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What, When, and Where: Phase Precession in Different Sized Fields

- Introduction

Spatial cognition consists of understanding the place and time of locations visited by a human/ animal. Despite the fact that navigating the environment seems like second nature, in actuality, the brain conducts great deals of isolation and integration of incoming sensory information in order to comprehend the basic makeup of the environment, as well as how to interact with it. Given that the timing of places visited is important in navigation, since an organism can only be at one location at one point in time, the brain must have underlying mechanisms that helps track the timing of places visited in addition to the spatial relationships of locations.

This project will analyze how *phase precession* in the brain changes rates for different lengths of routes run by rats. To explain this phenomenon, it is crucial to understand two important components: *place cells* and the *theta rhythm*. There are neurons in the cornu ammonis 1 (CA1) region of the hippocampus that fire when a rat is in a particular location in an environment. This location is often referred to as a *place field*, and the neuron that corresponds to this place field is called a *place cell*. The hippocampus generates a *theta rhythm* that is distributed throughout the cortex, where one second contains eight sine wave cycles (8 Hz).

Phase precession occurs when the CA1 place cell fires in the late phase of each cycle as the rat enters a place field, middle phases when the rat is in the center of a place field, and early phases when the rat is leaving a place field (as shown in Figure 1). The rate of this precession depends on the size of the place field, with smaller place fields requiring faster precession, and larger place fields requiring slower precession, as the place cell needs to have fired at all different phases (early, middle, late) to encompass the place field in a single theta cycle. If multiple overlapping place fields are approached by the rat in a particular order, the relationship of the phase precession of each place field helps encode when the rat visited which place field relative to the others; the emergence of the pattern of spiking activity for place cells within each theta cycle is called a theta sequence (Figure 2). By understanding when and how phase precession takes place in the brain, and what roles it may play in navigation, we can reach a better understanding of how the brain comprehends the temporal (timing) aspects of spatial cognition.

Figure 1: Place cell spiking in relation to phases of each theta cycle. Notice how the traversal of place fields as shown by the rat is colored in the same order as lines on the waveform, demonstrating how different regions of the waveform (phases) store information regarding, past, current, and future places visited. Adapted from “The Neurobiology of Mammalian Navigation,” by S. Poulter, 2018, *Current Biology*, vol. 28, no. 17. Copyright 2018 by Steven Poulter.

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Figure 2: Encoding of temporal relationships via theta sequencing. Each place field still demonstrates individual phase precession, but once put together, spike timing lines up in such a way that their order corresponds to which location the rat has visited, is currently at, and will

visit. Adapted from “Internal Operations in the Hippocampus: Single Cell and Ensemble Temporal Coding,” by G. Dragoi, 2013, *Frontiers in Systems Neuroscience*, vol. 7. Copyright 2013 by George Dragoi.

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- Literature Review
 - Place Cells and their Formation

The first major breakthrough in the neuroscience of spatial navigation is the discovery of place cells. They were first discovered by Dr. John O’Keefe, who called the areas in the environment for which these cells in the CA1 region of the hippocampus consistently fired “place units.” Even when he turned off the lights in the room (an environmental manipulation), these place units continued to exhibit firing activity which highly suggests that the brain can keep track of an environment and its various locations via a “cognitive map,” (O’Keefe 1976). After the discovery of place cells with corresponding place fields (as they are now called) in a single environment, further work was done to understand how the mechanisms of place cell activity works when presented with multiple environments. As it turns out, when a rat is presented with a cylindrical and rectangular environment, cells that contain place fields in the cylinder can be silent in the rectangle as other cells emerge with different place fields. One place cell may have multiple place fields in a single environment, and they can come in various sizes and/or can also conform to the distinct edges of the environment they are encoding (such as a linear place field along the edge of the rectangle). It was also discovered that place cells have higher firing rates as the rat approaches the center of a place field, which indicates that there may be a fine-tuning mechanism that allows the rat to specifically know where they are relative to a place field, in addition to knowing their general location in an environment (Muller et al., 1987).

