Role of the Hippocampus and Parietal Cortex in Rule-Based Environmental Fragmentation

by

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Abstract

The purpose of this paper is to investigate the hippocampal and parietal encoding of spatial information in rodents when the animals were trained to learn a behavioral rule of fragmenting (i.e., categorizing) an environmental space. Spaces are defined by the boundaries of the observable environment, relying on visual cues situated at various parts of an enclosure to create different frames of references. Through fragmentation, humans are capable of navigating a space using abstract, internal cues when visual, external cues are absent. It is possible that rodents are also capable of generating behaviors that are evident of fragmentation. The posterior parietal cortex and hippocampus are two regions of the brain that are known to be highly implicated in mapping out spaces in both humans and rodents, which make these the regions of interest for electrophysiological recordings because they map out the space of the route and the space of the environment, respectively. The behavioral data show evidence that the rodents can learn the rule to mentally fragment a space. The patterns of the single unit firing activity of the hippocampus and parietal cortex revealed both expected and unexpected findings, but still provided substantial evidence that the animals were implementing the behavioral rule. The population correlation matrices of both brain regions show high correlations when comparing the same points of a track despite the track occupying a different space in the room, indicating that both areas of the brain were mapping out the space of the track using the reference frame of the route. Taken together, the behaviors of the rodents as well as the firing patterns of the neurons were indicative of the animals having learned the rule of fragmentation.

Keywords: learning and memory, fragmentation, hippocampus, parietal cortex

In the early 1900s, psychology was dominated by the theory of behaviorism, which states that all behaviors were a result of stimulus-response associations (conditioning), and that behaviors can be explained in the absence of internal psychological processes (Watson, 2013). Thus, psychology could be studied through only observable behaviors. However in 1930, psychologists Tolman and Honzik discovered that learning can occur even when there is no reinforced exposure to a stimulus, which contradicted behaviorist interpretations that was dominating the field of psychology during that time. The design of Tolman and Honzik's study (1930) comprised of three different groups of rats that navigated from the start to the end of a complex maze: Group 1 was always given a food reward at the end of the maze; Group 2 was never given a food reward at the end of the maze; and Group 3 was given no food reward for the first ten days, but was given a food reward on the eleventh day. During the first ten days, the researchers observed that the rats in Group 1 had the lowest average error rate and the highest average speed when running from the start to the end of the maze. When the rats in Group 3 were first introduced with a food reward on the eleventh day, they immediately decreased their average error rate and increased their average speed when compared to those averages in Group 1 for the next following days during training. Tolman and Honzik's experiment provided evidence that because the rats in Group 3 were given ten days to wander to the end of the maze with no incentive, they were able to learn the configuration and route of the maze in the absence of a reinforcer (Jensen, 2006).

Tolman further supported this claim with another experiment (1938) that ultimately proved that rats were able to make a choice on a four-arm maze, and that this behavior was reflective of having a cognitive map in the brain for where the food reward was located on the maze. Initially, the rat was given time to freely explore the maze. When placed at a specific arm of the maze during training, the rat learned that every time it turned right, it found a food reward. From the perspective of behaviorism, it was predicted that after training on the same arm of the maze, the rat would continue to make a choice of turning right to “get to the reward” even after it was placed on a different arm of the maze that would require a left turn in order to receive the food reward. When Tolman placed the rat on a different location of the maze, the rat navigated directly to the reward with no errors. This result proved that the expected behaviorist outcome was incorrect and that the rat had learned the spatial layout of the maze.

A “cognitive map” was a term coined by Tolman and described the ability to commit the mental image of a learned environment to memory. This “map” could then be recalled later to navigate through the environment and embodied the knowledge or cognitive representation of spatial layouts (Klippel, Knuf, Hommel, & Freksa, 2004). A lot of the evidence gathered from the past century was behavioral data, which is important but cannot thoroughly explain the mental processes executed by the brain during psychological experiments. This discovery still left many questions unanswered regarding the mechanisms behind it—such as what cells, pathways, and brain areas allowed humans and other animals to have an internal map of our environment.

