 and 3) originate the main neocortical input to the hippocampus, and the deep layers of the EC (5 and 6) are the primary targets of neocortically bound hippocampal and subicular outputs (Witter et al., 1989).

(B) The W and U track environments. On the W track, the animal ran in the following pattern: center, left, center, right, center, and so on. The animal was rewarded in each of the arms when it made a correct choice. The small circles designate food wells and "CP" designates the choice point on the W track. On the U, the animal moved from one end of the U to the other. The individual arms of both tracks were 7 cm wide, approximately the same width as the animal's body, so the animals generally moved along the center of each arm.

(C) Two examples of histological sections through the EC. In each picture, the most ventral lesions are marked with large arrows and tetrode tracks with small arrows. The left picture shows the lesions from three tetrodes that terminated in the deep EC; going from left to right, the tetrodes were used to make one, four, and three lesions. Only two of the three lesions of the rightmost tetrode of the left picture are visible in this section. The right picture shows the lesions from two tetrodes that terminated in the superficial EC. One of these tetrodes was used to make one lesion and the other was used to make two lesions.

(D) An example of a cluster plot used to separate the units. The x and y axes of the main plot are the amplitude of spikes for two of the four channels of the tetrode. Two clusters are visible, and the mean \pm SD of the spike waveforms are shown next to the clusters. A third cell, visible in other projections, was also cut from this tetrode. Scale bars for spikes: 1 ms, 100 μ V.

We first characterized differences in the spatial selectivity of firing across these different regions by computing the values of two measures that quantify spatial firing properties, average field length and position information

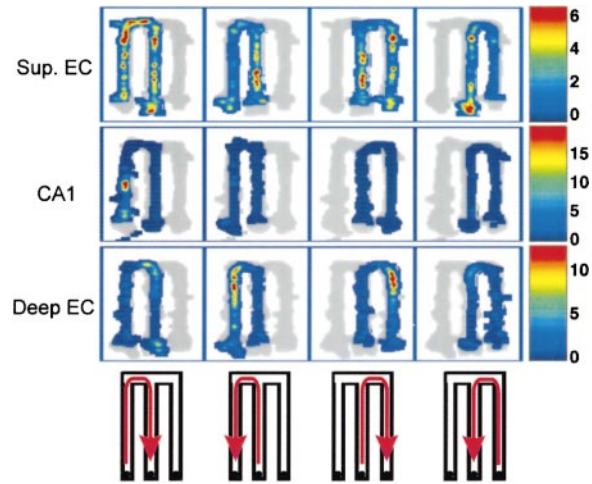


Figure 2. Firing Rate Maps by Path for Representative Neurons from Each Region

Row one shows a superficial EC cell, row two a CA1 cell, row three a deep EC cell, and row four shows the trajectory represented in each column. The color map indicates the firing rate. The section of the environment the animal did not traverse on a given path is shown in light gray.

(Skaggs et al., 1993) (Figures 3A–3D). These measures had similar distributions for the neurons that were recorded from on the U track (data not shown). Superficial EC neurons tended to have long fields, and their position information distribution indicates that very few, if any, had high place selectivity, a result consistent with earlier work (Mizumori et al., 1992; Quirk et al., 1992). CA1 principal cells, as expected, had small fields and firing that carried substantial position information. Deep EC neurons had fields that were, on average, not significantly different in length from those in superficial EC, but were significantly longer than those of CA1 cells. An examination of the distribution of position information coefficients for deep EC neurons indicates that while on average deep EC neurons displayed less position information than CA1 neurons, the firing of many deep EC neurons contained substantial information about the animal's position (Figure 3D).

Prospective and Retrospective Coding

If the hippocampus and EC play a role in representing and planning extended trajectories through the environment, neural firing patterns in these structures could reflect not only where the animal is, but also where it intends to go or where it has come from (Eichenbaum et al., 1999). To determine whether this pattern of activity was present, we analyzed the activity of each cell that had a field on the center arm. We examined both runs from the center arms out to an outside arm (outbound) and runs from the outside arms to the center arm (inbound). We calculated the firing rate of each neuron in a sliding 35 cm window and determined whether the firing rate could be used to predict the animal's future choice of outside arm (prospective coding) for outbound paths or correlated with the animal's past location (retrospective coding) for inbound paths. We use the terms retrospective and prospective coding to refer to the event (coming from or going to a particular outside arm) that was temporally closest to the cell's predictive or

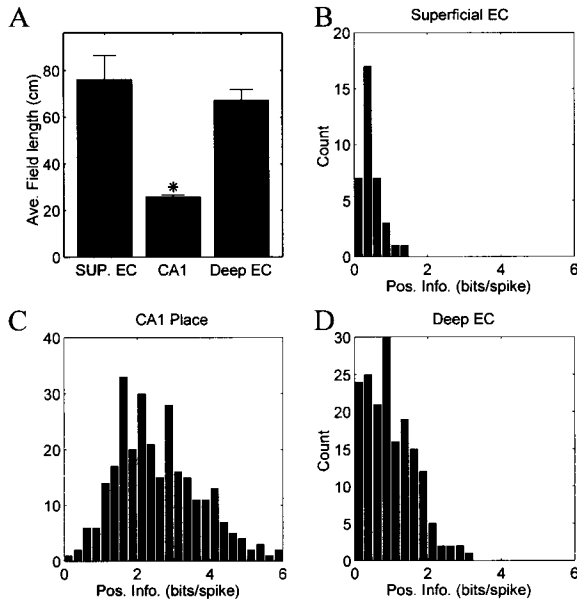


Figure 3. A Characterization of Spatial Information in CA1 and the EC

(A) The average field length for neurons from each region. Each bar represents a mean \pm SEM, and the asterisk indicates that the difference between mean hippocampal neurons' field lengths was significantly less than that of superficial or deep EC neurons ($p < 0.0001$, Scheffé S test). Superficial and deep EC neurons tended to have significantly longer fields than CA1 cells.