If place fields can be quickly formed and changed from environment to environment, it then begs the question for how these place fields are created/determined in the first place. It is hypothesized that the ability for synapses to be created in the brain (synaptic plasticity) in order to effectively transfer information among neural networks is highly dependent on a process called long-term potentiation (LTP). As it turns out, when the researchers stimulated the ventral hippocampal commissure with high frequency stimulation to mimic LTP, numerous changes in firing activity would occur. Cells that originally didn’t have a place field may acquire one, cells that originally had a consistent place field may only fire if the rat approached it in a certain direction, or cells may even shift their place fields completely. Despite these various local changes, global changes in firing rate remained the same, as the mean firing rate was maintained by increasing and decreasing firing rates of individual neurons within the network. It seems that with the mechanism of LTP to modify synaptic weights in the network, individual variables within an environment can be viewed differently without losing a sense of the wholistic view of

the environment, and it is with this mechanism that rats can learn to comprehend numerous environments with the same neural network (Dragoi et al., 2003).

After getting to piece together how spatial knowledge is acquired from a cognitive perspective, now we get to hypothesize how brain activity changes as more knowledge about an environment unravels. As previously discussed, LTP is the neural mechanism that allows new synaptic connections to form when a rat learns about a new environment. By integrating graph theory with the neuroanatomy of the hippocampus, place cells containing place fields are representative of place nodes, and the synapses between each pair of place cells represents direct paths of travel between these two place nodes. The strength of this synaptic connection most likely encodes the distance between the two place fields where if the place fields overlap, the two place cells will likely fire together, and through the mechanisms of LTP, their synaptic strength increases, and synaptic resistance decreases to indicate a shorter metric distance between the two place nodes. Having this algorithm in place helps explain to a certain degree how navigation of shortcuts or detour routes is possible, since having this neural representation of space in the hippocampus allows modifications to the cognitive map without distorting the global network. Following the graph model, it is safe to say that the closer two place nodes are together in space, the lower the synaptic resistance between the two place cells, allowing them to fire more closely together in time, and likely contributing to their sequential ordering emerging in the theta rhythm of the hippocampus (Muller et al., 1996).

- Phase Precession in Place Cells

There is also something to be said about the relationship of place cells and the hippocampal theta rhythm. The theta rhythm is defined as oscillating brain wave activity that has a frequency between 8-12 Hz. As previously recalled, place cells seem to exhibit slightly higher firing activity when the rat is in the center of a place field; it was also discovered that as the rat entered the place field, the place cell firing would be timed at the later phase of each theta cycle (towards the end), but as the rat approached the center, it would fire in the middle of the phase, and then finally at the earlier of the phase of each theta cycle as the rat leaves the place field. The shift in phases for the firing activity of the place cell in each theta cycle is called phase precession, and this phenomenon seemed to correlate more with the spatial location of the rat in the environment than the behavior the rat was executing. It seems that this fine-tuning way of tracking a rat's progression through a place field plays a critical role in encoding the temporal aspects of spatial navigation (when the rat visited which places) (O'Keefe and Recce, 1993).

Following the discovery of place cells, it was found that cells in the postsubiculum would fire consistently when the rat's head faced the preferred direction of that cell in the environment. The postsubiculum receives input from the subiculum and anterior thalamic nuclei, and outputs to the entorhinal cortex as well as dorsal and ventral anterior thalamic nuclei. It is theorized that since the subiculum receives information from the CA1 regarding place fields, it is able to relay that information to the postsubiculum in establishing the head direction cells. It is also worth noting that the thalamus is responsible for integrating sensory information from different sensory systems, and that the anterior thalamic nuclei is responsible for storing episodic memory. Each head direction cell would fire over a range of 90 degrees, with a section where the cell reached its peak firing (and in some instances, there may even be two peaks within that range). Head direction cells are similar to place cells in that they also don't seem to have any topological organization, but contrary to place cells, head direction cells each had only one preferred direction in a given environment, rather than multiple (as place cells may have multiple place

fields in an environment) (Taube et al., 1990a). Head direction cells also seem to change their preferred directions as the animal goes from environment to environment, but if that same environment is simply rotated relative to the animal, the angular coordinate system of the head direction cells also rotates with the environment, consistently firing at the same directions within the environment. This shows that these cells rely on visual cues in the environment for reference of how to set up the head direction angular coordinate system in order to contribute to the navigational process (Taube et al., 1990b).