Scientists today are able to compare the behaviors of rodents with the neural form of spatial maps using electrophysiological recordings. The discovery of the cognitive map spurred interest in how spatial environments were encoded by the brain, leading to the discovery of place cells in the rodent hippocampus in 1971 by John O’Keefe and his student, Jonathan Dostrovsky. The place cell is a type of neuron in the hippocampus that fires whenever the rat is in a particular place in the environment, which is called a place field. Place cells are located in the dorsal hippocampus, and place fields are approximately the same size as the rodents and are areas where firing of neurons occurs frequently (O'Keefe & Dostrovsky, 1971). Place cells fire for specific place fields, meaning there can be multiple place fields within an environment; thus, the place cell reconstructs the position of the rat in its environment and can be referred as a map of the animal's position (Derdikman & Moser, 2010). Decades later, grid cells were discovered in the medial entorhinal cortex of the hippocampus by Edvard Moser, May-Britt Moser and their students (Moser, Kropff, & Moser, 2008), but they could also be found in many areas of the brain, such as the parasubiculum. Grid cells are characterized by multiple firing locations that form a hexagonal grid or tessellated triangles over an open-field environment, and like place cells, they also map out the environment of the animal's position (Derdikman & Moser, 2010). This makes the place cell and grid cell known to represent the animal's universal map of the environment. Head direction cells are another type of cell in the hippocampus, specifically found in the presubiculum, and exhibit high firing only when the animal's head is allocated to the allocentric space of a given cell's preferred direction (Derdikman & Moser, 2010). Place, grid, and head direction cells all encode information about the environment using objects relative to the location of other objects in the same space, meaning they use the reference frame of the environment.

Earlier experiments found that damage to the right posterior parietal cortex led to hemineglect, a condition when patients neglect or fail to become aware of stimuli on one side of space (Kerkhoff, 2001). With damage to the parietal lobe, one could be clinically diagnosed with hemineglect and lose their ability to perceive space. Today, scientists know that the parietal lobe is also another brain region important for spatial navigation. Nitz (2006) recorded from the parietal cortex of rodents as they ran on the same route from different points in the room. When the track was moved, the firing pattern persisted for the same point on the route. The route was defined as inflection points in behavior or orientation, and the distances between the points. When the size of the track was doubled, Nitz found that the mapping was flexible because the size of the firing patterns also doubled. He concluded that the parietal cortex mapped out positions in the route, meaning the activity is in a route-centered frame of reference and the cells traveled with the route. Therefore, the parietal cortex encodes the spaces of the route whereas the hippocampus place cells encode the activity for the place in the environment; the parietal cortex neurons use the reference frame of the route while the hippocampal cells use the reference frame of the environment.

Another property of the parietal cells is the firing of activity as the animal executes a specific action. McNaughton et al. (1994) was the first to report that the sensorimotor and posterior parietal cortex cells have action correlates when they observed strong excitation as the animals performed distinct modes of locomotion. Like the parietal cells, these action specific cells also represented the body of motion of the animal through space, creating a “cognitive map” of the environment. Nitz (2009) found that from the posterior parietal cortex, there is a distinct and strong projection to a subregion of the prefrontal cortex called the medial precentral. This subregion is full of neurons with action correlates. Furthermore, this subregion projects to the primary motor cortex which is known to plan and execute motor movements. Nitz (2009) explained that there is a gradual transfer of information from the CA1 subregion of the hippocampus to the subiculum (picks out features of the track), which then projects to the retrosplenial cortex (maps the route in the room). The retrosplenial cortex then projects to the parietal cortex (maps out the route with the same tendency to encode actions), which has connections to the medial precentral (encode actions) that has a direct pathway to primary motor cortex. Nitz (2009) suggested that the parietal cortex and hippocampus are interacting together to create a route-based signal that is capable of guiding sensorimotor transitions.

Since the discovery that hippocampal and parietal cells can map out spaces, many scientists have contributed to the growing literature of the cellular basis of spatial cognition. More recently, there have been studies that tried to understand exactly how the hippocampus and parietal cortex interact together and contribute to the spatial information encoding process. Nitz (2011) found that place cells can fire at analogous locations of mazes that have the same routes that differed in scales. All the mazes used in the experiment were different and spiral-shaped. Routes can influence hippocampal firing because the repeating loops were technically the same routes, which meant that despite the different spaces of the differently scaled loops, the spaces were mapped similarly by the hippocampus. The path shape and scale of the maze also impacted the firing pattern of the neurons by altering their place fields; individual field lengths adapted to the different spaces with similar context. Nitz concluded that within certain bounds, the hippocampus can adjust to the place specifically. Another study by Wood et al. (2000) found that hippocampal place cells can be modulated by the route being taken. The activity of the neurons were recorded as the rodents made alternate left and right turns from the central stem of a remodeled T-maze. Each place the animal had visited was considered an experience or episode, and most of the cells, as many as two-thirds, fired at the same place on the track that had different episodes. The place cells seemed to map out more than just spatial information because they also fired according to the context of what route the animal was on.

