

A sense of direction in human entorhinal cortex

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Abstract

Finding our way in spatial environments is an essential part of daily life. How do we come to possess this sense of direction? Extensive research points to the hippocampus and entorhinal cortex (EC) as key neural structures underlying spatial navigation. To better understand this system, we examined recordings of single-neuron activity from neurosurgical patients playing a virtual-navigation video game. In addition to place cells, which encode the current virtual location, we describe a new cell type, EC *path cells*, whose activity indicates whether the patient is taking a clockwise or counterclockwise path around the virtual square road. We find that many EC path cells exhibit this directional activity throughout the environment, in contrast to hippocampal neurons, which primarily encode information about specific locations. These findings support the hypothesis that EC encodes general properties of the current context (e.g., location or direction) that are used by hippocampus to build unique representations reflecting combinations of these properties.

Introduction

Convergent evidence from electrophysiological, noninvasive-imaging, genetic, and lesion studies shows that the hippocampus plays an important role in spatial navigation and episodic memory, both in humans and in animals (Squire, 1992; Vargha-Khadem et al., 1997). An important example of this comes from recordings of rodent hippocampal “place cells” during spatial navigation (O’Keefe & Dostrovsky, 1971). During navigation, the network of place cells encodes the animal’s spatial location because each one activates when the animal is at a particular position in the environment, that cell’s “place field” (Wilson & McNaughton, 1993). However, in addition to location, subsequent studies show that hippocampal neurons may also be sensitive to other factors that can alter how they encode the location. These factors include the direction of movement (McNaughton et al., 1983), the phase of the behavioral task (Griffin et al., 2007), and the navigational goal (Ekstrom et al., 2003; Ainge et al., 2007).

In particular, the current direction of movement often has a dramatic effect on place-cell activity. When an animal’s movement is constrained to a narrow path, a place cell’s place fields usually occur at different locations according to the instantaneous direction (McNaughton et al., 1983; Muller et al., 1994; Markus et al., 1995). In contrast, in open, unconstrained environments, the location of each place field is typically fixed, regardless of the animal’s direction. The cause of these directional patterns is a mystery. Specifically, it is unknown whether directional place-cell responses are caused by an afferent directional signal outside the hippocampus, or whether they originate from local computation within the hippocampus. Examining this issue is important to better understand the role of the hippocampus in spatial and episodic memory.

A powerful way to examine hippocampal processing is to compare the neuronal activity observed in the hippocampus with activity recorded from its primary input, the entorhinal cortex (EC). Studies using this technique have shed light on various hippocampal phenomena. For example, the hippocampal representation of location has been informed by the discovery of EC “grid cells”, each of which indicate whether the animal is positioned at one of various equally spaced locations (Hafting et al., 2005). Computational models show that it is straightforward to compute the animal’s precise location from a population of grid cells (Fuhs & Touretzky, 2006; Solstad et al., 2006). This suggests that the hippocampal representation of location is the direct

result of EC grid cells. In the same way that grid cells help the hippocampus represent location, other EC activity may support various hippocampal phenomena (Hargreaves et al., 2005; Knierim et al., 2006), including direction-sensitive place-cell responses. However, direct reports of EC neuronal activity are relatively infrequent and patterns that could lead to direction-sensitive place cells are undiscovered.

In this study we set out to map the representation of location- and direction-related information in various regions of the human medial temporal lobe, including EC and hippocampus. We examine recordings of human single-neuron activity from neurosurgical patients playing a virtual-reality spatial-navigation video game. In each trial of this game, patients drive along a narrow square-shaped virtual road in either a clockwise or a counterclockwise direction to reach a destination landmark. We observe EC *path cells* that significantly vary their firing rate according to whether the patient is driving in a clockwise or counterclockwise direction, irrespective of the patient's location in the virtual environment. We suggest that this representation of directional information in EC is related to the phenomenon of direction-sensitive hippocampal place cells. This may be part of a general arrangement in which EC neurons encode attributes of the current context and the hippocampus uses this information to create conjunctive neural assemblies that represent combinations of features of the environment.

Results

We recorded a total of 1,419 neurons from widespread brain regions of neurosurgical patients implanted with intracranial depth electrodes for identifying seizure focus for potential surgical treatment for drug-resistant epilepsy. During each recording session, patients played *Yellow Cab*, a virtual-reality video game. In *Yellow Cab*, patients use a handheld joystick to drive a taxi cab through a virtual town to a randomly selected destination. The virtual town has six destination stores arranged along the outside of a narrow square road; the center of the environment is obstructed to force patients to drive in a clockwise or counterclockwise direction around the road to their destination (Fig. 1). In each trial, the destination store is randomly selected, ensuring patients repeatedly traverse every part of the environment across multiple deliveries.

To identify the patterns of neuronal activity used to perform this task, we examined the relation

between each neuron's firing rate and the patient's simultaneous behavior. Here we observed two patterns: First, some neurons varied their firing rate according to the patient's heading at certain locations in the virtual environment. We refer to these neurons as path cells. Some path cells exhibited this pattern throughout the environment in a manner that their spiking encoded whether the patient was currently travelling clockwise or counterclockwise around the virtual road. Second, we also identified place cells (O'Keefe & Dostrovsky, 1971; Ekstrom et al., 2003; Hori et al., 2005), each of which significantly increased their firing rate when the patient was positioned at a small set of virtual locations.

To quantify these observations systematically, we developed a statistical framework to classify each neuron as a path cell, a place cell, or neither (See Methods for details.) Briefly, we classified a neuron as a path cell if, in at least 10% of locations in the environment, the cell's firing rate significantly varied according to the current clockwise or counterclockwise direction of movement. We labeled a neuron as a place cell if it exhibited a significantly elevated firing rate in at least one contiguous set of locations, compared with the firing rate in the rest of the environment. We assessed the statistical significance of these phenomena via a resampling procedure.

Using this statistical framework, we identified a total of 81 path cells. As an example, Figure 2A depicts the activity of one path cell from patient 2's right EC (also see Supplementary Movie 1). Here we plotted the firing rate of this neuron as a function of the patient's virtual location and direction (clockwise or counterclockwise). This neuron's activity appeared to closely relate to patient's direction of movement because it was highly active during clockwise movement throughout the environment (left panel), but showed little activity during counterclockwise movement (left-middle panel). To examine this directional activity more specifically, at each location we directly compared the neuron's firing rate between clockwise and counterclockwise traversals. This analysis indicated that this cell's clockwise-specific directional activity was statistically robust at 94% of traversed locations in the virtual environment (middle-right panel). Thus, this neuron's activity encodes the patient's clockwise or counterclockwise direction of movement regardless of the current spatial location.

One possible explanation for this neuron's apparent clockwise-specific activity was that this cell was actually responding to right turns (cf. Sato et al., 2006), since right turns are prevalent in clockwise paths. To examine this possibility, we separately analyzed direction-related activity

during the portion of each trial when the patient was turning and during the portion of each trial when the patient was travelling straight (see Methods). We found that this neuron's robust clockwise-specific activity was present not only during turns, but also during straight movement (rank-sum tests, both $p < 10^{-12}$; Fig. 2A, right panel). Thus, this neuron's activity appeared to be a cognitive correlate of the "clockwiseness" of the current path, rather than a perceptual or motor-related response to right turns.

