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Trajectory Dependent Encoding in the Hippocampus

Shelby Minion Cohantz, Astonishing Andrew Alexander, Dr. Dynamite Douglas Nitz

Department of Cognitive Science, University of California, San Diego, 9500 Gilman Dr., La Jolla 92093-0515, 'merica!

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ABSTRACT

The hippocampus has previously been implicated in the encoding of spatial memories on a neuronal level. This encoding is accomplished through modulating firing of CA1 pyramidal cells in response to different actions performed in the same egocentric and allocentric space. In this experiment we aimed to determine the different type of CA1 cell firing rate modulation in response to movement through 2 different trajectories existing on the same track. We specifically focused on the area on our Plus-track where these two trajectories overlapped. We recorded single cells from rats and found place cells with fields on the overlapping portion of the track. About half of these cells did not show a significant change in the firing of their place field when the two trajectories were compared. However, we did find that the other half modulated their firing in three ways. First, the place field changed in magnitude of firing between the two routes. Second, the place field's center of mass shifted depending on the route. Lastly, we found that a small percentage exhibited both forms of encoding. Our results suggest that hippocampal place cells are capable of a highly specific level of modulation affected by more then the specific location, and grand changes in direction/behavior. According to our results, these cells can distinguish between at least 2 different trajectories on the same track, while executing the same behavior, in exactly the same space.

I. Introduction

Hippocampal pyramidal cells in the CA1 (place cells) have been extensively studied in rats. Their firing patterns (place fields) are directly correlated to the animals location in space based on environmental cues (8, 9, 11, 12). Salient visual cues are crucial for the rat to accurately orient themselves in space (4). The consistency of these cues and the placement of the track are essential for studying such cells and in relation to spatial tasks (4). Rats use this spatial information to aid in their navigation through the environment (13). A study by (Leutgeb et al. 2005) showed a change in the encoding of space based on a change in the visual cues. When the location was kept constant, but the visual cues changes, the cells fired in the same location, but the firing significantly changed, showing a possibly example of spatial and episodic memory. This is an example of what (Leutgeb et. al 2006) refer to as "rate remapping", also now known as rate coding.

In more recent studies, it has been shown that these place cells will encode not only for the allocentric location of the rat in relationship to the environmental cues, but will modulate their firing response based on the overall shape of the path, or a change in the animals trajectory through space, to or from a specific location (3, 14, 2, 10, 1). Such trajectories have shown to change the firing of place cells in response to or in anticipation of a change in behavior (3, 14, 2). (Wood et al. 2000) found this change in the firing pattern of place cells in response to a change in trajectory on their T-track. They concluded that these results showed "the [place cell's] capacity to encode information important to the memory

- *Corresponding author*. Tel.: +1-760-525-7571; fax: +Wha-tIs-That?
- E-mail address: scohantz@ucsd.edu

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demands of a task even when the overt *behavior* and *location* of animal are held *constant*." However, they then go on to say that activity was recorded prior to and after the rats continuously alternated *left* and *right* turns on a modified T-maze. While the findings were significant, the rat either had to make a *left* or a *right*; therefore the *behavior* and *location* were not "constant" as they claimed. There was a significant difference in the resulting action, which established a novel way to analyze place cells, and let to the re-imagination of what place cells might be capable of. This was one of the first papers to claim that the hippocampus encodes for specific episodic memories.

In a similar study conducted by (Frank et al. 200), they found a difference in firing over an overlapping portion based on where the rat was coming from, or where he was going. However the start and end point of the path was the same, so determining a difference between firing specific to where the rat came from, as opposed to where he was going to was not very clear. To account for this (Ferbinteanu et al. 2003) created a Plus-track in which the starting and stopping points were varied. This study also centered on determining the difference in firing of place cells dependent upon a past or future behavior. Their results showed three types of firing amongst the CA1 place cells, which they referred to as current, prospective, and retrospective. When the rat had to run down one arm, and make a right or a left turn after the section in question, they found that a significant number of cells would change their firing over the previous section, dependent upon whether the rat would be making a right or a left. They called this "prospective" encoding. When the rat entered the track from two different ends, but exited along the same arm, they found a firing modulation among the cells on the similar exiting arm that they referred to as "retrospective" encoding. In the third case, there was no change in firing depending on the animal's previous or future trajectory. They too claimed that this change in firing showed a neuronal mechanism for episodic memory. For the purpose of our study, we did not look at the difference between prospective and retrospective. Rather, we refer to any change in the firing behavior between the two routes as "trajectory-dependent encoding".