Many of the studies that were just described concerned the spatial information encoding process with its relation to physical boundaries in the environment. A study by Muller and Kubie (1987) asked whether a rat can learn to a fragment, or categorize, a room space in the absence of a marker that divides the room space. In their experiment, they investigated how place specific firing would change when a physical barrier was placed in the place field of specific cells they found. The experimenters discovered that when a clear, plastic barrier was placed in the spot where the place field exhibited strong firing activity, the spatial activity of the cell respected the activity of the other space that was divided by the barrier. Therefore, place cells could respond to the environmental manipulations as if they were encoding abstract spatial information.

One may ask how humans and animals can navigate spaces with arbitrary or abstract boundaries. While this topic in spatial cognition is more recent, one experiment by Freedman and Assad (2006) investigated this problem about mental fragmentation in animals and discovered that animals can learn to categorize objects based on an abstract behavioral rule. They recorded from the lateral intraparietal and middle temporal lobes of monkeys while the animals performed a task to mentally fragment arrows into two categories. The monkeys were trained to group arrows of twelve different trajectories of motion into just two types, which were split by a "learned category boundary" that was neither visible nor physically there. The monkeys were successful in this task and the recordings provided neural evidence that the animals have indeed learned this categorical boundary.

Humans are also capable of using their spatial memory for planning routes and navigating through mentally fragmented spaces. One very distinct example is the ability to divide environments into regions that lead to hierarchical encoding of space (Wiener & Mallot, 2003). This behavior was observed when people were asked to judge where Reno is located relative to San Diego. Most will say Reno is northeast of San Diego, since they know Nevada is east of California. If one were to find the cities on a map, they would find that Reno is actually west of San Diego. This distortion in people's estimation of directions and distances between cities is due to people having a hierarchical organization of space, which is what helps us plan our routes and navigate our environment. Given that humans can learn to mentally fragment spaces in our daily encounters, it is interesting how mental fragments contribute to information encoding process in the hippocampus and parietal cortex. Therefore, the purpose of this research is to determine what is happening at the cellular level in the brain as we move through mentally fragmented spaces. The objectives of the study involve generating the behavior evident of fragmentation, and finding where in the brain these mentally fragmented spaces are encoded.

Method

Subjects

Three adult, male Sprague Dawley rats served as the laboratory subjects. The reported data of from the neural analyses were only from two rats.

Apparatus

Function. A T-shaped maze (T-maze) was used for the purpose of meeting two functions: 1) to impose the behavioral rule by satisfying the simplest conditions for the rule; and 2) for practicality in giving only two choice-point possibilities which allowed for splitting the training room into two compartments. The T-maze also allowed the rats to run many repeated trials of every position throughout the training day.

Dimensions. The T-maze was constructed with a round platform attached to end, where the animal was placed during the first trial of every block of a new position and where the animal also returned to after each trial. There were two L-shaped barriers placed at the corners of where the stem of the T and the side of the T met. The purpose of having the L-shaped barriers on the maze was to prevent the animal from physically cutting corners to one choice of the T, and to ensure the animal would run straight the entire time he was on the stem of the T without leaning right or left. The dimensions of the L-shaped barriers measured 2.5 in. x 2.5 in. (length x width) on the side of the T, and measured 3.5 in. x 2.5 in. on the stem of the T (Figure 1A). The entire T-maze was 1.75 in. above the ground, since there were five 1.75 in.-high wooden wedges glued underneath the stem of the T and the sides of the T to give the maze support. The sides of the T measured 26.38 in. x 4 in. x 1.7 in. (length x width x height). The stem of the T measured 4 ft. x 4.25 in. x 2 in. (length x width x height) (Figure 1B). The diameter of the circular platform was 7.25 in., making the circumference about 22.77 in. and the area about 41.48 in.^{2}

Design

Task. The rodents were trained to perform a decision making task of turning either left or right as it ran up the stem of the T-maze. There were five trials within each block, which represented the four different positions the T-maze was placed in the training room. The number of blocks was slowly increased from 4 to a total of 28, making a total of 140 trials a day that the animal had to run. The 28 blocks were split into 7 randomized iterations of 4 blocks within each such set.