(B–D) The distributions of the position information measure for neurons from each region. The firing of CA1 cells clearly contained more information about the animal's current location than that of deep EC neurons, but many more deep EC than superficial EC neurons displayed relatively high position information in their firing patterns. The mean and standard deviation for the position information coefficient for each region/cell type are as follows: superficial EC, 0.46 ± 0.26 ; CA1, 2.34 ± 1.25 ; and deep EC, 0.99 ± 0.66 . The distributions of the EC neurons are non-Gaussian, so we tested for significant differences between means with a Wilcoxon rank sum test with a criterion of $p < 0.01$ to correct for multiple comparisons. All of the means were significantly different. As deep EC cells show significantly more place specificity than superficial EC neurons, it is likely that the strong position coding present in CA1 contributes to the increased spatial specificity seen in the deep EC as compared to the superficial EC. The mean \pm SD of the firing rates for neurons from each of the three regions were as follows: maximum rates (as measured on the smoothed firing rate over distance plots): CA1, 21.9 ± 12.5 Hz; superficial EC, 18.9 ± 10.2 Hz; deep EC, 21.8 ± 22.4 Hz; average rates: CA1, 1.1 ± 1.1 Hz; superficial EC, 6.5 ± 10.7 Hz; deep EC, 5.3 ± 10.0 Hz.

correlative activity. In total, a window with significant prospective or retrospective coding was found in 20 of 60 superficial EC units (33%), 79 of 218 CA1 units (36%), and 96 of 196 deep EC units (49%). Examples of cells whose firing showed significant retrospective or prospective coding are shown in Figure 4. These plots show the firing rate of the cell along two paths, one involving the left and center arms and the other the right and center arms. In each plot a region on the center arm where the cell fired at a significantly higher rate on one path as compared to the other is highlighted by a blue box.

To ensure that this activity is truly reflective of a representation dependent on past or intended future location,

we examined the relationship between path dependence and other variables that are known to affect hippocampal firing rates. CA1 place cell activity is known to be influenced by position, head direction, and velocity (McNaughton et al., 1983). To eliminate the potential contributions of those factors we performed a test on the lateral position of the animal on the track, the head directions, and the velocities associated with the firing of a given neuron in each window where path dependence was found. Using criteria based on the known tuning properties of head direction cells (Taube and Muller, 1998) and the degree of velocity dependence of CA1 place cells (McNaughton et al., 1983), we excluded those cells where prospective or retrospective coding was associated with differences in lateral position, head direction, or velocity between the two paths (number excluded: superficial EC, 9; CA1, 48; deep EC, 60). We also excluded those cells that showed prospective or retrospective coding for the left arm in one window and the right in another, as that pattern of activity is difficult to interpret (number excluded: superficial EC, 3; CA1, 6; deep EC, 4). The proportion of cells showing significant prospective or retrospective coding in each window tested is displayed in Figures 5A–5C, and the proportion of cells that show significant prospective or retrospective coding at the $p < 0.01$ level is shown in Figure 5D. Thus, even after applying a very stringent criterion that eliminated all cells whose prospective or retrospective coding was associated with lateral position, head direction, or velocity differences, more cells than would be expected by chance show prospective or retrospective coding. In addition, the number of neurons showing prospective coding differed significantly among the different cell types and regions ($p < 0.02$, logistic regression, χ^2 test), suggesting that prospective coding is most prevalent in the deep EC.

We should note that there is a superficial similarity between the prospective and retrospective coding observed here and the observation by Muller et al. (1989) that the firing of CA1 place cells was best correlated with a position ~ 120 ms in front of the animal when the animal was randomly foraging for food pellets. In our case, however, a number of cells showed retrospective coding near the food wells on the center arm, and the time required for an animal to run from an outside arm to the edge of the window containing the food well averaged 1.6 s. That, combined with the observation that animals running on the W track generally move much faster than during random foraging, suggests that the phenomena observed here are fundamentally different than those previously observed.

These findings may have important implications for understanding the representation of the relationship between positions and behaviors. Other studies have suggested that, at least for CA1 and CA3 place cells, many neurons can code for behavioral or task-related variables (Ranck, 1973; Deadwyler et al., 1996; Wood et al., 1999). This suggests that a cell showing prospective or retrospective coding on the center arm can be understood as representing the same position differently, depending on the phase of the task in which the animal is about to engage or the phase of the task it has just completed. In other words, our results suggest that CA1 and the deep EC can represent the connection between the animal's current position and its past and future behavior along a path through the environment.