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In addition to the theta rhythm playing a significant role in phase precession, another mechanism that is related to it is the theta sequence. Since phase precession encodes when a rat enters and leaves a place field, if multiple place fields located close to each other are taken into consideration, the rat can visit those place fields in a particular order. This is reflected in the theta rhythm since the place cell precesses through the theta phase backwards (place cell spike-time occurring later in the phase corresponds to early in the field). In this case, the temporal order of the place fields visited by the rat are stored in a compressed fashion in each theta cycle, helping solidify the understanding of the relationships between each place field and the different ways the rat can approach them (Foster and Wilson, 2007). What is key to piecing together how the theta rhythm helps specify the temporal order of the place fields visited is to see how the theta sequence is formed when the rat experiences a novel environment. As it turns out, theta sequences only clearly emerge after the rat's first run of the maze; however, phase precession for each individual place field is present even in the first run. What may very well be the case is that a new undiscovered mechanism is at play in managing theta sequences, as the emergence of the theta sequence is an indicator that the rat comprehends the relationships between the place fields he has visited. It is highly suspected that this experience-dependent encoding relies on synaptic plasticity within the hippocampus in order to allow such a fast emergence of a firing pattern to occur (Feng et al., 2015).

- Single Cell vs. Network Theories of Phase Precession

When considering how phase precession is able to be created and maintained in the brain, there are two theories that approach this question from opposing directions. The single cell theory proposes that the phase precession mechanism is dependent on the individual cell that is phase precessing, while the network theory proposes that it is the network of cells surrounding the phase precessing cell that allows phase precession to take place. Most likely, the truth about this phenomenon lies somewhere in the middle of these two theories. In one experiment, mice were

head-restrained, and then asked to carry out spatial navigation tasks in a virtual environment. By taking whole-cell recordings of place cells, it was observed that when a mouse approached a place field, the place cell's membrane potential continuously increased until the mouse passed the place field. Despite the asymmetric quality of the ramp of depolarization in the membrane potential, the firing rate of the place cell when the mouse was in the place field remained consistent. The amplitude of the intracellular theta oscillation of the place cell significantly increased, however, the spike times of the place cell did not precess with the intracellular theta oscillation as they did with the LFP theta rhythm. This suggests that a phase shift may have occurred between the intracellular theta rhythm with the local LFP theta oscillation. Because of the disconnect between intracellular dynamics with the LFP theta, with the intracellular theta oscillations occurring at a higher frequency than the LFP theta, this suggests that the place cell itself may have mechanisms that allow it to phase precess rather than relying on the outside network of neurons (Harvey et al., 2009).

Simultaneously, phase precession seems to be network supported since even after quieting neurons that displayed phase precession for a short amount of time, they still maintained phase precession when their activity returned. In another experiment, as rats ran on a U-shaped maze, when the theta oscillation was shut off by sending single-pulse signals to the commissural pathway, after the re-emergence of the theta oscillation, spikes would fire in more advanced phases compared to the phases they fired at before the silencing of the theta oscillation. This indicates that even with the reset of the theta oscillation, phase precession was preserved within the place cells. This may be explained by the theory that extrahippocampal inputs update how the place cell will phase precess at the beginning of each theta cycle, further supporting that phase precession is a network phenomenon rather than being solely that dependent on the single neuron exhibiting phase precession (Zugaro et al., 2005).

- Head Direction Cells in Postsubiculum

Cells in the postsubiculum rely on visual cues in the environment to set up the head direction angular coordinate system in order to contribute to the navigational process. Following the discovery of place cells, it was found that cells in the postsubiculum would fire consistently when the rat's head faced the preferred direction of that cell in the environment. The postsubiculum receives input from the subiculum and anterior thalamic nuclei, and outputs to the entorhinal cortex as well as dorsal and ventral anterior thalamic nuclei. It is theorized that since the subiculum receives information from the CA1 regarding place fields, it is able to relay that information to the postsubiculum in establishing the head direction cells. It is also worth noting that the thalamus is responsible for integrating sensory information from different sensory systems, and that the anterior thalamic nuclei is responsible for storing episodic memory. Each head direction cell would fire over a range of 90 degrees, with a section where the cell reached its peak firing (and in some instances, there may even be two peaks within that range). Head direction cells are similar to place cells in that they also don't seem to have any topological organization, but contrary to place cells, head direction cells each had only one preferred direction in a given environment, rather than multiple (as place cells may have multiple place fields in an environment) (Taube et al., 1990a). Head direction cells also seem to change their preferred directions as the animal goes from environment to environment, but if that same environment is simply rotated relative to the animal, the angular coordinate system of the head direction cells also rotates with the environment, consistently firing at the same directions within the environment (Taube et al., 1990b).