Training. Animals were gently handled and taught to eat a cereal reward (Honey Nut Cheerios®) for 5-10 weeks prior to training. The animals were familiarized to the track environment in a dimly lit room and habituated to the maze apparatus by being placed on it for several times with scattered cereal pieces on the surface of the maze. They were trained to run from one end of the maze to the other end by having experimenters slowly removing available cereal pieces from the central portions of the track. They were trained by approximation to make ballistic runs from the bottom of the maze stem to the arm of the T-maze, where the animals were trained to perform a decision making task. The training consisted of random blocks of five trials at one of four positions: position 1 (Pos1), position 2 (Pos2), position 3 (Pos3) and position

4 (Pos4). The food reward was given contingent on the animal choosing the correct side of the crossing T to go to. Pos1 and Pos2 demanded a left turn, whereas Pos3 and Pos4 required the animal to make a right turn (Figure 2). Thus, the behavioral rule determined the receipt or non-receipt of the reward, and effectively split the room into two fragments; this created an invisible rule-determined boundary line that lied directly between Pos2 and Pos3.

Two experimenters were required to train the animals in the room. One experimenter sat in the middle near the end of the T-maze, indicating on a run sheet whether the animal had made the correct turn and giving the rat a half reward based on the status of the turn. The first experimenter also moved the T-maze after the end of all trials for a block. The other experimenter sat in the middle near the end of the platform of the T-maze and gave the rat a quarter of a food reward as it returned to the platform, depending on the rat's performance on that trial.

Electrophysiology

Surgery. Once the rats' performance achieved a hit rate that was significantly above chance at all positions, the animals were prepared for the electrophysiology component of the experiment. During surgery, a microdrive with tetrodes was implanted to the animal's head. The electrodes were placed stereotaxically centered on posterior 3.8 mm, lateral 2.2 mm over the course of recording days. We recorded from the posterior parietal cortex and the CA1 subregion of the hippocampus. The electrodes picked up the firing activity of single neurons from the parietal cortex, and were slowly descended over the course of recording days to pick up single neurons from the hippocampus.

Track Recordings. The recording sessions were scheduled once per day for each of the three rodents in the training room. The rodent was prepared prior to each recording by having the electrodes slowly lowered as needed, and the electrodes were connected with a cord to a system that displayed and recorded the firing of action potentials of the cells that could be detected. LED lights were attached to the head of the animal and tracked the positions of its head and body orientation, which was captured by three cameras placed in different positions of the room that allowed for the creation of three-dimensional videos. Similar to what would occur during a training session, two experimenters sat on the ends of the T-maze, but a third experimenter was present for the track recordings to hold the cord as the animal ran along the maze throughout the recording session.

Behavioral Data Analysis

The behaviors of the animals captured during each of the recording sessions were saved into files, and were scored using a Graphic User Interface in MathWorks® MATLAB. The data was plotted as pixels on a time-dependent window that represented the points of the track space traveled by the animal. Scorers clicked the start and end of each outbound run from each file, and labeled the runs accordingly (whether they appeared to be clean or dirty runs). The clean runs were turned into a vector of points for a path shape for the averaged position along the track, which was later used as a template in the neural analysis.

All Trials. If the animals did not learn the abstract fragmentation rule, then their performance should be at chance (50%). The average percentage correct for the three animals' individual performance for turning correctly (i.e., left turn for Pos1 and Pos2, and right turn for Pos3 and Pos4) at all four positions was assessed. The percentage correct for the animals' performance averaged together was also assessed.

First Trials. The first trial of each block was important in determining whether the animals have learned the fragmentation rule. It revealed whether the animals were guessing what to do every time they were placed in a new position. If they were simply guessing, it should make their performance for the first trials at chance. If they made an incorrect turn at the first trial, the animals could learn to switch to a different turn for the subsequent trials—making the performance for the averaged trials above 50%. In order to testify against the argument that the animals were using a switching strategy, we separated the trials to specifically analyze the performance for the average percentage correct at all positions for just the first trials of the three rodents. The percentage correct for the animals' performance averaged together was also assessed.