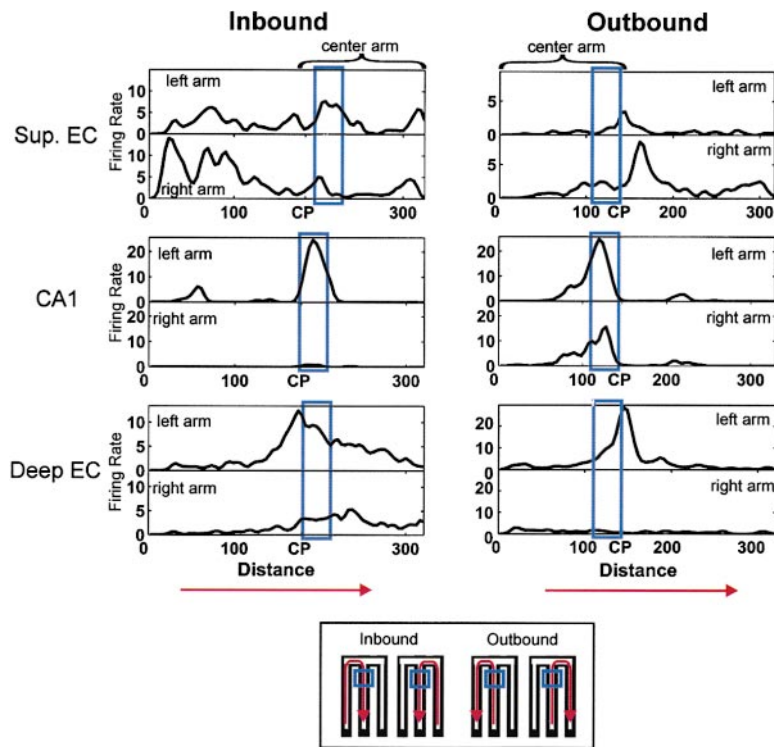


Figure 4. Prediction of Past or Future Location by Firing Rate on the Center Arm

Each row contains plots of firing rate versus distance for two different neurons from each region, one showing retrospective coding (inbound) and the other prospective coding (outbound). Within a row, the left and right top plots show firing from the left arm to the center arm and the center to the left arm, respectively, while the left and right bottom plots in the row show firing from right to center and center to right arms. The location of the choice point “CP” (Figure 1B) is shown on the x axis. The blue box highlights a window on the center arm where the firing rate of the neuron significantly ($p < 0.05$, GLM model) predicted future position or correlated with past position. While CA1 and deep EC neurons showed large differences in firing rate on the center arm across different paths, superficial EC neurons showed only modest firing rate changes. Overall, the degree of position specificity in trajectory-dependent neurons across regions reflected the general trends across regions, as superficial EC neurons were active across many large regions of the environment (Figure 4, top row) while CA1 and deep EC neurons were more place specific (Figure 4, center and bottom rows).

Path Equivalence

While both CA1 and the EC appear to represent position in the context of the path the animal is on, it is not clear how these structures might represent the relationships among different paths through the environment. Marr (1971) hypothesized that the hippocampus acts as a simple memory system, storing single events, while the neocortex both stores and classifies events. The neocortex, then, would perform an essential role in an event memory system, representing the similarities among different events. That would allow the animal to learn regularities in its environment and use those regularities to guide behavior. If the EC plays a role in a system that represents categories of events, we would expect that locations along different trajectories could be represented similarly if those locations were associated with the same phases of the task. In other words, the same cell could be active in different places if the animal was engaging in the same action in both places (e.g., two locations where the animal is turning left toward a food well). If the EC is supporting that sort of generalization, then not only could a single cell fire in behaviorally related locations in one environment, but it could also fire in behaviorally related locations across environments.

We identified a number of cells showing that pattern of activity both within the W track and between the W and the U tracks. We first noted that a number of cells fired in the same relative location and in the same direction on two different paths on the W track. An example of that type of firing pattern is shown in the bottom cell of Figure 2 where the cell fires preferentially as the animal approaches a food well on the outside arms of the track. Many neurons also showed a similar pattern of firing across paths from the W and U tracks (Figure 6A). We labeled this pattern of activity “path equivalence” because the cells fired in the same location relative to the

start and end food wells on two or more different paths. The firing patterns of neurons that showed strong path equivalence can be described in terms of a combination of a path or set of paths and the specific action or behavior the animal was engaged in, such as “moving away from the food well on the rightmost arm” (Figure 5A, top cell) or “moving away from the food well and performing a left turn” (Figure 6A, bottom cell). These patterns of activity cannot be explained as simple behavioral or spatial correlates, as the cells only fire when the animal is executing a specific action in a specific set of locations. Similarly, an explanation based on simple visual responses does not easily account for these patterns of activity. Those cells that were active primarily on the outside arms of the W track (e.g., Figure 2, bottom cell), for example, cannot be responding to a single visual cue and, more generally, as the deep layers of the EC receive the majority of their input from hippocampal structures that do not show simple visual responses, a visual cue-based explanation is unlikely to be correct.

We quantified the prevalence of those patterns of activity across the brain regions studied by developing a path equivalence coefficient. This coefficient captures the extent to which a cell fires at the same relative location while not firing at different or nonequivalent locations on two paths. We examined the distributions of these coefficients for superficial EC, CA1, and deep EC cells by calculating the value of the coefficient for pairs of paths from the W track (W versus W) and for pairs of paths where the first path was from the W track and the second from the U track (W versus U; see Figure 6B). The results from both comparisons were similar. Superficial EC showed a moderate tendency to fire in a path-equivalent manner. The average W versus W and W versus U CA1 path equivalence coefficients are significantly greater than zero (t test, $p < 0.01$), and the distribution of coefficients for CA1 cells appears to be slightly