- Route-specific Firing in the Posterior Parietal Cortex

There is also a type of firing exhibited by the posterior parietal neurons called “route-specific firing.” Cells would consistently fire at the same locations within a route if the rat travelled the same route (can be seen as executing the same sequences of behaviors from an egocentric point of view), regardless of the location of the route in the environment. This discovery illustrates that the brain is capable of navigating from a more abstract perspective rather than solely relying on physical cues and may pave the way for how physical aspects of an environment can be translated into a cognitive map (Nitz, 2006). Further exploring the role of the posterior parietal cortex (PPC) in spatial cognition, it is able to identify the same route of travel even if the scale has increased or decreased in size. When rats were trained to run a squared spiral track starting from the outside and then running towards the center, the spiking activity of the neurons in the PPC would repeat the same pattern for each arm of the square for every iteration of a square even as it shrinks. Since the arms of each square was decreasing as the rat ran inwards, the duration of neuronal spiking activity for each repeating arm would also decrease to reflect the shorter distance on each iteration of the loop. The three reference frames that the PPC was able to simultaneously keep track of was the position of the rat in terms of which arm of a square he was running, which loop iteration he was running, and how far along the entire spiral track he has traversed. In order to do this, the PPC needed to consider repeating behaviors on the rat’s behalf for turning onto each arm (egocentric navigation), repetition of the square route on each loop iteration (route-centric navigation), and his location in the environment to understand where he is on the entire track (allocentric navigation). Due to the fact that PPC neuron repeated its spiking activity for each iteration of the loop, a correlation between the spiking activity can be made with the behavior of the rat regarding distorted routes (Nitz 2012).

- Firing Activity of Subiculum Neurons

Another mechanic of spatial navigation that also contributes to the integration of allocentric and egocentric space are axis-tuned neurons in the subiculum, a major output area from the CA1 of the hippocampus. These neurons would fire whenever the rat traversed a particular axis of travel, but when the maze would be rotated, rather than the subiculum neurons firing at the same spot they did previously on the maze, they would fire at new spots on the maze that corresponded with the previous positioning of the maze’s axis of travel it originally fired for (if previously the cell fired when the rat was travelling from north to south, even after the maze rotation, the neuron would still fire whenever the rat travelled north to south relative to the environment, not the maze). However, if the rat was placed in an open field environment with no clear directions of travel indicated by a structure (like the maze paths), axis-tuning activity of subiculum neurons would disappear! This demonstrates that most likely, axis-tuning of subiculum neurons helps integrate route-centric and allocentric navigation, helping the rat identify what kind of relationship a route will have with the environment if it is displaced (Olson et al., 2017).

Even though the subiculum neurons can fire selectively for axis of travel in an environment, they can still fire selectively in space as well as display theta precession. What is interesting is that along the proximal (closer to hippocampus) - distal (further from hippocampus) gradient of the subiculum, the firing rates of these subiculum neurons go from sparse to distributed, almost as if it were taking the sparse activity of place cell activity in the CA1 and generating more activity so that it may carry more information about the rat’s location in space to the rest of the brain. In terms of theta phase precession, despite the differing amounts of firing activity in the proximal

vs. distal subiculum, theta phase precession occurs in all areas of the subiculum, indicating that information regarding the past, present, and future places the rat visits is all being distributed to the rest of the cortex (Kim et al., 2012).

- Direction of Current Project

Our current project seeks to explore how the phase precession rate of neurons in the subiculum may change when the length of the route increases or decreases. Since subiculum neurons are known to exhibit axis-tuning, route-specific firing, and spatial firing, we wanted to see how phase precession may display itself in these firing modalities, and how phase precession in the subiculum compares to CA1 hippocampal neurons. Therefore, the rats were trained to run the same routes scaled to different sizes, as well as run these same routes from different locations in an environment. This allows for a multitude of different sizes and directions of each route to be explored by the same rat. The analysis afterwards seeks to find if there are any particular relationships between the phases of each spike exhibited by a neuron and the progression along the route the rat had executed at the time of the spike. If there is a negative correlation between the phase and progression along the route, we can definitively conclude that the selected neuron phase precesses. If the slope of linear regression for a phase precession neuron is steeper for shorter routes compared to longer routes, we can conclude that the rate of phase precession adjusts according to the length of the route.