Proximity to the Boundary Line. If the animals did not learn to the behavioral rule of fragmentation, they should have about equal performance and equal rate of error at each of the four positions. If the animals did learn the rule, then the positions closer to the invisible boundary line (Pos2 and Pos3) would debilitate the rats' performance because these positions were more difficult to distinguish which side of the fragment the positions occupied. This also meant that performance should be higher for Pos1 and Pos4 because they were farther from the invisible boundary line, making it easier for the rats to perceptually distinguish which side of the boundary line these positions occupied. Thus, to further support our claim that the animals have learned the fragmentation rule and not just learned four independent associations, difference in performance between Pos1 and Pos4 to Pos2 and Pos3 was tested using a paired *t*-test.

New Positions. A final way to further prove that the rats have learned the fragmentation rule was to assess the performance for the first time the rats were introduced to new potions. When the performance for all trials of each of the four positions was found to exceed 80% correct for nine consecutive days, further platform positions were brought into play. Three new positions were introduced: position 5 (Pos5), which was placed up from Pos4; position 6 (Pos6), which was placed right laterally to Pos1; and position 7 (Pos7), which was placed right perpendicularly to Pos5 (Figure 3). These positions were experienced by all three animals in that order. Thus, the initial reactions and turns the rats made at Pos5, Pos6, and Pos7 would be informative as to whether they have learned the behavioral rule to mentally fragment the room.

Neural Data Analysis

Waveform discrimination into single neurons was carried out manually using the Plexon OfflineSorter™ software. The process started with identifying single neurons from clusters of cells that shared similar relative spike amplitudes picked up by the electrodes, followed by removing artifacts to refine clustering, which included differently shaped action potentials. After behavioral scoring was completed and single neurons have been identified, the two sets of data were used to create rate maps. Rate maps displayed the amount of firing of each neuron each time the animal occupied a position on the track. The following analyses involved looking at the patterns generated by the single unit firing activity across cells in each of the two brain regions of interest, as well as looking at the patterns of the population of neurons as a whole in those areas.

Parietal Cortex. We analyzed the single unit activity of the parietal cortex and expected to find cells that were consistent with previous findings that the parietal cortex maps out space as the animal progresses through the route or track space. The goal was to examine the patterns of firing activity in the parietal cortex as the animals traveled through mentally fragmented spaces.

Hippocampus. For the single unit activity of the hippocampal place cells, we expected to find neurons that were consistent with previous findings that they would fire according to the reference frame of the environment. The goal was to examine the patterns of firing activity in the hippocampus as the animals traveled through mentally fragmented spaces.

Population Correlation Matrices. In addition to examining the firing activity of single units, we also looked at the population of the neurons as a whole. This type of analysis was used to assess whether the encoding of spatial information in the population activity reflected that of the single unit activity, which could provide evidence of how the hippocampus and parietal cortex differed in their firing patterns. Correlation matrices were used to carry out the analyses for the population of neurons. We divided the track space into 100 sections or bins that were the same points for each of the four positions. The Pearson correlation coefficient was calculated for the average firing activity of all the cells in each bin for one position to the average firing activity of all the cells in each bin for another position, ultimately creating a matrix of correlations. The diagonals of a specific matrix represented the comparison of the correlation of the same bin on the track for two different positions. All possible combinations of comparisons of two positions were assessed, creating six matrices for each of the brain regions.

Comparison of Correlations. The final component of the neural analysis involved plotting the correlations of the diagonals for each of the possible combinations used in the population correlation matrices analysis. This would compare where and when the point of pattern activity would diverge on the track position (0-100 bins) for both the parietal cortex and hippocampus. This would show the Pearson correlation of population activity pattern as a function of where the animal was on the track position.

Results

Behavioral Data

All Trials. If the animals did not learn the abstract fragmentation rule, then the results would show performance at chance (50%) for all the animals at all the positions. The results showed that all the animals performed above chance at all of the four positions (Figure 4A).

When they were averaged together, the animals also showed performance above chance (Figure 4B).

First Trials. We separated the trials to specifically assess the percentage correct at all positions for just the first trials. If the animals did not learn the abstract fragmentation rule, then the results would show performance at chance for all the animals at all the positions. When the first trials were assessed, the animals performed slightly worse at Pos2 and Pos3 (averaging 84.92%) than at Pos1 and Pos4 (averaging 100%), but the animals were still performing above chance (Figure 5A). When the data was averaged across the three animals, the percentages correct were still above chance for all positions (Figure 5B).