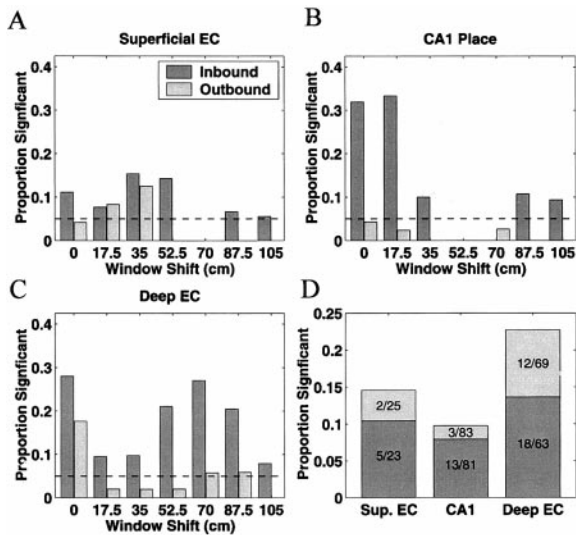


Figure 5. Proportion of Neurons from CA1 and the EC Showing Prospective and Retrospective Coding

(A–C) The proportion of neurons in each region with fields on the center arm that showed either prospective or retrospective coding in each window where cells were tested. The x axis shows the different distances the window was shifted away from the choice point, so 0 represents a window whose edge is at the choice point and 105 represents a window whose closest edge is 105 cm from the choice point. The number of cells that would be expected to show prospective or retrospective coding by chance alone is shown by the dotted line at 0.05 on the y axis. It is clear that retrospective coding was more prevalent than prospective coding and that prospective coding is more frequently seen close to the choice point. It is important to note that the number of superficial EC neurons tested in each window was relatively small (~25), so the actual proportion of units showing path-dependent coding in the superficial EC may differ somewhat from that shown here.

(D) The proportion of all of neurons from each region whose firing patterns showed prospective or retrospective coding as determined by the χ^2 test on the GLM model on all windows ($p < 0.01$). The number of neurons showing prospective or retrospective coding is shown inside each bar. The proportions were normalized to adjust for the different numbers of neurons tested in the inbound versus the outbound direction. The proportions of neurons showing prospective coding were not homogeneous ($p < 0.02$, logistic regression, χ^2 test), suggesting that prospective coding is most prevalent in the deep EC.

positively skewed in both the within and between environment measure, suggesting that a small number of CA1 cells show a tendency to fire in a path-equivalent manner. Overall, however, most CA1 cells did not show path-equivalent firing. In contrast, deep EC neurons showed a strong tendency to fire in a path-equivalent manner both within the W track and between the W and U tracks. The strength of path equivalence for the W versus W measure was not significantly correlated with field length for superficial EC and CA1 ($p > 0.1$), and the correlation was negative for deep EC ($r = -0.17$, $p < 0.02$). There was, in the case of the W versus U path equivalence coefficient, a small but significant correlation between path equivalence and field length for CA1 cells ($r = 0.2$, $p < 0.01$), but the correlation was not significant for either deep or superficial EC cells ($p > 0.1$), so differences in field length cannot explain our results. At the same time, the prevalence of path-equivalent firing in the deep EC may partially explain the lower

position information coefficients as compared to CA1 neurons, as deep EC cells were more often active along multiple paths and thus convey less information about a single position.

In addition, in many cases, path equivalence was associated with prospective/retrospective coding in the deep EC. The cell whose firing is shown in the third row of Figure 6A, for example, coded prospectively for a left turn as the animal came out of the center arm but also fired as the animal approached a left turn from the right arm of the apparatus. We determined that, on average, cells from the deep EC that showed significant prospective or retrospective coding often fired in a path-equivalent manner in the W track, while the same path equivalence was not clearly present in prospective or retrospective coding superficial EC or CA1 cells (mean \pm SD, W versus W path equivalence coefficient for prospective/retrospective cells in each region: superficial EC, 0.05 ± 0.26 ; CA1, 0.05 ± 0.36 ; deep EC, 0.31 ± 0.40).

Conclusion

In summary, our findings strongly suggest that neurons in both CA1 and the EC code for more than just the animal's position in two important respects. First, many cells in CA1 and the deep EC fired at significantly different rates in the same position on the center arm of the track depending on where the animal had come from or where it was going. This differential activity supports prospective or retrospective coding, connecting the animal's current location with past and future positions and behavior. This result is consistent with electrophysiological data from the monkey EC where neural responses to a stimulus were related to subsequent behavior (Suzuki et al., 1997). In addition, in the deep EC, the fields were longer, suggesting that these neurons may represent longer trajectories through the environment.

Second, deep EC neurons, but not CA1 neurons, had a strong tendency to fire at the same relative location along two different paths, even when those two paths were in physically different environments, a pattern of activity we labeled "path equivalence." Superficial EC neurons also showed a tendency to fire in a path-equivalent manner, perhaps as a result of the substantial feedback connections from deep to superficial EC (Witter et al., 1989). The activity of these EC neurons can therefore represent the similarities between locations on different trajectories the animal moved along. Furthermore, there appears to be a connection between prospective and retrospective coding and path equivalence in the deep EC, as many deep EC neurons that showed prospective or retrospective coding also showed strongly path-equivalent firing. That connection supports the hypothesis that deep EC path equivalence is an expression of a representation that relates locations and behavior, as deep EC neurons were found that were both active across locations associated with the same behavior and differentially active in the same location depending on the animal's past or intended future behavior.