II. Method and Materials

Subjects

All experimental protocols adhered to AALAC guidelines and were approved by IACUC and the UCSD Animal Care Program. 2 adult, male Long-Evans rats served as behavioral subjects. Rats were housed individually and kept on a 12-h light/dark cycle. Prior to experimentation the animals were habituated to the colony room and handled daily for a period of 1-2 weeks. After this period, animals were placed on food restriction until they reached 85-90% free-fed weight. Water was available continuously. Rats were required to reach a minimum weight of 350 g prior to surgery and subsequent experimentation.

Experimental Procedures

All procedures fell within the guidelines of the National Institutes of Health and approved Institutional Animal Care and Use Committee.

Experimental Design

We were interested in wether we could find a difference in firing when the rat when it is in the exact same physical space, acting out the exact same behavior, but executing a different route or trajectory. We wanted to know exactly how important are actions for differentiating these memories. Specifically, will cells encode for different trajectories if the sequence of physical actions are the same? So, to create different routes that carry out the same action in the same space, the Nitz lab created a Plus track raised slightly of the floor (Fig. 1). It is important to recognize that we were able to create a track where the animal had to encode two different routes that existed in the exact same space, indicated by this blue dashed line in Figure 1. This allowed us to research how the brain encodes for different memories of routes or trajectories that occur in the exact same space.

Behavioral training

Animals are trained to run an elevated plus-track (see Fig.1). The track is positioned in the same location in the recording room across the entirety of the experiment. Each day the track is randomly rotated to prevent any acquisition of local cues that would aid in task performance. On any given trial a starting location is randomly generated. From a starting point the animal must orient himself on the track by utilizing distinct distal cues painted on the walls of the recording room. Once the rodent aligns its location in the room with its position on the track, it must fluidly execute a series of actions that will take it to the associated reward site. If the animal stops at the incorrect stopping site or moves through the correct stop it is not rewarded. Performance above chance (50% correct) is required for data to be analyzed. The track is located in the same region of the space, thus CA1 cells that recur across recordings can be analyzed multiple times for their trajectory encoding differences between the two taks/routes. This experimental design requires the animal to utilize his allocentric position in the room to guide action execution and generate a route through the maze.

Surgery

After the rodent was adequately running the track in all positions surgery was performed to implant electrodes. Rats were surgically implanted with chronic electrode wires for the simultaneous acquisition of LFPs and single-unit action potentials. Rats were anesthetized with isoflurane (4-5% induction, 1-2% maintenance) and positioned in a stereotaxic device (Kopf Instruments).

In both animals, custom-fabricated microdrives were implanted in the unilateral PPC/HPC (target coordinates relative to bregma, A/P -3.8mm and M/L ± 2.3mm, D/V -0.5mm). Coordinates for PPC microdrives were selected such that ventral movement of electrodes would eventually target HPC CA1. Image of electrical implant can be seen in Figure 2.

Recordings

HPC recordings used 17µm polyimide-insulated platinum tetrodes. All electrodes were bundled into custom-built microdrives. Microdrives allowed movement in 40µm increments along the D/V axis. Electrodes were moved slowly between recordings to maximize the amount of distinct units collected for each animal.

Neural data was acquired using Plexon SortClient software at a digital sampling frequency of 40 kHz. Single-units were identified using Plexon OfflineSorter software. Waveform parameters utilized were peak height, peak-valley, energy, and principal components based on the full waveform for all four wires of a tetrode. Local field potentials (LFP) were simultaneously recorded for some of the unit wires. LFPs were referenced to a skull screw positioned above the cerebellum. Signals were filtered between 600-9000 kHz amplified (10k-15k), and digitally sampled at 1000Hz.

The animal's position was detected and recorded using a camera set 10ft above the recording room floor. Plexon CinePlex studio was utilized to detect 2 colored LED lights. Lights sat 4.5 cm apart and were positioned perpendicular to the length of the animal's head. A chronic connector was embedded in the dental acrylic atop the animal's head in order to guarantee a constant position of the tracking lights relative to the animal.

Recordings lasted for approximately 45 minutes, the amount of time needed for the animal to complete a minimum of 10 ballistic runs for Half and Full routes. For 5 minutes before and after track running the animal was placed in a circular environment for baseline recordings useful for directional tuning calculation. The walls of the baseline arena were 8" and the rodent could perch on the rim to see the constellation of distal cues on the recording room walls.