Proximity to the Boundary Line. When the average percentage correct for the performance comparing Pos1 and Pos4 to Pos2 and Pos3 were assessed across all three rats, the results showed performance above chance (Figure 6A). When Pos1 and Pos4 were averaged together, we see that the performance for Pos1 and Pos4 was statistically significantly higher ($p < 0.05$) than that of Pos2 and Pos3 averaged together (Figure 6B).

New Positions. If the animals did not learn the abstract fragmentation rule, then the results would show performance at chance for all the animals the first time the tracks were placed at a new position. All the animals made the correct turn for Pos5 (turn right) and Pos6 (turn left). Only one of the rats made an incorrect turn at Pos7 (turn right), which was arguably more difficult than Pos5 and Pos6 because it involved a change in position as well as a change in rotation of the room. The rats performed 8 out of 9 trials correctly, which was 88.89%, for the first trials of the introduction of these new positions.

Neural Data

Parietal Cortex. We found three examples of cells in the parietal cortex with expected and unexpected firing activity patterns that were consistent across all cells. We expected to find cells in the parietal cortex that mapped out the route as the animals progressed through the track space, which was the case seen in the activity pattern of one of the examples of neurons we found. We also examined action specific cells as well as action planning cells, which planned out the action of the animal. Of the three example types of neurons examined in the parietal cortex, the action planning cells were the unexpected finding.

One type of neuron we examined followed the space of the track, which was consistent with previous findings in the literature that parietal cortex neurons use the reference frame of the route to map out space. This particular neuron peaked in its firing activity when the animal ran at the beginning of the stem of the T-maze, but dropped in its firing activity as the animal continued along the straight path of the stem (Figure 7A). The location of the firing activity is consistent across the four different positions of the maze.

Another type of neuron we examined fired only when the animal was executing a specific action. The action in this case would be turning either left or right. These action cells carried information for where the animal was in the route. This particular cell fired as the animal made a right turn, which was made clear when the overall mean activity was much higher after the animal had made a turn at only Pos3 and Pos4 (Figure 7B).

The third type of neuron found in the parietal cortex had an unexpected firing pattern. Rather than firing as the animal made an action, this cell appeared to fire just before the animal made the action. This suggests that the animal was planning what type of action to execute as it approached the choice point in a track position that required the correct turn, which was a right turn in this example because the firing occurred at Pos3 and Pos4 (Figure 7C).

Hippocampus. Surprisingly, we found three examples of place cells with unexpected and unusual firing activity patterns that were consistent across all cells. These included neurons with high firing activity for just the first trials of each position, place cells that actually fired with the reference frame of the route instead of the room, and neurons that changed in their in-field firing rates as the positions were moved. These three new findings are the first to be reported and shown in this study.

We examined one example of a place cell that had higher firing rates for the first trials than the overall mean at all four of the positions (Figure 8A). This suggests that the animals were processing what they needed to do on the first trial while they were performing the task, and that allowed them to subsequently perform the same behavior on trials 2-5.

The place cells we found did not map out the space of the environment or room, which was the prediction made prior to completing the neural analysis for the hippocampus. We were surprised to see that the place cells were actually mapping out the space on the track (Figure 8B), similar to what the cells in the parietal cortex were doing. This second piece of surprising evidence suggests that the hippocampal place cells were doing the same thing as the cells that map out route in the parietal cortex.

Despite this surprising finding in the hippocampus, it appeared that many of the cells that traveled with the track differed in their in-field firing rates. What we saw was that when the animal was entering a different space, such as traveling along the four different locations where the positions were placed, the hippocampus could change in how much the cells were firing. One place cell that we examined fired low to high as the animal moved from Pos1 to Pos4 (Figure 8C). We also examined other cells with patterns that could range from high to low firing activity as the animal moved from Pos1 to Pos4.