Our results are consistent with earlier work on the superficial EC (Mizumori et al., 1992; Quirk et al., 1992), where neurons were reported to show relatively little spatial specificity. In addition, Quirk et al. (1992) reported that superficial EC neurons had similar firing patterns in a square and a cylindrical environment when the animal was foraging for food pellets. We hypothesize that

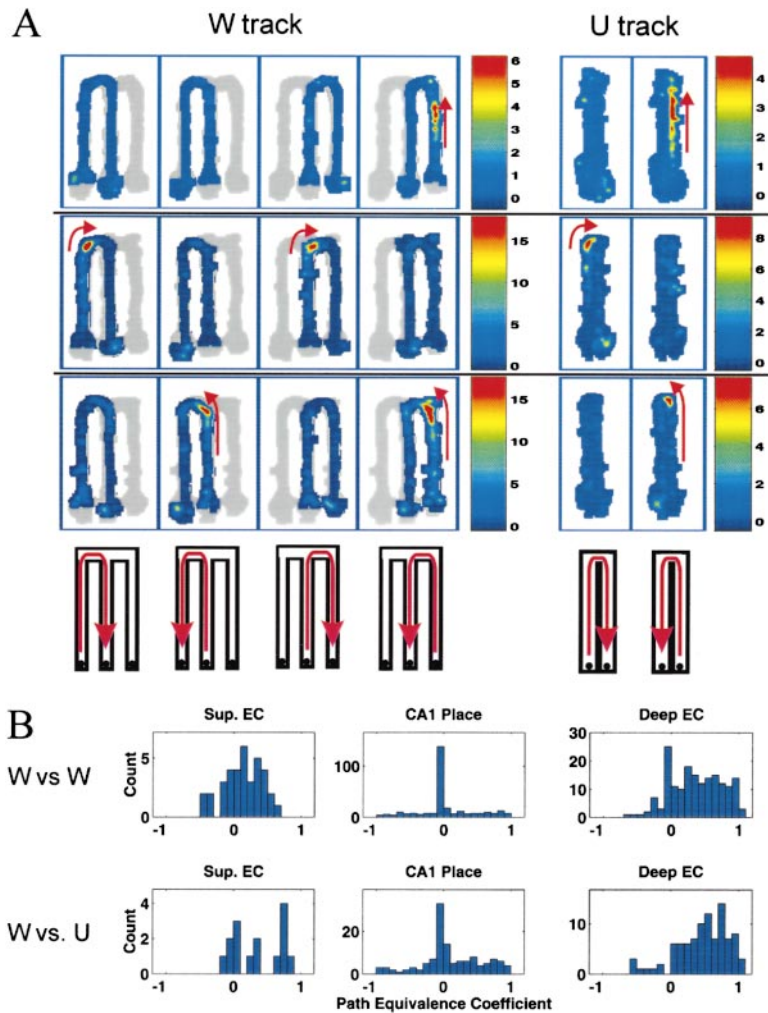


Figure 6. Path-Equivalent Firing in CA1 and the EC

(A) Three deep EC neurons that show strong path equivalence across environments. Each row represents the firing of one cell across the different paths of both the W track and the U track, and the red arrows highlight the path-equivalent activity. The path the animal was on in each panel is pictured below the firing rate maps. The first row shows a cell that fired only in the right arm of both tracks as the animal moved away from the food well. The second row and third rows show cells that had high path equivalence both within and between environments, firing as the animal executes a right turn (second row) or a left turn (third row) as the animal ran away from a food well. The cell in the bottom row is particularly noteworthy because it shows both path-equivalent firing and prospective coding. Note that these cells do not fire during every right or left turn but only during the first turn of the paths on which they are active. The within-environment (W only) path equivalence coefficients for the three cells pictured are 0.0, 0.97, and 0.84, respectively, while the cross-environment (W versus U) path equivalence coefficients are 0.84, 0.92, and 0.46.

(B) The distributions of W versus W and W versus U path equivalent coefficients for superficial EC, CA1 place, and deep EC cells. Beginning with the W versus W path equivalence coefficients, deep EC cells clearly show the strongest tendency to fire at the same relative location on different paths. The means and SDs for the three regions were: superficial EC, 0.13 ± 0.28 , CA1, 0.06 ± 0.40 , deep EC, 36 ± 0.38 . These path equivalence coefficients were all significantly different from one another (Wilcoxon rank sum test, $p < 0.001$). The same pattern of results was evident in the distributions of path-equivalent coefficients measured across the two envi-

ronments. Deep EC cells again had the strongest tendency to fire in a path-equivalent manner. The means and SDs for the three regions were: superficial EC, 0.36 ± 0.37 ; CA1, 0.12 ± 0.41 ; deep EC, 0.50 ± 0.38 . The deep EC coefficient was significantly greater than the CA1 coefficient (Wilcoxon rank sum test, $p < 0.0001$). Thus, the deep EC appears to represent general relationships between positions and specific behaviors both within and across environments.

this similarity across environments may be an expression of something akin to path equivalence, but further studies are needed to understand the relationship between their work and our results. While our findings for superficial EC are consistent with previous reports, Mizumori et al. (1992) reported that deep EC neurons had about the same position specificity as superficial EC neurons, while our results indicate that deep EC neurons' firing contains more position specificity than that of superficial EC neurons. The difference may be due to differences in the measure of position specificity used. We should also note that one of the neurons shown in their paper fires in what we would describe as a path-equivalent manner, as it is active selectively on runs outward from the center on an eight-arm radial arm maze (Mizumori et al., 1992; Figure 5, bottom right).

More generally, our results suggest that neurons in CA1 and the EC can combine information about location with other variables that represent the relationship between past or intended future position and behavior, a result consistent with previous findings (Ranck, 1973; Berger and Thompson, 1978; O'Keefe and Nadel, 1978;

McNaughton et al., 1983; Markus et al., 1995; Deadwyler et al., 1996; Wood et al., 1999). While the computations that underlie the representation of those variables are not yet understood, one explanation offered for the correlates seen in a spatial context (e.g., direction) is that they are the result of differences in the reference frame used by the animal to construct its spatial map (McNaughton et al., 1996; Touretzky and Redish, 1996). Those theories suggest that different sets of neurons are active in different directions in linear environments because the animal uses a different reference frame for constructing a map for each direction. Our results are not easily explained by a reference frame-based theory, however. Most neurons that showed prospective or retrospective coding, particularly those in the deep EC, were active at above baseline level along both paths, and thus it was not the case that entirely different sets of neurons were active along the center arm depending on which path the animal was on. Furthermore, the presence of path equivalence in the deep EC is not consistent with a reference frame-based theory, as no set of fixed reference frames could account for the presence

of path equivalence across different sets of paths for different cells in the same animal.