- Methodology/Theoretical Framework

Behavioral Apparatus: This project intends to tackle phase precession in the subiculum by having the rat run different sized routes facing different directions. Rats ran on a hexagonal maze split into small and large equilateral triangles as well as small and large diamonds pictured below. In order to see how egocentric and allocentric navigation may be integrated by the rat, the rat learned how to run the triangle routes from each of the six vertices and six midpoints of the sides of the hex maze (Figure 3). The location of the placement of the rat on the maze served as a cue to indicate which route he was to run.

Figure 3: Rats will be performing their navigation task on this hex maze. Triangles outlined in red consist of large equilateral triangles starting from the corner and were assigned an even numbered route. Triangles outlined in green consist of small equilateral triangles starting from the midpoint of each edge of the maze and were assigned an odd numbered route. In the analysis portion of the project, large triangles were designated 80 bins while small triangles were designated 35 bins.

Figure 3: Rats will be performing their navigation task on this hex maze. Triangles outlined in red consist of large equilateral triangles starting from the corner and were assigned an even numbered route. Triangles outlined in green consist of small equilateral triangles starting from the midpoint of each edge of the maze and were assigned an odd numbered route. In the analysis portion of the project, large triangles were designated 80 bins while small triangles were designated 35 bins.

Behavioral Training: Rats are first placed on the maze with black cans that block them from running paths other than either the small triangle, large triangle, small diamond, and large diamond. Each time the rat completes one run, they are rewarded with half of a Honey Nut

Cheerio. After 2 weeks of training them with cans as guidance, cans were removed 2 at a time, until finally the rats were able to execute the runs without any cans on the maze. Each rat ran a total of twenty-four different routes on the hex maze, consisting of a combination of small triangles and large triangles.

Full Task: Rats were placed either at a vertex of the hex maze or a midpoint along an edge of the hex maze. Placement at a vertex always indicated the rat was to run either a large triangle or diamond, whereas placement at a midpoint always indicated he was to run either a small triangle or diamond. After each run of the specified route, rats were rewarded with half of a Honey Nut Cheerio.

Data Acquisition: Microdrives were surgically implanted into three rats' brains (DN20, DN21, and RP5) starting at the cortex level. The microdrive contained four tetrodes (each with four channels of insulated wires, and coiled around a screw), each of which will obtain extracellular recordings of spiking activity of neurons. After the rats have recovered from surgery, brain activity of the rats recorded from the implanted microdrive were connected via wires to a computer as they carried out their task. Tetrodes were then be extended into the brain to targeted regions CA1 and subiculum by turning the screws of each respective tetrode. The local field potential, consisting of the summation of population activity in a region of the brain, was also recorded by the inserted tetrodes to provide the theta oscillation to conduct phase precession analysis on. From this methodology, a total of 507 subiculum neurons and 283 CA1 hippocampal neurons were recorded. In addition to recording their brain spiking activity, an LED mount fixed to their microdrive will be recorded by cameras on the ceiling of the recording room to track the rats' position in the room throughout the recording. The cameras will automatically take the average x and y coordinates of the two LED lights to give the x and y coordinate of the center of the rats' head.

Data Analysis: A Matlab program was used to perform behavioral scoring of the rat, where only clean runs (runs with no errors) executed by the animal will be included in further analysis for this project. The average of the pooled together clean runs of the rat for each route were used as the template of each route. 2D Rate maps (Figure 4) were generated by overlaying the spiking activity of neurons onto the position of the rat recorded by the cameras tracking the LED head lights.

Figure 4. 2D rate maps of a CA1 neuron (a), and 3 subiculum neurons (b, c, and d). Warmer colored portions on the maze corresponds to higher firing rates, with yellow representing the highest firing rate. CA1 neurons have more clearly defined fields compared to subiculum neurons, whereas subiculum neurons display a wider variety of firing activity, including axis-tuning (c), and route-specific firing (d).