In addition to this cell, we observed that many other neurons followed this motif in which they encoded the patient's clockwise or counterclockwise direction of movement throughout a large subset of the environment (e.g., Figs. 2B–F). Next, we aimed to quantify the prevalence of this phenomenon and measure its properties across the entire dataset. To do this, we calculated the proportion of the traversed locations in the environment where each path cell exhibited significant directional activity, A_{dir} , and the proportion of these directional locations that were in the path cell's preferred clockwise or counterclockwise direction, D_{pref} . We found that 38% of the path cells we observed had $D_{pref} > 0.95$ (Fig. 3A), indicating that they encoded either a clockwise heading or a counterclockwise heading throughout at least 95% of the environment—we refer to these as *clockwise path cells* or *counterclockwise path cells*. In one notable patient's right EC (Fig. S2) we observed a number of both clockwise path cells (e.g., Figs. 2A, & 2C) and counterclockwise path cells (e.g., Fig. 2B). Notably, across Yellow Cab sessions conducted in this patient on different days, we often found that the path cells recorded from the same microelectrode maintained their same clockwise or counterclockwise preferred direction (see inset plots in right panels of Fig. 2A–C).

In addition to clockwise or counterclockwise path cells, our statistical framework also identified a set of *complex path cells* which exhibited directional activity at widespread locations in the virtual environment but had different preferred directions across these locations. Thus, complex path cells had large values of A_{dir} , but had $D_{pref} < 0.95$ (see Figure S1 for examples.) Unfortunately, in our limited dataset it is difficult to distinguish the behavior of complex path cells from other navigation-related neuronal phenomena, such as head-direction cells (Taube et al., 1990), goal-related place cells (Ekstrom et al., 2003; Ainge et al., 2007), and spatial-view cells (Rolls, 1999). For example, a "north" head-direction cell might appear as a complex path cell that is active at clockwise headings in the west part of the environment and active at counterclockwise headings in the east part of the environment (e.g., Fig. S1D).

Next, we aimed to determine, across our entire dataset, whether path cells were significantly clustered in particular brain regions or if they were homogeneously distributed throughout the brain. We computed the probabilities of observing path cells in different brain regions (Fig. 3D), and we found that path cells were not distributed uniformly ($\chi^2(5) = 90, p < 10^{-13}$). Rather, they were especially prevalent in the EC where 21% of the neurons we observed were path cells—this was significantly greater than the Type 1 error rate of our statistical framework (one-sided binomial test, $p < 10^{-12}$). We also observed significant numbers of path cells in hippocampus ($p = 0.003$) and in parahippocampal cortex ($p = 0.002$), and also many path cells in frontal cortex ($p = 0.06$).

One possibility is that path cells from different brain regions have divergent properties. To examine this, we compared the properties of the path cells from hippocampus, EC, parahippocampal cortex, and frontal cortex (Fig. 3A–C). We found that path cells in each of these regions exhibited significantly different areal extents of directional activity, as measured via A_{dir} (Kruskal–Wallis nonparametric ANOVA, $H(3) = 18.6, p = 0.0003$). Specifically, EC path cells had significantly larger values of A_{dir} than path cells in hippocampus, parahippocampal cortex, and frontal cortex (post-hoc rank-sum test, $p = 0.0003$). This indicates that EC path cells exhibited directional firing at a greater proportion of the locations in each environment than path cells in other regions. We also tested for regional differences in the tendency for path cells to have a single clockwise or counterclockwise preferred direction, as assessed through D_{pref} (Fig. 3C). Here we found that path cells in EC had significantly larger values of D_{pref} than path cells in other regions (nonparametric ANOVA, $H(3) = 9.9, p = 0.019$; post-hoc rank-sum test, $p = 0.004$). Thus, EC path cells were more likely to encode a single clockwise or counterclockwise preferred direction across much of the environment (e.g., Fig. 2), whereas complex path cells were more prevalent in other brain regions (e.g., Fig. S1).

Although most place-cell researchers focus on spatial coding in excitatory cells, in recent literature the functional role of interneurons has received increased interest. Historically, interneurons were often excluded from navigation studies because they did not show a clear relation to spatial navigation (e.g., Christian & Deadwyler, 1986). However, recent studies that used more sensitive statistical techniques show that some interneurons robustly encode location-related information (Ego-Stengel & Wilson, 2007; Wilent & Nitz, 2007). In our dataset, we estimated that 9.9% of neurons were putative interneurons (see Methods). To test for potential differences in the properties of

interneuron and noninterneuron path cells, we conducted a separate analysis of the interneurons that fulfilled our path-cell criteria. The only brain region where significant numbers of path cells were found was EC (30%). This proportion was more than the expected by chance (one-sided binomial test, $p = 10^{-4}$) but it did not significantly differ from the proportion of EC path cells observed among putative principal cells ($p > 0.1$). Furthermore, we compared the properties of interneuron and non-interneuron EC path cells and found that they did not significantly differ in terms of A_{dir} (rank-sum test, $p = 0.93$) or D_{pref} (rank-sum test, $p = 0.36$). Because we did not observe any significant differences between these groups, we pooled all cells for our statistical analyses (Fig. 3).

In addition to path cells, we identified a total of 124 place cells (see Methods). Each place cell was especially active when the patient was at a particular location and facing in a certain direction, compared with all other locations. Thus, the activity of these place cells was much more spatially focused than path cells. For example, Figure 4A depicts the activity of one hippocampal place cell which had a significantly elevated firing rate when travelling clockwise through a location in the southwest corner of the environment. Like this example, all the place cells we observed were unidirectional, meaning that their increased activity at a particular location was contingent on the patient facing in a certain direction. These direction-sensitive place cells have not been reported before in humans (cf. Ekstrom et al., 2003). However, they are often observed in the hippocampus of rodents navigating along thin tracks that constrain the direction of movement, similar to the narrow virtual road in our task (McNaughton et al., 1983; Muller et al., 1994; Markus et al., 1995). We identified statistically significant quantities of place cells in hippocampus, in EC, in parahippocampal gyrus, and in frontal cortex (one-sided binomial tests, all $p < 0.02$, Fig. 5).