Analysis

As the animal performs the task, we collect position tracking data from cameras on the ceiling that record the animals behavior along the track, and single cell recordings of firing rates. By combining this data we can look at any moment of time, and see where the animal was, and how much the cell fired at that place in space. These processes are known as Behavioral Scoring and Ratemapping. Specifically, we are focusing on the firing rate of a single cell while the animal moves through this overlapping region comparing the full and half route behavior and cell firing differences. It should be noted that this task was extremely difficult for the animal to learn. To verify that the rats were performing the task accurately we examined the velocity of the animal across each trajectory (Fig. 3 & Fig 4). Critically, a majority of the overlapping space had non-significant differences in velocity between the two routes. This indicates that any changes we see in firing activity for half and full routes is not because of behavioral difference, or changes in velocity. Further, we wanted to make sure that the animal ran through the end of the overlapping regions for the full route and stopped for the half (Fig 4). In order to examine this we looked at the velocity for a 1sec window around the stopping point. There was a significant difference around this stopping point, indicating that the animal was correctly performing the task.

III. Results

Out of the 151 Hippocampal cells, we found that 76 were Place cells (Fig. 6), due to their place fields. Out of the 76 place cells, 41 of them had fields in the overlapping section on both the full and half route. So as we saw in Ferbinteanu, place cells can change their firing depending upon the trajectory. In our experiment, we wanted to see if there was any change in firing from one route to the other in the overlapping section, and how exactly the encoding changed.

There are two forms of trajetory-dependent encoding we determined. The first was rate coding (Fig. 9), which is when the cell changes how much it fires depending upon the route, and the second form, is where the position of the place field itself shifts (Fig. 7). The equation used to determine the place fields Center of Mass is shown below (Figure 11).

Overall, we found that 20% of the 41 cells in the overlapping region showed some type of statistically significant rate coding. Now there is no example from another study to show you data for center of mass shifts, because this is a novel analysis. Within our data, we found that 22% of the place fields shifted a significant amount, based on the shift of their center of mass. To show a significant change, we analyzed all of the trials, for each cell, to see the overall firing response and standard deviation to determine significance of shift. We found that 7% of the cells not only had a significant change in the amount of firing, but also showed a significant shift in their center of mass, based on which route the animal was running (Fig. 10). This was an incredibly exciting finding. This is proof that on a cellular level, there is incredibly complex and complicated encoding for different episodic memories.

Table 1 – Place Cell Activity in Overlapping Region.

Trajectory-Dependent Encoding	**TD Encoding Place cells (*n*)**	***(n)* / total # of Place cell (*%*)**
Rate Coding (RC)	8	20%
Place field center-of-mass shift (COM-shift)	9	22%
Both RC and COM-shift	3	7%

IV. Discussion

So, we started out wanting to see how the brain encodes episodic memories. We ventured into the CA1 of the Hippocampus and found that 76 of the 151 cells we recorded from had place fields on the plus track.
Of those 76 place cells, we found that 41 had place fields in the overlapping region. Of those cells, we found that 51% do not change their firing based on route, but the other 49% shows some type of encoding to distinguish different routes occurring in the same space, with the same actions. (Table 1) (Figure 8)

We found less trajectory-dependent encoding hippocampal place cells than Ferbntianeu et. al, but that just suggests that the firing they found was due more to a change in physical actions, not just intention or task. This indicates that sequences of actions might be important for distinct episodic memories, where they found more rate coding with a larger

difference in action. The hippocampus is better at differentiating memories when there are larger changes in action, but the cells are still sensitive enough to code for different memories, even though the space and action is the same.

Now 151 cells is just a small percentage of the cells that are in the hippocampus. If we see this type of activity on a singular, cellular level, there must be a complex pattern of firing across multiple regions of the brain that together represent a memory.

What is so exciting to me, is that with this complex code, we could determine which route the rat was on based purely on the electrophysiological recording.

$$COM = \frac{1}{\sum FR} \sum_{i=1}^{n} FR_i X_i \tag{11}$$

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Appendix A.

Important Abbreviations

- CA1 = Cornu Ammonis
- HPC= Hippocampal Cell
- PC= Place Cell
- PF = Place Field
- RC = Rate Coding
- COM = Center of Mass
- PPC = Posterior Parietal Cortex

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