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Next, each spike was assigned a phase by relating the spiking activity of the neurons to the local

theta rhythm. One theta cycle was defined as the duration of time between two peaks in the theta rhythm. Since the theta cycles were not necessarily symmetrical, the halfway point of each cycle was considered to be the time the trough occurred between the two peaks of each theta cycle. Phases were then defined as the duration of time from the first peak to the current timestamp divided by the duration of time between the two peaks. The phases were also scaled so that they would go from $-\pi - 0$ from the first peak to the trough, and $0 - \pi$ from the trough to the second peak. Therefore, the entire phase range was 2π long. Spike times of neurons were then assigned a phase that had the closest timestamp to them in the LFP recording. Due to the fact that the theta wave recorded and used in this calculation may not be in line with the actual theta oscillation occurring at the location of the recorded neuron, I doubled the values of the calculated phases for each spike and added them to the original dataset. Afterwards, the phase for each spike was assigned an integer between 1-36, and thus each spike contained a position bin integer and a phase integer (Figure 5).

Figure 5. (Left) Raw dataset of position vs. phase for each spike of a subiculum neuron. Phase range extends from $-\pi$ to π . (Right) Dataset contains double the number of spikes, and phase goes from 1 to 36. Each 18 range window along phases for the plot on the right can be potentially representative of one theta cycle.

Figure 5. (Left) Raw dataset of position vs. phase for each spike of a subiculum neuron. Phase range extends from $-\pi$ to π . (Right) Dataset contains double the number of spikes, and phase goes from 1 to 36. Each 18 range window along phases for the plot on the right can be potentially representative of one theta cycle.

Each route was segmented into positional bins (indicated by an integer starting from 1 to whatever desired value) that could be assigned to each spike to demonstrate the rat's progression through a route at the time a spike occurred. The small triangle was allocated 35 positional bins and the large triangle was allocated 80 positional bins. By looking at the timestamp of each spike, the positional bin with the closest timestamp to the timestamp of the spike was assigned to the spike, taking into account which particular route the rat was running.

In order to see any correlation between the location of the rat and phase precession, each spike's assigned positional bin and phase for each of the twenty-four routes were plotted on a 2D map with the positional bins on the x-axis and phases on the y-axis. To objectively identify any fields, a density-based spatial clustering of applications with noise (DBSCAN) was used on the position by position, taking inputs Epsilon () of 7, representing distance between points in a cluster, and Nmin of 10, the minimum number of points in a cluster. Afterwards, the identified fields were then exported extracted based on their assigned labels by DBSCAN for further analysis (Figure 6). Statistics that were recorded for each cluster include the positional bin range, phase range, slope and mean square error of the linear regression line of the cluster, and correlation coefficient.

Figure 6. DBSCAN was applied to the plot on the left, which contains the position bin of each spike on both the x and y axis. The labels assigned to each spike were then translated over to the position vs. phase dataset on the right to conduct further analysis on.

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position vs. phase dataset on the right to conduct further analysis on.

To isolate the phase window that we wanted to conduct our phase precession on, after obtaining desired field based on position using DBSCAN, we calculated the correlation coefficient of all the points that were included within a range of 18 bins of phase (representative of 1 theta cycle) and incremented the window once a total of 18 times (going from 1-18, 2-19, etc... until 19-36). The phase range with the most negative correlation coefficient was considered further in analysis (Figure 7).

Figure 7. Extracting the phase window (18 bins) with the most negative correlation coefficient. The plot on the left spans from 5 to 23, and the extracted field contains a correlation coefficient of -0.4298. It was extracted from the modified doubled dataset displayed on the right, as indicated by the dotted green box.

Figure 7. Extracting the phase window (18 bins) with the most negative correlation coefficient. The plot on the left spans from 5 to 23, and the extracted field contains a correlation coefficient of -0.4298. It was extracted from the modified doubled dataset displayed on the right, as indicated by the dotted green box.

In order to confirm statistical significance for phase precession in a field, a criteria was set that if a field contained a correlation coefficient that was beyond two standard deviations from the mean of correlation coefficients generated by chance, that field would be considered to be significantly phase precessing. To determine correlation coefficients of fields that could be generated by chance, phases for each field were randomized 1000 times with the same positional bins, with each randomization taking into account the correlation coefficient of the linear regression line. The mean and standard deviation of the 1000 randomizations for each field were calculated, so that the original correlation coefficient for each field could be compared to the randomized data sets. The boundary correlation coefficient calculated from the randomized set turned out to be -0.4152, and so any of the original fields that contained a correlation coefficient smaller than that -0.4152 was considered to be phase precessing.