While it is relatively straightforward to construct a model that explains the longer place fields of deep EC neurons as a result of input from a number of CA1 and/or subicular neurons whose place fields overlap, a model explaining the existence of prospective and retrospective coding and path equivalence is more difficult to generate. Retrospective coding can, at least in principle, be generated by associative connections among CA3 place cells that would connect the representation of locations on the outside arms to locations on the center arm. An explanation of prospective coding, however, appears to require more than simple place cells, as there must be inputs to the superficial EC and/or the hippocampus that provide information about the animal's intended trajectory. As prospective coding has been observed in the primate prefrontal cortex (Rainer et al., 1999), we hypothesize that prefrontal inputs may contribute to the prospective coding we observed.

Similarly, as CA1 cells show little path equivalence, the predominance of that type of coding in the deep EC suggests that the inputs from structures other than CA1 may provide information about the elements of the behavioral task. We hypothesize that this task-related information is combined with place specificity from CA1 in the deep EC to create path equivalence. Sharp (1997) and Sharp and Green (1994) demonstrated that, like superficial EC neurons, subicular neurons had similar firing patterns in a square and a cylindrical environment during random foraging. As such, the subiculum may provide something akin to path equivalence information to the deep EC. Although the activity properties of subicular neurons have not been explored in the tasks used here, we suggest that the activity of these neurons may be related to generalization across environments, and that if the animals were performing a complex behavioral task, these neurons would reflect the similarity of locations across environments based in large part on the similarity of the behaviors associated with them.

If an animal is to successfully find a goal, it must understand the relationship between where it is, where it has been, and where it wants to go, and it must know what behavior it should execute to get where it is going. Furthermore, if the animal is to use its past experiences to guide its behavior, it must be able to generalize across those experiences to extract frequently encountered relationships between locations and behaviors. We hypothesize that the hippocampus refines the relatively weak place and retrospective coding present in the superficial EC to create a representation of position in the context of a trajectory that combines current position and past or intended future position. The deep EC would then extend these representations to relate positions to one another over longer distances and times. Furthermore, while CA1 cells clearly distinguished between the two environments the animal was exposed to, deep EC neurons appear to capture regularities across those environments in a manner that may support the animal's ability to generalize across different experiences and extract useful associations between locations and the behaviors relevant to them.

Experimental Procedures

Behavioral and Electrophysiological Methods

Four adult male Long-Evans rats, three to six months of age, were food deprived, handled, and trained to run along a U-shaped track,

moving from one end of the U to the other and back again, for hot chocolate mix reward. Once the animals were running smoothly (~3–7 days) they were also trained to run in an alternation task for liquid chocolate reward on a W-shaped track. The animals ran in the following pattern: center, left, center, right, center, etc., and were rewarded each time they arrived at a food well in the correct arm. The first two animals were trained on a half-length W track (75 cm long), while the other two were trained on the full-sized track. The animals were trained until they achieved a criterion of 85% correct (~4–7 days), at which point the electrode array was surgically implanted. After recovering from surgery, the animals were run on both the W and U tracks for 20 to 30 min a day with an intervening rest period of ~30 min. The two tracks were supported 60 cm off the floor and located next to each other in the recording room, separated by ~20 cm. The food wells of the two tracks were all on the same side of the room. The U track had a 15 cm high blue vertical divider down the middle, while the center arm of the W track had black walls that angled outwards at ~45°, and the two outside arms of the W track had 2 cm high edges but were otherwise open to the room. The two environments were therefore readily distinguishable. The recording room contained a rack, two banks of computers, and various other clearly visible cues. During the experiment animals performed at between 70% and 100% correct, with a mean of 90% across all four animals.

After each animal had been trained, it was anesthetized and a twenty-tetrode microdrive array was implanted. All surgical procedures were carried out following Massachusetts Institute of Technology and National Institutes of Health guidelines. Of the eighteen tetrodes, thirteen targeted the lateral aspects of the EC (AP -7.8, L 4.8) and seven targeted the CA1 subregion of the hippocampus (AP -3.6, L 2.2). One channel of one tetrode in each region was used as a reference electrode. Once the animal had recovered from surgery (4–7 days), recordings were begun. Neural activity was sampled and stored to disk for offline assignment of spikes to single neurons using the AD software package (M. W. and L. F.). Position information from two infrared diodes mounted on a boom on the animal's head was sampled at 30 Hz and stored to disk. The location of the rear diode, which was directly above the back of the animal's head when the animal was in motion, was used as the position indicator for the animal.