One potential explanation for the clockwise or counterclockwise directional activity of path cells is that they are encoding the right or left direction of a future or past turn. Indeed, recent studies showed that rodent EC neurons performed this prospective coding (encoding future events) or retrospective coding (encoding past events) (Frank et al., 2000; Lipton et al., 2007), and one path cell in our dataset appeared to have an especially elevated firing rate when approaching a clockwise turn (Fig. 2C). To examine this issue, we conducted a separate set of analyses to determine whether prospective or retrospective coding might explain the path-cell activity we observed. To examine the potential role of prospective coding in path-cell activity, we separately analyzed each path

cell's activity in the final, straight approach to the destination store (i.e., after leaving the corner of the environment; see Fig. 6A). During this final approach, the patient is driving directly to their intended destination where they will deliver their passenger and then make a pause before the next delivery. Here, they have no information regarding future, post-delivery turns and thus neuronal activity during this interval is not likely to be related to prospective coding of an upcoming turn. We calculated the firing rate of each path cell during this final approach and found that this activity was consistent with path cells' overall clockwise or counterclockwise preferred directions (sign-rank test, $p < 10^{-8}$; Fig. 6B). Thus, it appeared that path cells still encoded their preferred direction even after accounting for the potential influence of prospective coding. We also conducted an analogous analysis to determine whether retrospective coding could account for our results. To do this, we examined neuronal activity at the beginning of each delivery before reaching the environment's corner (Fig. 6C). Again, we found that path cells significantly encoded their preferred direction during this period (sign-rank test, $p < 10^{-8}$), indicating that retrospective coding does not account for the path-cell activity we observed. Based on both of these analyses, we suggest that the direction-dependent activity of path cells reflects a different representation of direction than would be predicted on the basis of either prospective or retrospective coding.

Discussion

The primary finding in the current study is that the human EC contains path cells that encode information about the direction of the current route. Below we comment on the relation between path cells and the current body of knowledge on the electrophysiology of spatial navigation, and we discuss the role of the EC in theories of human cognition.

Studies analyzing recordings from the EC of navigating rodents have produced two primary classes of findings: the discovery of grid cells, which encode pure location and (sometimes) compass-like heading information (Hafting et al., 2005; Sargolini et al., 2006), and the discovery of retrospective- or prospective-coding cells, which encode information about past or future movements when the animal is at certain spatial locations (Frank et al., 2000; Lipton et al., 2007). Our discovery of EC path cells shows that the human EC also encodes a new type of information—the circular direction of the current path—that is independent of the current spatial location. Because

the activity of clockwise path cells is insensitive to the precise spatial location, it is fundamentally different than the representations encoded in hippocampus, which are typically closely linked to the current location. Our findings support the view that the hippocampal–entorhinal system operates in this manner: Each EC neuron encodes a perceptual, spatial, or cognitive attribute of the current environmental context. This occurs in a nonspecific manner so that similar, but nonidentical, contexts have comparable EC representations. Path cells may be part of this phenomenon such that each path cell encodes circular direction in the same manner across many environments. Then, in the hippocampus, this information is used to create a unique representation of the current context that is highly specific to the precise combination of currently active EC neurons (Norman & O'Reilly, 2003; Howard et al., 2005).

The phenomenon of retrospective- and prospective-coding (Frank et al., 2000) is broadly related to the clockwise and counterclockwise path cells we observed because in both phenomena cells encode information that is correlated with the direction of an upcoming or previous turn, and because both may do so at more than one spatial location. The critical difference between path cells and neurons performing prospective- or retrospective-coding is that clockwise and counterclockwise path cells provide a directional signal throughout the entire environment. In contrast, neurons performing retrospective or prospective coding only encode information about past or future actions at certain locations, such as before or after turns (Frank et al., 2000) or at locations where navigation decisions are made (Lipton et al., 2007). One way of conceptually relating path cells, place cells, and retrospective- or prospective-coding cells is to organize the information they encode in terms of their locational and directional content: Path cells encode only direction information, place cells encode only location information, and retrospective- or prospective-coding neurons encode a combination of directional and location information. Thus in terms of their information-coding properties, retrospective- and prospective-coding cells can be regarded as a hybrid between path cells and place cells.

At first glance, the spatial firing pattern of the path cell depicted in Figure 2C is reminiscent of a grid cell (Hafting et al., 2005) because it was especially active near the corners of the environment, during clockwise movement. (A similar pattern could appear from a grid cell that had its grid vertices aligned to the square track's corners (Derdikman et al., 2006)). However, we do not believe that our results directly correspond to grid cells. There are two critical differences between

this neuron's activity and the behavior of grid cells (Hafting et al., 2005) or grid \times direction cells (Sargolini et al., 2006). First, whereas grid \times direction cells are active when certain locations are traversed at a fixed bearing (e.g., when driving north), the direction at which this cell activates varies depending on the instantaneous location (e.g., driving south in the east part of the environment and driving north in the west part of the environment). Second, whereas grid cells are typically silent when the animal is between the grid's vertices, this cell also had a robust directional signal between the locations exhibiting peak activity. One possibility is that the spatial activity pattern seen in Figure 2C reflects increased attentional demands when approaching a turn. One might imagine that a patient attends to the task more closely when nearing locations where they must execute a turn. Therefore, this cell's especially increased clockwise activity before turns may reflect an attention-related gain modulation (Salinas & Thier, 2000) of the underlying directional activity.

We note that Hough and Bingman (2004) reported neurons in the pigeon hippocampus that they also identified with the term "path cells" because they exhibited elevated firing throughout large sub-regions (paths) of the constrained environment, similar to place cells with especially large place fields. The directional properties of these pigeon path cells were not reported and thus Hough and Bingman's (2004) findings qualitatively differ from the path cells we describe.

One significant difference between our current findings and recent literature concerns place cells. Whereas most studies report that place cells are especially prevalent in hippocampus, here we found place cells in various brain regions (Fig. 5). This relatively low number of hippocampal place cells, relative to previous studies (cf. Muller, 1996; Ekstrom et al., 2003) may be explained by potential differences in the navigational demands of this task. Previous human studies show that the use of a spatial or nonspatial strategy to perform a particular task can dramatically affect the resultant hippocampal activity (Maguire et al., 2003). Because our task involved a relatively simple, mostly one-dimensional environment, participants may have relied more heavily on nonspatial navigational strategies, such as learning the relative ordering of adjacent stores, to perform the task. This could account for the relatively small number of hippocampal place cells we observed. A further difference in our results was that all the place cells we observed are unidirectional, meaning that they are only active when their place field was traversed in a certain direction. In contrast, a previous study from our group indicated that human place-cell responses in an unconstrained virtual city were not sensitive to the direction of movement (Ekstrom et al., 2003).

However, our observation of directional place cells is consistent with findings from studies in which rodents navigated narrow tracks like the road in our task. In these studies, the track constrains the rodents' movements to one of two directions, and different place fields appear in each direction (McNaughton et al., 1983). These results are often explained by the hypothesis that different "cognitive maps" are used for each direction of movement (Markus et al., 1995). Here we suggest the same phenomenon may be occurring because the road in our task is surrounded by high walls and thus appears narrow and constraining on the laptop screen (Fig. 1A). Therefore, this environment may induce patients to use different cognitive maps for clockwise movements than for counterclockwise movements. The activity of EC neurons, such as clockwise or counterclockwise path cells, may be one of the primary factors in determining which directional map is utilized by the hippocampus during navigation.