Population Correlation Matrices. By looking at the populations of cells as a whole, it would provide an opportunity to see how the brain works through patterns of activity rather than by individual firing of cells. As seen in the analyses of the single unit activity of the parietal cortex and hippocampus, both brain regions used the frame of reference of the route. When we look at the population correlation matrices for the hippocampal neurons, the color maps show that the there was a strong correlation across the diagonals of the matrices for the comparisons of positions that were within the same fragment. The correlation remained high for the comparisons of the positions that were from different fragments just until before the turn, which was where the pattern began to diverge (Figure 9A). When we look at the neurons for the parietal cortex, we saw that there were similar patterns in the color maps as we saw in the hippocampus. We found high correlations as the animal ran through the progression of the track for the comparisons of positions that were within the same fragment. However, the correlation began to drop right before the animal made a turn for the comparisons of positions that were from different fragments (Figure 9B).

Comparison of Correlations. The correlation of the diagonals of the matrices was plotted as a line for each of the position comparisons, which showed the Pearson correlation of population activity pattern as a function of where the animal was on the track. For the hippocampus, the correlation remained high for comparisons of positions that were within the same fragment while the correlation dropped for the comparisons of the positions that were from different fragments just until before the turn, where the pattern began to diverge (at bin 76). Similar patterns for the comparisons of correlations were also seen for the parietal cortex. The correlation remained high for the comparisons of positions within the same fragment but the correlation dropped just before the turn for comparisons of positions from different fragments.

For the parietal cortex, we see that the point of pattern divergence happened earlier on the track (at bin 66) than it does in the hippocampus (Figure 10). This proposes an idea that the animal was recognizing when he was making a choice and also suggests the rat seemed to know which side of the boundary line it was occupying.

Discussion

This study's objective was to find a measure for generating a behavior that would satisfy the conditions for learning a fragmentation rule, as well as to find which brain areas would map out these fragmented spaces. The results of the behavioral analyses revealed that it was very likely that all three animals had successfully learned the rule to mentally fragment the room. We found both expected and unexpected findings from the electrophysiological recordings of the parietal cortex and hippocampus, some of which were new findings that were the first to be reported in this study. We also asked whether the population of neurons as a whole reflected the activity seen in the single unit activity in both the hippocampus and parietal cortex. Using population correlation matrices, we found that both brain regions used the frame of reference of the route to map the animals' position in the room. By plotting the correlations, we found that the pattern of the correlations of positions from different fragments dropped just before the turn on a track, indicating that the animals have learned the behavioral rule of fragmenting the room.

Prior to the study, it was unknown whether the performance of the animals would reveal any evidence of fragmentation. To our surprise, the performance of the animals for all trials at all four initial positons was a strong suggestion that the animals have learned the fragmentation rule. The results of this behavioral analysis showed promising evidence of fragmentation, but the behaviors could have also been explained by the animals learning four independent associations with no relationship to having learned the rule. That was why further analyses of the behaviors were required to provide results that would counter that argument. The performance on the first trial was the most important because it revealed whether the animals just guessing what to do every time they were placed in a new position. If they were simply guessing, it would make their performance at the first trials at chance (50%), which was not what we found in the results. Additionally, if the animals did not learn to fragment the room, they should have about equal performance and rate of error at each of the four positions. However, we found that the animals had worse performance when the positions were closer to the invisible boundary line (Pos2 and Pos3) because it was more difficult to distinguish which side of the fragment the positions occupied. This was a strong piece of evidence that the animals have actually learned the rule. One final component of the behavioral analysis was adding new positions to the study and observing how the animals initially react to them. That is, we recorded whether they made the correct turn for these new positions (Pos5, Pos6, and Pos7) when they were placed on these positions for the very first time. We found that the animals' performance exceeded the chance level. Because the animals performed above the chance level on the first trials, on positions with proximity to the invisible boundary line, and on the three new positions, we can make a strong claim that they have actually learned the behavioral rule.

Given that we asked where in the brain areas these mentally fragmented spaces were encoded, electrophysiological recordings were required to analyze the patterns of firing activity of the neurons from the parietal cortex and the hippocampus. From the parietal cortex, we found one type of neuron that followed the space of the track, which was expected and seen in the literature. This type of cell could reveal where the animal was along the path of the track by simply observing the firing activity patterns of these neurons. We also found action correlate cells that fired whenever the animal was making a left or right turn at the choice point on the T-maze, and these type of cells also carried information for where the animal was on the route. The unexpected finding from the results of the neural analysis of the parietal cortex was the discovery of cells that predicted the upcoming action of the animal, essentially planning the animal's decision before the execution of the action. It could be argued that the example we chose to describe was actually a "right leaning" cell, but this cell stopped its firing at the turn. If it really was a "right leaning" cell, it would have continued to fire at the turn. The L-shaped blockers on the T-maze would have prevented the animals from leaning one way or another, making the argument that it was a "right leaning" cell unlikely. Because this third example of a cell from the parietal cortex only fired prior to the animal making a turn, it is apparent that this cell corresponds to action planning.