Each day the EC electrodes were advanced so as to sample different neurons. Once single neurons in the EC could no longer be isolated, the animal was deeply anesthetized and between one and four lesions (30 μ A for 3 s) separated by a vertical distance of 325 μ m were made on each electrode. The animal was perfused, the brain sectioned at 50 μ m, and the slices stained with cresyl violet. For the EC electrodes, the number of lesions made with each tetrode, the relative depths of each tetrode, the relative locations of each tetrode in the array, and the degree of shrinkage that the slices underwent during the fixing process was used to reconstruct the location of each tetrode on each day of recording and to thereby assign neurons recorded on that electrode on that day to either the deep or the superficial EC. The penetration through the EC was at a ~45° angle to the ventral cortical surface, so the layers were relatively thick along the tetrode tracks. In addition, layer 4, a cell-poor layer that separates deep from superficial EC, was relatively thick along the axis of the penetration (~100 μ m) and provided a natural separation between deep and superficial layers. Furthermore, we made a concerted effort to ensure that the electrodes emerged from the microdrive parallel to one another. As such, we believe that the potential for misassignment of electrode location was relatively low. Most EC electrode tracks terminated above layer 2 of the EC, so most recordings from superficial EC neurons were from layer 3. In addition, many electrodes were not advanced past the deep layers of the EC, so we are confident that these electrodes sampled only deep EC neurons.

Data Analysis

After each recording session the data were transferred to Linux work stations for analysis. A custom software package (Xclust, M. W.) was used to cluster the spike waveforms from each tetrode into putative single neurons. Only data from the run periods of the recording are examined here. We estimated that the background noise level was

~35 μ V, and we eliminated all units with a mean peak waveform amplitude of <70 μ V, resulting in an average signal-to-noise ratio of at least two to one. In general, only a few neurons could be isolated from each EC tetrode. For tetrodes in CA1, we examined the spatial firing properties of every cell from each tetrode and, for each cell that appeared to have been recorded from across multiple data sets, we eliminated the cell from all but one of the data sets.

We examined the spatial firing properties of the units by examining the firing of each unit along the paths between food wells. For the W track, the four paths the animal ran along were examined (center to left arm, left to center arm, center to right arm, and right to center arm) while for the U track, the two paths from the left to right arm and from the right to left arm were examined. We separated the four paths by defining a bounding box around each food well and determining the times the animal exited from that box on the way to another food well. A path was defined as the set of positions between box exits and therefore includes the time spent at the end food well of each path. We then defined a set of line segments that were located along the center of each path and, for each position sample, found the point along those line segments closest to the animal's position and assigned the position to that point. That linearized each path so that all of the positions were situated along the line segments. We then determined the distance from the first point of the first line segment (the start food well) to each linearized position, thereby translating a set of positions into a set of distances along the path for each path. We then binned those distances into 4.2 cm bins. The animals often ran at speeds over 1 m/s, and this relatively large bin size was chosen to ensure that there was at least one position sample in each bin for each run along a path. We computed the firing rate of each unit in each bin. To further reduce the effect of high velocities on position sampling, each unit's binned firing rate was smoothed with a six point Gaussian window with a standard deviation of one.

We analyzed the activity of each unit along each path by computing two measures of position specificity: average field size and position information. We defined a "place field" as the set of adjacent locations at least 10 cm long over which the firing rate of the neuron was above one-fourth of its maximum firing rate. Adjacent fields where two high-firing rate regions were separated by firing at above one-eighth of the neuron's maximum rate were considered to be a single field. This definition is somewhat similar to that used by Muller et al. (1987) and produced field boundaries that generally agreed with the experimenter's intuitions. We calculated the maximum firing rate as the peak rate across all binned, smoothed path firing rates. Only cells with at least one field with a maximum firing rate greater than 3 Hz were included in our analyses. Average field size was computed as the mean of the lengths of all fields for each unit.

Position information was calculated as the number of bits per spike, treating each path as a separate set of locations. That result, in terms of position information, is the same as if the four paths were joined end to end into one long path. Position information was calculated according to the formula of Skaggs et al. (1993):

$$\text{Position information} = \sum_i P_i(R_i/R) \log_2(R_i/R)$$

where i indexes over the position bins in the environment, P_i is the probability that the animal was in bin i , R_i is the mean firing rate in bin i , and R is the mean firing rate over the environment. It is important to note that the pairs of outbound (center to outside arms) and inbound (outside to center arm) paths on the W track contain the same section of the center arm in the same direction. We treated those paths as distinct because subsequent analyses (see below) demonstrated that a large fraction of CA1 and EC neurons fire differently on the center arm depending on where the animal has come from (inbound paths) or is intending to go (outbound paths), and thus the fields on the center arm for the inbound or outbound paths were often different.

After characterizing the basic spatial firing properties of units in CA1 and the EC, we examined the relationship between the activity of CA1 or EC neurons on the center arm of the track and the animal's previous or subsequent presence on one of the outside arms of the track. These analyses were designed to determine if CA1 or EC firing patterns can reflect not only the animal's current position but

also where it has been or where it intends to go. We defined the choice point (CP; see Figure 1) to be several centimeters (~4-7) from the "T" junction at the end of the center arm, so that between the food well on the center arm and the choice point, the animal's lateral position did not differ substantially depending on which outside arm it was heading toward or coming from. We calculated the firing rate of each neuron in a 35 cm window beginning at the CP and extending toward the food well on the center arm and determined whether firing rate could be used to predict the animal's future choice of outside arm (prospective coding) for outbound paths or correlated with the animal's past location (retrospective coding) for inbound paths. The window was then shifted by multiples of 17.5 cm from 0 to 105 cm along the center arm toward the food well, and each window that overlapped with the neuron's field was examined. That allowed us to examine where along the track prospective or retrospective activity was present.