Our discovery of EC path cells provides a further striking demonstration of the broad range of information that may be encoded in EC. In addition to the directional information encoded by path cells, the EC contains neurons encoding various other cognitive variables, including characteristics of the current behavioral task (Griffin et al., 2007), future or past actions (Frank et al., 2000; Lipton et al., 2007), the contents of working memory (Suzuki et al., 1997), the objects currently being viewed (Ekstrom et al., 2003; Quiroga et al., 2005), and the current spatial location (Quirk et al., 1992; Hafting et al., 2005). Taken together, these findings support the view that the EC plays a pivotal role in memory formation because EC neurons encode attributes of memories that are subsequently stored by the hippocampus (Norman & O'Reilly, 2003; Howard et al., 2005). Going forward, we suggest that more fully characterizing the diverse information-coding properties of EC neurons is an important step to understanding human cognitive representations.

Methods

Behavioral Task. The data for this study came from 13 patients undergoing surgical treatment for drug-resistant epilepsy (surgeries performed by I.F.). This study was in compliance with the guidelines of the Medical Institutional Review Board at UCLA. We examined data from thirty-four 30–50-minute testing sessions which were conducted in patients' spare time between standard clinical procedures. (Individual patients completed between 1 and 4 testing sessions.)

In each testing session, patients played *Yellow Cab* (Ekstrom et al., 2003; Newman et al., 2007), a taxi-driver video game, on a laptop computer in their hospital room. In *Yellow Cab*, patients use a handheld joystick to drive through a virtual three-dimensional environment delivering passengers to their requested destinations. At the beginning of the first testing session, patients completed a short four-delivery practice session to familiarize them with the game. (We did not analyze the data from this practice session.) In the main part of the experiment, the taxi cab were placed in a large virtual environment that contained six possible destination stores arranged on the perimeter of a narrow square-shaped road (Fig. 1). Each destination store is marked by a brightly colored storefront with a sign displaying its name. The patient delivers a passenger by driving the cab into the front of the store. After each delivery, text appears on-screen displaying the name of the next destination.

In the virtual environment, two stores are located on each of the east and west walls, and one store was on each of the north and south walls. Between stores, the wall consisted of pale, non-descript buildings. The center region of this environment was obstructed by buildings, so patients had to choose to drive in either a clockwise or counterclockwise direction around the road. (As a result of this obstruction, we found that patients indeed drove in a clockwise or counterclockwise direction 99.92% of the time, excluding store approaches). Defining the total size of the virtual environment as 100×100 VR units, the width of the road was 25 VR units and the obstructed area in the center of the road was 50×50 VR units. During navigation, patients had a 60° field of view. Patients pushed the joystick forward to accelerate forwards (maximum forward speed=12.5 VR units/sec) and pulled the joystick back to accelerate backwards (maximum backwards speed=5 VR units/sec). The patients could turn by pushing the joystick left or right (maximum angular velocity= $40^\circ/\text{sec}$). To encourage patients to take the shortest route to each destination, patients received fifty points for each successful delivery and had one point deducted for each second they spent navigating. The running point count was displayed on-screen at all times and patients were verbally instructed to maximize their point total.

Electrophysiology. In each patient, we recorded spiking activity at 28–32 kHz using 40- μm platinum–iridium microwire electrodes (Fried et al., 1999) connected to a Cheetah recording system (Neuralynx Inc., Bozeman, MT). Nine microwires extended from the tip of each clinical depth

electrode. The first eight wires were insulated except for their tip and were used to record action potentials. The ninth microwire had its insulation stripped for \approx 1 cm and served as the voltage reference for the other eight microwires. Action potentials were manually isolated using spike shape, clustering of wavelet coefficients, and inter-spike intervals (Quiroga et al., 2004).

Electrode locations were planned by clinical teams in order to map the seizure focus for potential subsequent resective surgery. Thus the planned recording sites were distributed across widespread brain regions (1,419 total neurons: 301 in hippocampus, 176 in entorhinal cortex [EC], 93 in parahippocampal gyrus, 270 in amygdala, 335 in frontal cortex, and 244 in temporal and parietal cortices). We designated cells with mean firing rates above 10 Hz as putative interneurons (Viskontas et al., 2007). We found that 9.9% of all cells were putative interneurons (hippocampus, 7.6%; EC, 19%; parahippocampal gyrus, 2.2%; amygdala, 6.7%; frontal cortex, 8.1%; temporal and parietal cortex, 16%). To visually depict the location of the right EC neurons from patient 2 (Figs. 2A–C), we studied a computed-tomography image taken after electrode implantation and superimposed the electrode's location onto a pre-operative magnetic-resonance image (Fig. S2).

Data analysis. In order to determine how neuronal activity related to the patient's behavior in Yellow Cab, we computed the firing rate of each neuron in 100-ms epochs throughout the task. We then labeled each epoch according to the patient's instantaneous location, whether they were currently pointed in a clockwise or counterclockwise direction, and whether they were turning or driving straight. The patient's direction (clockwise, counterclockwise, or neither) was calculated from the patient's bearing and location. For example, if a patient was pointed south in the east part of the environment then their direction was clockwise. We discarded epochs when the patient was not driving clockwise or counterclockwise (e.g., if the patient was facing the outer wall while turning around) or when their speed was below 5 VR units/sec. A patient was considered to be turning if their angular velocity exceeded 15°/sec. When the patient's velocity, direction, or turning status changed in the middle of an epoch, we labeled that epoch with the most frequent value of the variable during the epoch.

Our initial visual observations revealed *path cells* that significantly varied their activity depending on the patient's direction at certain locations in the virtual environment. To examine this phenomenon in detail, we designed a statistical framework to identify path cells and measure their

detailed spatial firing characteristics. We defined path cells as neurons that exhibited direction-related activity throughout large areas of the environment. First, at each location we measured whether each neuron was active at a significantly different rate depending on whether the patient was moving clockwise or counterclockwise. This analysis was conducted separately at each location in a 100×100 VR-unit grid across the virtual environment. For each pixel, we identified all nearby epochs as those occurring within 10 VR units of that location. If there were at least 10 nearby clockwise epochs and at least 10 nearby counterclockwise epochs, we then used a two-tailed rank-sum test to compare the firing rates between these two sets of epochs. A neuron was determined to exhibit a significant effect of directionality at that pixel if this test indicated ($p < .01$) that the median firing rate at that location significantly differed between these distributions.

Next, to summarize the areal extent of each cell's directional activity, we computed A_{dir} , which is the fraction of locations in the environment which exhibited significant directionality, out of all traversed locations. Next, we used a reshuffling procedure to estimate the distribution of A_{dir}^{shuf} , a random variable we created to indicate the fraction of spatial locations at which this cell would exhibit significant directional activity by chance. To perform this reshuffling, we randomly time-shifted the neural firing rates relative to the behavioral epochs and computed A_{dir}^{shuf} on the reshuffled dataset; this was repeated 100 times. This reshuffling procedure is important because it estimates the prevalence of spurious directional activity that could appear as a result of temporal correlations in a neuron's spike train or in the patient's behavior. We determined whether each neuron was a path cell by comparing the distribution of A_{dir}^{shuf} estimated via the reshuffling procedure with the actual value of A_{dir} . A neuron was considered to be a path cell if A_{dir} exceeded the 95th percentile of A_{dir}^{shuf} (i.e., $p < 0.05$). Furthermore, we established the additional criterion that A_{dir} must be greater than 0.1 for a cell to be considered a path cell, to ensure that a bona fide path cell exhibits directional activity throughout a large fraction of the environment.