In order to translate the position vs. phase relationship back to physical space, phase range of each cluster was converted to degrees, and position range to centimeters. This was done using the following equation:

Because one cycle is equivalent to a phase range of 18 in the modified dataset, by taking the proportion of the phase range for each cluster, dividing by 18, then multiplying it by 360, we can derive what portion of 360 degrees that field goes through. Each position bin corresponded to roughly 3 cm on the hex maze, so the conversion from position bin to cm only involved multiplying the position bin range by 3. Finally, by dividing the range of degrees of phase by range of position in cm, we get the rate of phase precession of the field in degrees of phase per cm in space.

IV. Results

Of the 8,624 fields picked up by DBSCAN, 644 of those fields were considered to be

significantly phase precessing. This indicated that ~7% of fields significantly phase precess. Compared to the hippocampus, subiculum neurons phased precess at a much lower rate, as of 2,196 fields picked by DBSCAN in the CA1, 260 of those fields were considered to be significantly phase precessing (~12%) (Figure 8).

Figure 8. Distribution of correlation coefficients of randomized datasets. Red line indicates boundary correlation coefficient of -0.4152. Green line indicates mean correlation coefficient of subiculum neurons, -0.5196. Yellow line indicates mean correlation coefficient of hippocampal neurons, -0.5580.

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- CA1 neurons display stronger phase precession relationship

Because the firing fields of CA1 neurons are much more clearly defined compared to subiculum neurons, it would only be natural that phase precession is easier to observe in their fields, which is quantifiably justified by having on average a stronger negative correlation coefficient compared to subiculum neurons and their fields (Figure 9).

Figure 9. CA1 neuron (left) and subiculum neuron (right), position vs. phase (top) and linear firing rate map (bottom). CA1 neuron shown has correlation coefficient of -0.6951, whereas subiculum neuron has correlation coefficient of -0.4231. The firing rate map of the CA1 neuron also follows more closely a standard firing field rate, with highest firing rate in the center, compared to the subiculum neuron.

Figure 9. CA1 neuron (left) and subiculum neuron (right), position vs. phase (top) and linear firing rate map (bottom). CA1 neuron shown has correlation coefficient of -0.6951, whereas subiculum neuron has correlation coefficient of -0.4231. The firing rate map of the CA1 neuron also follows more closely a standard firing field rate, with highest firing rate in the center, compared to the subiculum neuron.

- Rate of phase precession adjusts according to size of the field

There was a variety of phase precession displayed by subiculum neurons. Since there was a wide variety of field sizes, the rate of phase precession would adjust according to the duration of the field (Figure 10). Smaller fields would contain faster rates of phase precession, whereas larger fields would contain slower rates of phase precession. This ensures that as the rat traverses every field, the neuron would only phase precess once.

Figure 10. Slope (rate of phase precession) vs. position bin range (duration of field). The smallest field (yellow star) spanning over 7 position bins has a slope of -2.755, whereas the largest field (red star) spanning over 55 position bins has a slope of -0.182. The rate of phase precession through a field slows down if the field spans for a longer duration of space.

Figure 10. Slope (rate of phase precession) vs. position bin range (duration of field). The smallest field (yellow star) spanning over 7 position bins has a slope of -2.755, whereas the largest field (red star) spanning over 55 position bins has a slope of -0.182. The rate of phase precession through a field slows down if the field spans for a longer duration of space.

- The same subiculum neuron may contain different sized fields with different rates of phase precession

Of the 507 recorded subiculum neurons, 132 of them contained more than one significantly phase precessing field. These fields would all contain correlation coefficients that passed the boundary of -0.4152, so they would be considered significantly phase precessing fields from the same neuron that are different in size. Consistent with the previous finding, the smallest fields of each neuron had a faster phase precession rate on average compared to the largest fields of each neuron (Figure 11). There was also a wide array of differences in ratios between the rate of phase precession for the smallest field vs. largest field of each neuron (Figure 12).