To determine whether a unit's firing rate in a single window on the center arm showed prospective or retrospective coding, we used a generalized linear model (GLM) in Splus (MathSoft, Cambridge, MA) with a binomial link function (McCullough and Nelder, 1989). This model is a binomial version of regression and uses the formula

$$\log\left(\frac{p}{1-p}\right) = \alpha + \sum_{i=1}^n \beta_i r_i$$

where p is the probability that the animal came from or was going to the left arm, α is an intercept term, n is the number of windows being included in the model (1 for the single window analyses), β_i is a coefficient for the rate variable for the i th window, and r_i is the rate in the i th window. The GLM estimates the α and β_i coefficients and yields T values that can be used to evaluate the probability of the hypothesis that the rate variable r_i predicts p , the outside arm the animal is going to or coming from. For a few neurons the binomial model did not converge, so a Gaussian model was used instead. The GLM with the Gaussian model is identical to normal regression. If a unit had a field on the center arm both outbound and inbound, the two directions were considered independently as two different neurons. A neuron was considered to show prospective or retrospective coding in a particular window if the T value from the firing rate coefficient β_i in the model corresponded to a $p < 0.05$ in a two-tailed test.

The firing rates of CA1 units are known to be modulated by position, head direction, and velocity (McNaughton et al., 1983). We determined whether the observed prospective or retrospective coding was associated with variations in lateral position on the track, head direction, or velocity as follows: for each window where rate was a significant predictor of future or past location for a given neuron, the positions at which spikes occurred and the distributions of lateral positions, head directions, and velocities at the times spikes occurred were found. The distributions of lateral positions, head directions, and velocities at the same positions on the non-preferred trajectory were then found and the distributions on two trajectories compared. A prospective or retrospective coding neuron was determined to show a firing pattern not dependent on head direction or velocity in a given window when the following criteria based on known cell tuning properties (McNaughton et al., 1983; Taube et al., 1990, 1996) were met: (1) the lateral position distributions were statistically indistinguishable at the $p < 0.05$ level, (2) the two edges of the 95% confidence intervals for the distributions of head direction were not more than 10° apart, and (3) the edges of the 95% confidence intervals for velocity were not more than 5 cm/s apart.

The above analyses examine each window independently so that the proportion of cells showing prospective or retrospective coding in each window could be examined. To determine the total proportion of cells showing prospective or retrospective coding anywhere on the center arm, we used the GLM model with $n = 7$ to include all windows and applied a χ^2 test with a criterion of $p < 0.01$ to the model with each r_i term added sequentially. That yielded a p value for each r_i variable, and the neuron was regarded as showing significant prospective or retrospective coding if any r_i corresponded to a $p < 0.01$ and at least one single window from the first GLM model showed significant prospective and retrospective coding that was

not associated with differences in the animals lateral position, head direction, or velocity. That ensured that the error rate for a false positive for a given neuron was 0.01.

We also computed a "path equivalence" coefficient to measure the extent to which a unit fired in the same relative location along different paths. For each cell, the pattern of activity on each path was compared to the pattern of activity on all other paths and normalized by an estimate of the expected similarity based on the place field structure. Only paths that contained a field were included in the analysis. We calculated the coefficient as

$$PEC = \max_{\substack{i = 1...3, \\ j = i + 1...4}} (\text{corr}(P_i, P_j)) - \max_{\substack{i = 1...3, \\ j = i + 1...4}} (\text{corr}(P_i, S_j))$$

where max takes the maximum of its arguments over the specified range of i and j , corr is the Pearson product-moment correlation, i and j reference path number (one through four for the four possible paths for the W track), P_i and P_j are the binned and smoothed firing rates along the entirety of the i th and j th paths, and S_j is a shuffled version of P_j . This measure finds the maximum correlation of the activity on two paths across all pairs of paths and subtracts from that the maximum correlation between activity on a path and a shuffled path across all (path, shuffled path) pairs.

The shuffling was designed to disrupt path-equivalent structure while maintaining as much as possible of the overall place field structure. The shuffling was done on the raw, unsmoothed path firing rates and, after shuffling, the resulting firing rate over distance plot was smoothed with a six-point Gaussian with a standard deviation of one to match the smoothed path firing rates. We shuffled each path by dividing the path in half, mirror reversing the first half, and then switching the order of the two halves. Thus, a path that started as A...BC...D, where A marks the beginning of the path, B and C the center of the path, and D the end of the path, would become C...DB...A. The similar D...CA...B shuffle produces a similar distribution of coefficients. That shuffling is preferable to other possible shuffles where the environment is divided into more than two sections because those shuffles excessively disrupt place field structure. In addition, it is preferable to two other obvious shuffles that minimally rearrange the environment: D...CB...A and C...DA...B. In the case of the D...CB...A shuffle, many cells were active around the turns in the middle of multiple paths, a legitimate path-equivalent pattern, and thus the region around CB was often similar to the region around BC. That resulted in a high (path, shuffled path) correlation and a low path equivalence coefficient. The C...DA...B shuffle is undesirable because some cells did not fire near the beginning or end of a path, and the DA combination therefore produced a large downward deflection in the firing rate. That results in uniformly low (path, shuffled path) correlations, and thus an excessively high value of the path equivalence coefficient. For pairs of trajectories that contained the same direction of motion on the center arm (e.g., center to left versus center to right) the center arm section was omitted from both the normal path and the corresponding section was removed after shuffling from the shuffled path.

To calculate the cross-environment path equivalence coefficient, the paths from the W and U track were scaled to be the same length. The lengths of the paths were generally no more than 5 cm different, so this scaling did not substantively distort the locations of the fields. The W versus U path equivalence coefficient was computed as above with the following modifications to the formula: P_i was the firing rate from a path on the W track, P_j and S_j were the firing rates along a path and a shuffled path from the U track, and thus the index i went from 1 to 4, and the index j went from 1 to 2.

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