We identified a set of path cells that had a consistent clockwise or counterclockwise directional preference across nearly all the locations that exhibited any directional activity (Fig. 2). To measure this phenomenon, we first identified the preferred clockwise or counterclockwise direction of each path cell according to which direction exhibited higher neuronal activity across a larger area of the environment. Then we calculated D_{pref} , which is the fraction of spatial locations that had a significant preference for the preferred direction, out of all locations that exhibited a significant di-

rectional effect in either direction. This quantity varies between 0.5 (half of significantly directional locations are clockwise, half are counterclockwise) and 1 (all significantly directional locations are in the preferred direction).

For cells that did not meet our path-cell criteria, we also conducted a series of analyses to identify place cells. In contrast to our path-cell analyses, where we identified neurons that exhibited directional activity throughout large areas of the environment, here we aimed to find specific locations where the cell's firing rate significantly increased. Thus, each place cell encodes the patient's presence at this virtual location (the cell's "place field"). To do this, we used a statistical criterion such that a neuron classified as a place cell must have at least one set of contiguous spatial locations where the cell's firing rate is significantly elevated compared with the baseline firing rate in the rest of the virtual environment. At each location across the environment, we used a one-sided rank-sum test to compute the probability P_{place} that the firing rate at nearby locations (distance ≤ 10 VR units) was significantly greater than the firing rate at distant locations (> 10 VR units). We conducted separate place-field analyses for clockwise and counterclockwise movements because rodent navigation studies show that place fields in constrained environments (like the road in our task) are unidirectional (McNaughton et al., 1983; Muller et al., 1994; Markus et al., 1995). (We also conducted a separate, albeit unsuccessful search for omnidirectional place cells.) We assessed the statistical significance of each place field via 100 iterations of a time-shifting reshuffling procedure (see above for details). For each reshuffled dataset, we calculated $P_{first_field}^{shuf}$, the smallest p threshold yielding at least one place field. (Here we defined a place field as a contiguous region of $\geq 2\%$ of the environment that exhibited significantly increased spiking activity compared with distant locations, according to a one-sided rank-sum test.) We then took the 5th percentile of $P_{first_field}^{shuf}$ and used it as the threshold for assessing the statistical significance of place fields based on the values of P_{place} from the original dataset. This reshuffling procedure ensured that the Type 1 error rate for identifying a place cell in the original dataset is fixed at 5%.

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Figure Captions

Figure 1. The *Yellow Cab* virtual-navigation video game. **A.** A patient's on-screen view of the environment during Yellow Cab. **B.** Overhead map of the environment. Possible destination stores are brightly colored and outlined in red. Pale-colored buildings form the remainder of the outer and inner walls of the environment.

Figure 2. Clockwise and counterclockwise path cell activity. **A.** The firing rate of a clockwise path cell from patient 2's right entorhinal cortex (microelectrode 13) during testing session 2. Left panel depicts neuronal firing rate during clockwise movement superimposed on an overhead map of the virtual environment. Color indicates the mean firing rate (in Hz), gray lines indicate the path of the patient. Middle-left panel depicts neuronal activity during counterclockwise movements. Middle-right panel indicates whether firing rate at each location was statistically greater (rank-sum test) during clockwise movements (red) or during counterclockwise movements (blue). (Spatial statistics: $A_{dir} = 0.94$ and $D_{pref} = 1$; see Methods for details.) Right panel shows the firing rate of this neuron combined across all regions of the environment for clockwise ("CW") and counterclockwise ("CCW") movements during straight movements and turns ("Turn"). Inset plot shows the activity of a neuron recorded from this same microelectrode in a different testing session. **B.** Activity of a counterclockwise path cell recorded from patient 2's right entorhinal cortex (microelectrode 15). ($A_{dir} = 0.78$ and $D_{pref} = 1$.) **C.** Activity of a clockwise path cell recorded patient 2's right entorhinal cortex (microelectrode 9). ($A_{dir} = 0.81$ and $D_{pref} = 1$.) **D.** Activity of a counterclockwise path cell recorded from the left entorhinal cortex of patient 5. ($A_{dir} = 0.29$ and $D_{pref} = 0.99$.) **E.** Activity of a counterclockwise path cell from patient 4's right parahippocampal gyrus. ($A_{dir} = 0.33$ and $D_{pref} = 1$.) **F.** Activity of a counterclockwise path cell from patient 13's right orbitofrontal cortex. ($A_{dir} = 0.45$ and $D_{pref} = 1$.)

Figure 3. Characteristics of path cells. **A.** Each path cell is portrayed as a dot positioned to indicate its value of A_{dir} , the fraction of the environment at which it exhibited directional firing, and D_{pref} , the proportion of the exhibiting directional firing that were in the cell's overall preferred direction (see Methods for details). Each dot's color indicates the brain region where the cell was observed. Black dashed line separates clockwise or counterclockwise path cells ($D_{pref} > 0.95$)

and complex path cells ($D_{pref} \leq 0.95$). Only path cells from brain regions where significant numbers of path cells were observed are included (see text). **B.** Mean value of A_{dir} for all path cells in each region; error bars denote 95% confidence intervals. **C.** Histogram for values of D_{pref} from path cells in each region. Horizontal axis (D_{pref}) has the same scale for panels A and C. **D.** Regional distribution of *path cells*: Bars depict the percent of neurons observed in each brain area that were path cells (see Methods for total region-wise cell counts). Dark shading indicates clockwise or counterclockwise path cells, light shading indicates complex path cells. Region key: H, hippocampus; EC, entorhinal cortex; PHG, parahippocampal gyrus; A, Amygdala; Fr, Frontal cortices; Cx, parietal and temporal cortices. Black dashed line and 'x's indicate the percent of path cells expected by chance (Type 1 error rate). Asterisks indicate regions in which the number of observed path cells significantly exceeds the Type 1 error rate (one-sided binomial test; black asterisks, $p < 0.05$; gray asterisk, $p = 0.06$).

Figure 4. Example place cells. **A.** Activity of a place cell from the hippocampus of patient 1. Left panel shows this cell's activity during clockwise movement, middle panel shows this cell's activity during counterclockwise movement. Color scale indicates each cell's firing rate (in Hz). Right panel indicates the computed place field of this cell (see Methods). The red place field indicates that this cell was active when the place field was traversed while travelling clockwise. **B.** Example place cell from the hippocampus of patient 5. **C.** A place cell from the hippocampus of patient 13. **D.** A place cell from the entorhinal cortex of patient 5. In the right panel, the blue-colored place field indicates that this cell was active when the place field was traversed while moving counterclockwise.