Figure 11. Rates of phase precession for smallest fields of each neuron (blue) vs. largest fields (red). Smallest fields phase precessed on average at 2.549 degrees/cm. Largest fields phase precessed on average at 1.5878 degrees/cm.

Figure 11. Rates of phase precession for smallest fields of each neuron (blue) vs. largest fields (red). Smallest fields phase precessed on average at 2.549 degrees/cm. Largest fields phase precessed on average at 1.5878 degrees/cm.

Figure 12. Ratios of differences in rates of phase precession between the smallest field and largest field for each subiculum neuron. The ratios were organized in increasing order. The smallest ratio was 1.0162, whereas the largest ratio was 9.6704.

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Figure 11. Ratio of rate of phase precession for smallest field vs. largest field of each subiculum neuron. X-axis indicates which of the 507 neurons has multiple fields, and y-axis indicates the value of the smallest field's rate of phase precession divided by the largest field's rate of phase precession. Largest difference was neuron 115, with ratio of 9.67.

Figure 11. Ratio of rate of phase precession for smallest field vs. largest field of each subiculum neuron. X-axis indicates which of the 507 neurons has multiple fields, and y-axis indicates the value of the smallest field's rate of phase precession divided by the largest field's rate of phase precession. Largest difference was neuron 115, with ratio of 9.67.

- The same subiculum neuron may contain fields that phase precess can also have fields that don't phase precess

Neurons that contain fields that significantly phase precess may also contain fields that do not significantly phase precess. This would explain how out of 8,624 fields scanned from only 507 neurons, only ~7% of them are considered to be significantly phase precessing. In some cases, fields from the same neuron on the same path may contain a mixture of which ones significantly phase precess, and which ones do not (Figure 13).

Figure 13. Neuron 123 on Path 6 with three different fields. Top plot shows linear rate map of

this subiculum neuron, with position on the x-axis and firing rate in Hz on the y-axis. Fields 1 and 2 (left and middle plots, respectively), significantly phase precess, with correlation coefficients of -0.7511 and -0.6481. Field 3 (right plot) does not significantly phase precess, with correlation coefficient of -0.2326. This figure demonstrates how the same neuron may contain some fields that phase precess, while others do not.

Figure 13. Neuron 123 on Path 6 with three different fields. Top plot shows linear rate map of this subiculum neuron, with position on the x-axis and firing rate in Hz on the y-axis. Fields 1 and 2 (left and middle plots, respectively), significantly phase precess, with correlation coefficients of -0.7511 and -0.6481. Field 3 (right plot) does not significantly phase precess, with correlation coefficient of -0.2326. This figure demonstrates how the same neuron may contain some fields that phase precess, while others do not.

- Discussion

Phase precession does occur in subiculum neurons, however, only a small percentage of the fields of these neurons significantly phase precess (~7%). This percentage is slightly smaller compared to CA1 neurons that contain fields that phase precess (~12%). Among the neurons that exhibit phase precession in the subiculum, they may contain fields of different sizes, for which the rate of phase precession will adjust to accommodate the duration of time it takes for the rat to fully traverse the field. The same neuron that contains phase precession in certain fields may not phase precess in other fields. Based on the findings in this analysis, future directions should address the question of in the instances that subiculum exhibit phase precession, is there a pattern to the circumstances of the fields that the neuron chooses to phase precess? It could be the case that subiculum neurons phase precess when the field is created due to route-specific firing / axis-tuning, but chooses not to phase precess in other instances of activity.

Phase precession may be a widespread mechanism not limited to the CA1 of the hippocampus, however, due to the scarcity of its occurrence in the subiculum, further research is needed to understand the role of phase precession in the subiculum. Due to the numerous different types of firing activity exhibited by subiculum neurons (which includes route-specific firing, axis-tuning, and spatial firing), there is potential in narrowing down if phase precession occurs only when subiculum neurons exhibit a particular kind of firing activity. Ultimately, phase precession holds the potential for being a key component in how synapses in the brain are strengthened in order to create and preserve the memory of which locations are traversed along a route. If we are able to clarify the instances that phase precession occurs in the subiculum, it will shed more light on the role of the subiculum in spatial navigation. By understanding phase precession in the subiculum, we can better understand how time is conceptualized in the brain for spatial navigation, adding more towards the knowledge of how space, time, and memory are encoded in the brain.

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