Figure 5. Regional distribution of place cells. Bars depict the percent of neurons in each region that were place cells (see Methods for total region-wise cell counts). Region abbreviations and colors same as in Figure 3. Black dashed line and 'x's indicates expected the percentages of path cells to be expected by chance. Black asterisks indicate regions in which the the number of observed path cells significantly exceeds the number expected by chance (binomial test, $p < 0.05$).

Figure 6. Analysis of retrospective and prospective coding as an explanation for path-cell activity. **A.** Overhead map of the environment, superimposed with example path in one trial. Blue portion of path indicates the initial path segment that is analyzed in our retrospective-coding analysis; red portion of path indicates the final path segment analyzed in our prospective-coding analysis (see text). Black cross-hatched region indicates the corner turn region of the environment. **B.** Histogram of mean path-cell directional firing during the initial path segment. The median of this distribution (0.61 Hz) is greater than 0 (sign-rank test, $p < 10^{-8}$). **C.** Histogram of mean path-cell directional firing during the final path segment (median= 0.55 Hz ; sign-rank test, $p < 10^{-8}$).

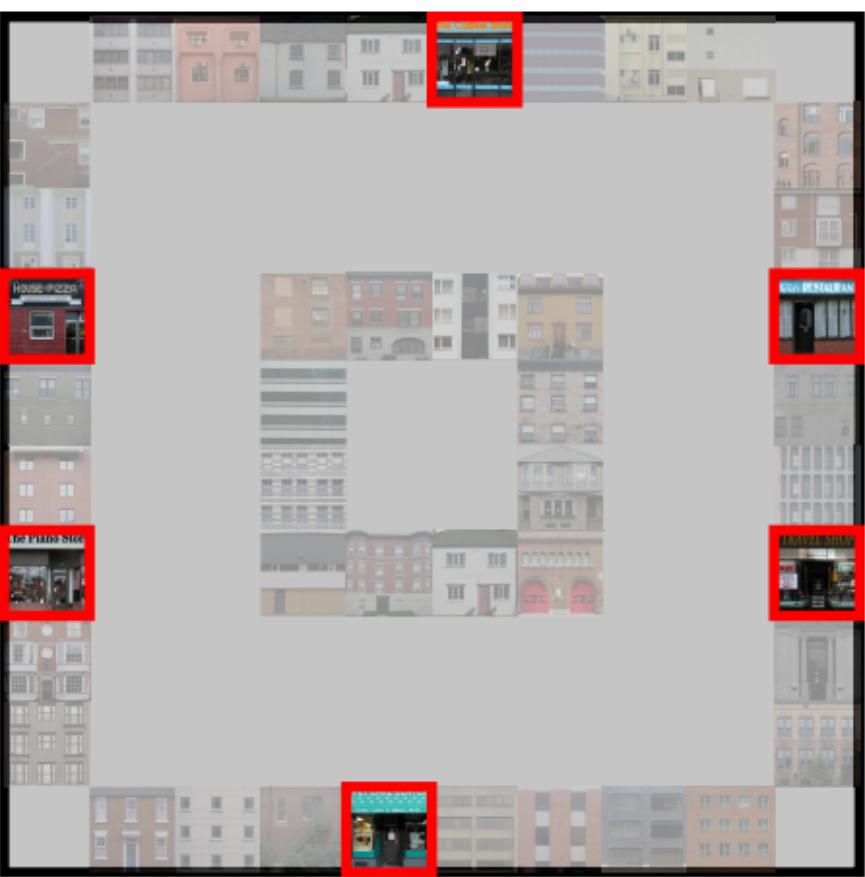
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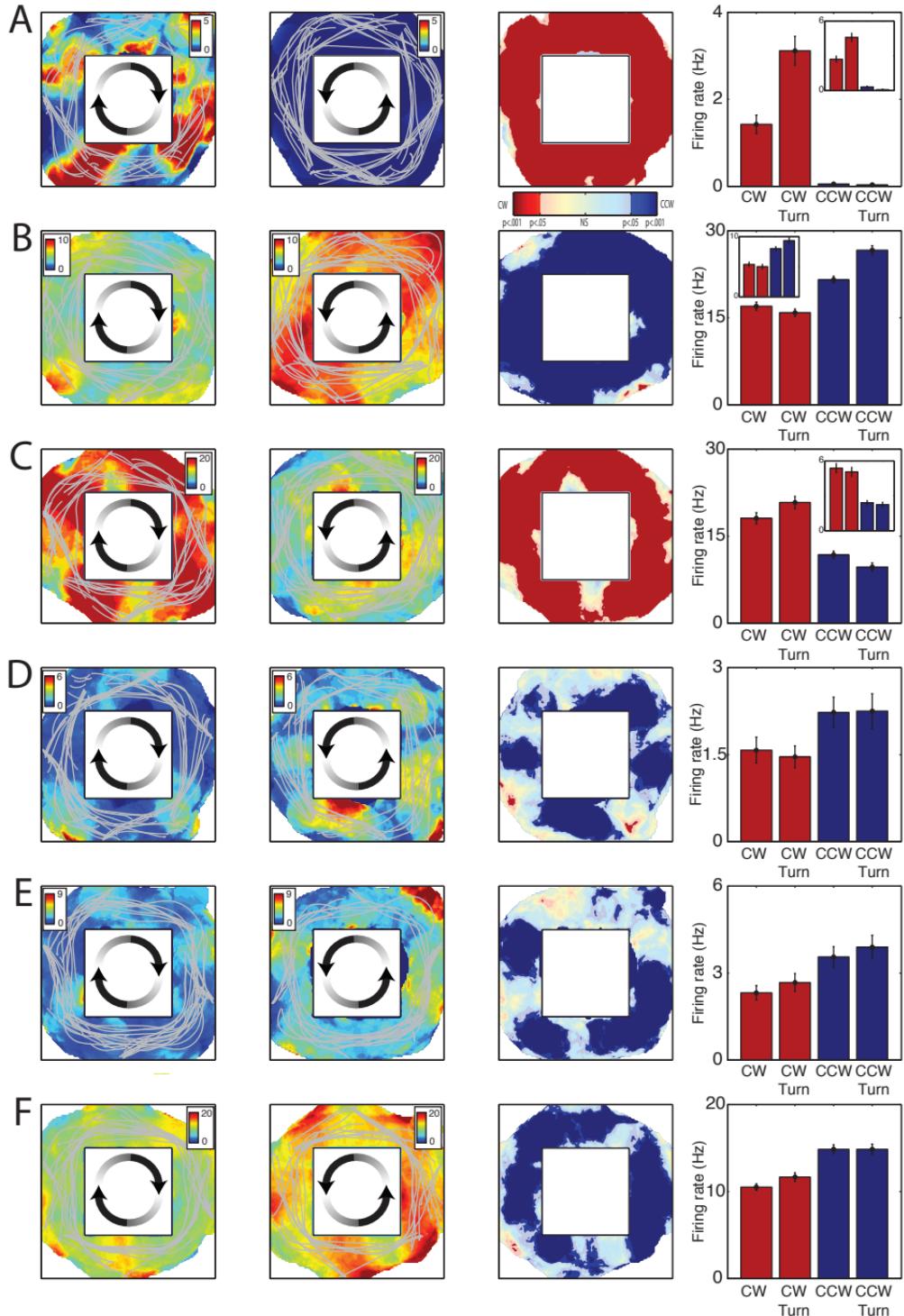
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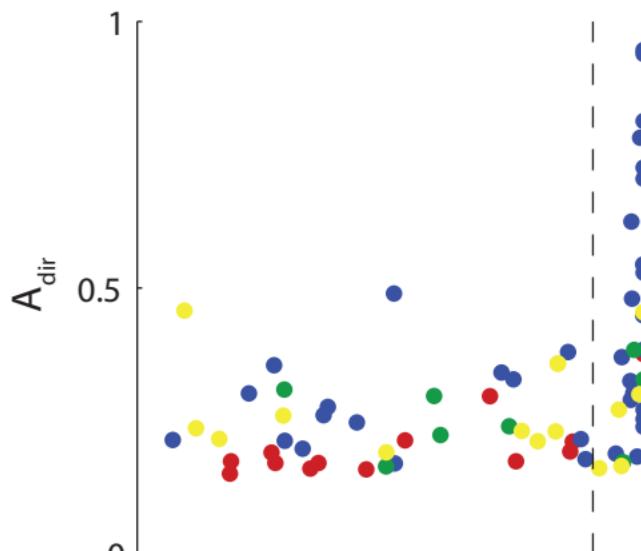
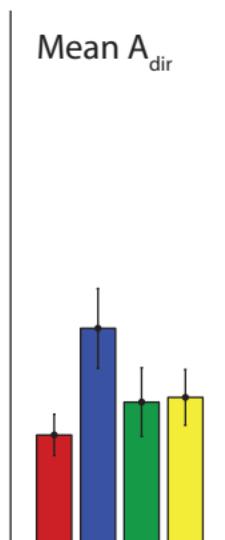
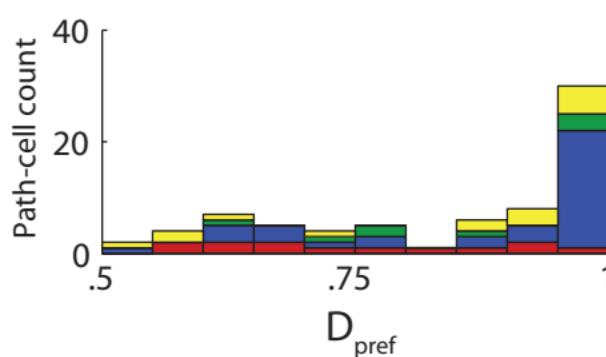
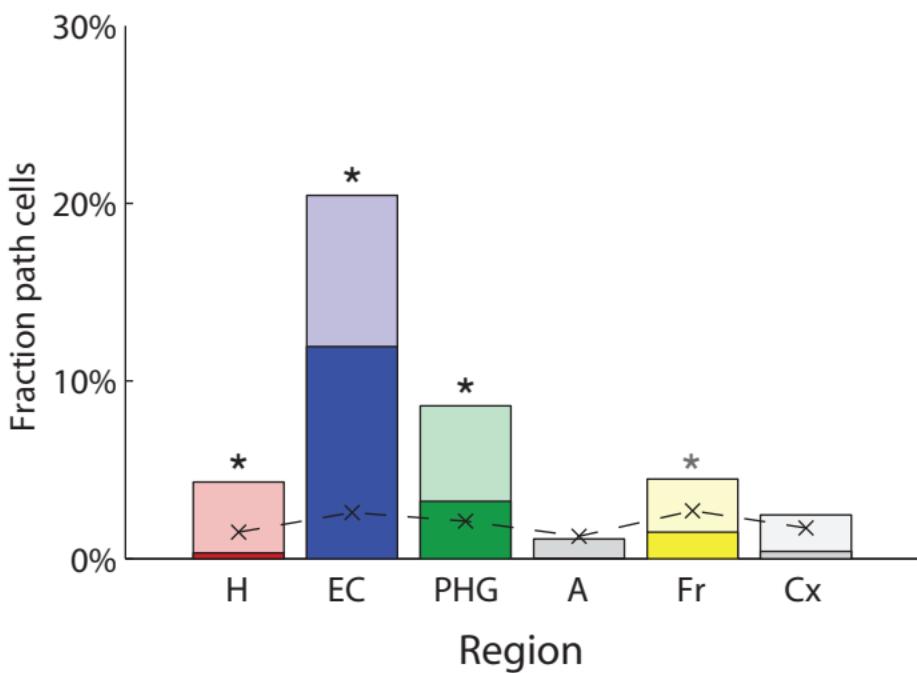
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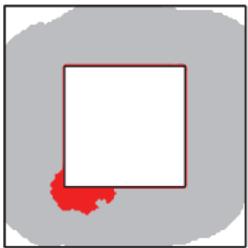
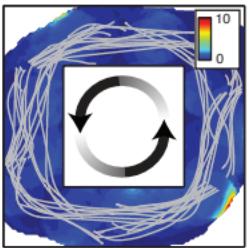
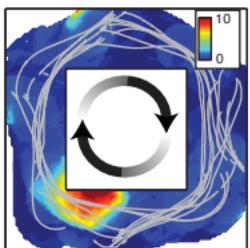
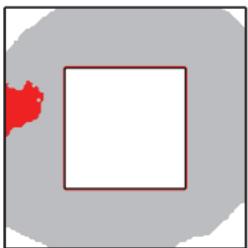
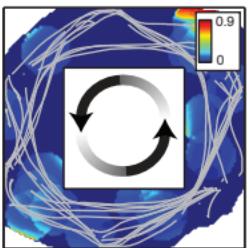
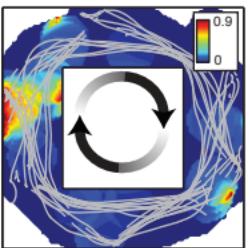
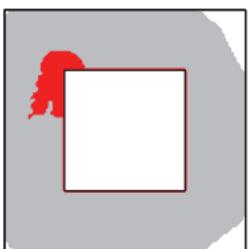
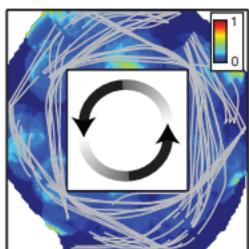
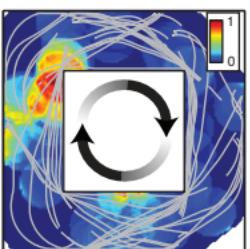
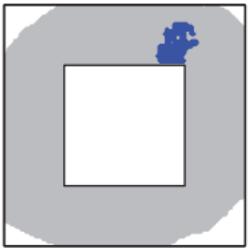
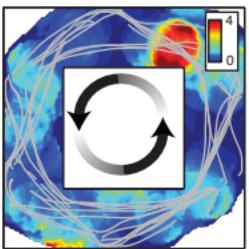
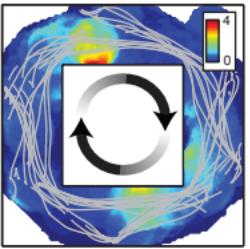


B





A**B**Mean A_{dir} **C****D**

A**B****C****D**

Percent place cells

20%

10%

0%

H

EC

PHG

A

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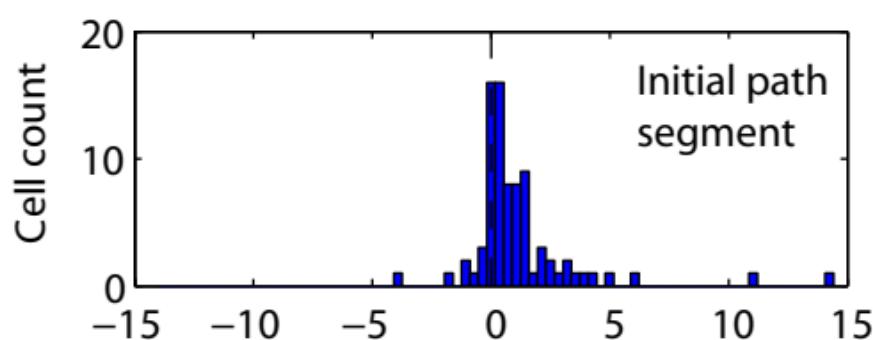
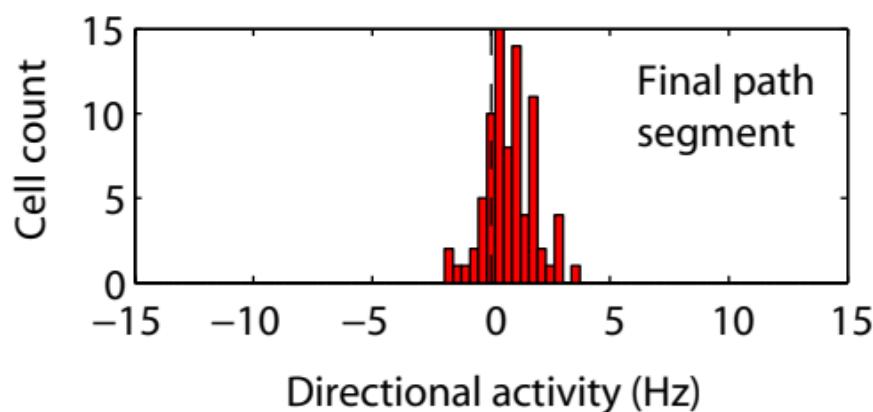
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A**B****C**

A sense of direction in human entorhinal cortex:

Joshua Jacobs, Michael J. Kahana, Arne D. Ekstrom,
Matthew Mollison, & Itzhak Fried

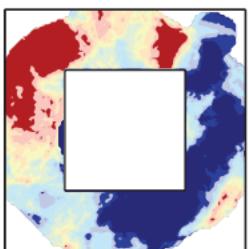
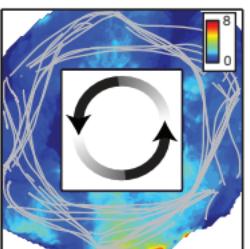
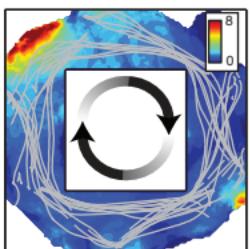
Supplementary Figure Captions

Supplementary Movie 1. Activity of one example path cell. This movie depicts the activity of the path cell from Fig. 2A during part of the testing session. Left panel shows a computer reconstruction of the subject's view of the virtual town. Top right panel indicates the smoothed firing rate of this cell. Bottom right panel indicate the patient's current virtual location on an overhead view of the environment. The movie's soundtrack indicates when spikes occur via audible tick-like sounds.

Figure S1. Example complex path cells. These cells all exhibited significant directional firing across various locations in the environment, but there was not a single clockwise or counterclockwise preferred direction (cf. Figure 2). **A.** The behavior of a path cell from patient 5's left entorhinal cortex. Left panel indicates this cell's activity during clockwise movements, middle panel indicates activity during counterclockwise movements. Gray lines indicate the path of the subject. Right panel indicates whether firing rate at each location was statistically greater (rank-sum test) during clockwise movements (red) or during counterclockwise movements (blue). (Spatial statistics: $A_{dir} = 0.3$ and $D_{pref} = 0.61$; see Methods for details) **B.** The behavior of a path cell from patient 2's right entorhinal cortex. ($A_{dir} = 0.46$ and $D_{pref} = 0.76$.) **C.** The behavior of a path cell from patient 4's right entorhinal cortex. ($A_{dir} = 0.21$ and $D_{pref} = 0.94$.) **D.** The behavior of a path cell from patient 6's left entorhinal cortex. ($A_{dir} = 0.21$ and $D_{pref} = 0.65$.) **E.** The behavior of a path cell from patient 2's right entorhinal cortex. ($A_{dir} = 0.27$ and $D_{pref} = 0.69$.)

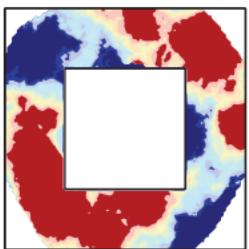
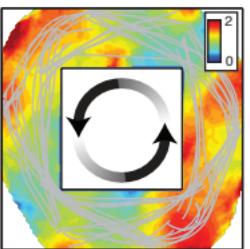
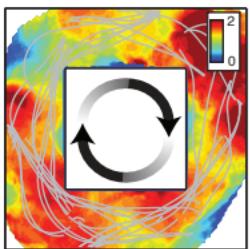
Figure S2. Location of microelectrode bundle in patient 2's right entorhinal cortex. Brain image in neurological convention (i.e., right side of image depicts the right brain hemisphere).

A

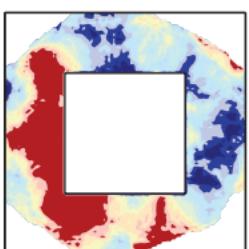
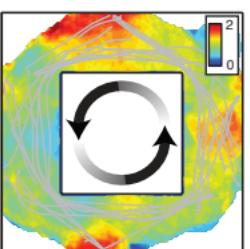
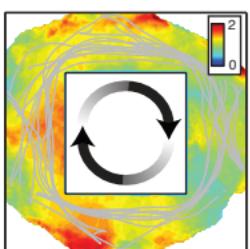


CW p<.001 CCW p<.05 NS p<.05 p<.001

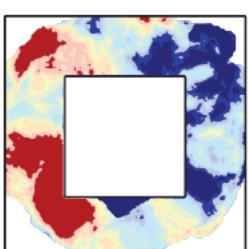
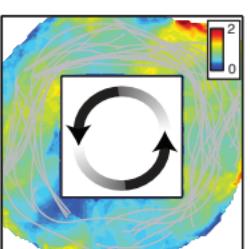
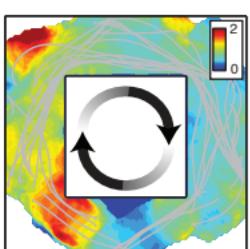
B



C



D



E