We found surprising and unusual results as we analyzed the single unit activity of the hippocampus. We found hippocampal place cells that had higher firing rates for the first trials than for the overall mean of the trials. This suggests the animal was processing what it needed to do on the first trial as it performed the task, which allowed it to subsequently perform the same behavior on trials 2-5. However, we are still unclear about this finding and its relationship to this study. Another example of a neuron we examined contradicted what we knew beforehand from the literature on place cells; we expected the hippocampal cells to use the reference frame of the environment. What we found instead were hippocampal cells that were mapping out the space of the track. This result suggests that the parietal cortex and hippocampus were interacting together in some way because the hippocampus was being influenced by what the parietal cortex was doing, which was using the reference frame of the route or track. Finally, we found cells that changed in their in-field firings rates, which suggests there was some kind of interaction between the parietal cortex and hippocampus. This result showed that even though the hippocampus was mapping out the space on the track, it was still encoding information for where the animal was in the room. Therefore, this indicates that the hippocampal place cells were mapping out both the room and the route simultaneously.

The results of the population correlation matrices show that for both the hippocampus and parietal cortex, the brain regions were using the reference frame of the route to map out the animals' position in the room. The color maps showed a strong correlation across the diagonals of the matrices for the comparisons of positions that were within the same fragment. However, the correlation remained high for the comparisons of positions that were from different fragments just until before the turn, where the patterns began to diverge. When we plotted the correlations of the diagonals for each of the possible combinations used in the population correlation matrices analysis, we found that the correlations for the comparisons of positions from different fragments diverged from their pattern earlier. This is true for both the hippocampus and parietal cortex, with the latter's firing pattern diverging earlier on the point of the track. This suggests that the two brain areas were interacting together as the animals performed the task. The results of the neural analyses complement the results of the behavioral analyses, and provided further evidence that the animals have learned the rule of fragmentation.

One limitation of this study was that there were only three rats in our sample, and the reported results from the neural analyses were from only two rats. Though we found consistent results across all the animals, more data would be needed to confirm our claims. It is necessary to increase the sample size so that we can have more data and sample means to carry out other types of statistical tests, such as a chi-square test or sign test. Even though the data we currently have were from a small sample size, we were still able to find promising evidence of the animals having learned the behavioral rule by looking at just their performance. The addition of more subjects would only confirm the reported results and strengthen our claim of the animals having learned the abstract spatial rule.

The future directions of this study would be to continue training and recording the animals to gather and analyze more data. Of the unexpected findings discovered though the neural analyses, such as the first trial cells of the hippocampus and the cells that changed in their in-field firing rate, it would be important to continue investigating these types of cells so that we can find a reasonable interpretation for what causes these unusual activity patterns.

It may be interesting to make a few adjustments to how the current study is being done to confirm that the rats have learned the abstract spatial rule. For example, it may be meaningful to add some more track positions in the room. So far, it appears that the fragmentation happened along one dimension since Pos1, Pos2, Pos3, and Pos4 were aligned and moved along the same axis. It can be the case that the fragmentation can occur in two dimensions. By adding an addition track position next to Pos6, and recording the animal as it traveled there after visiting Pos1 and Pos6, it could be possible to find cells that would fire along this other axis and have the animals learn to fragment the room in a different way. This proposal could help explain the changes in in-field firing rates that were observed in the hippocampal cells. Perhaps observing the changes from low to high, or high to low, firing rates of the cells as the animals traveled along two dimensions would provide some answers as to whether the fragmentation happens gradually. Another suggestion to this study would be to move the spatial cues on the walls to different places in the room. By moving them, we can observe whether the rats were relying on certain cues to perceive their current position that would have helped them make their decision of choosing to make a left or right turn.

We suggested that there could be an interaction between the hippocampus and parietal cortex as the animals were traveling through these fragmented spaces. To support this claim, we would need to have unilateral lesion experiments to examine how much each brain region contributes to the ability of fragmentation when one of them is damaged or completely absent. Because we found that both brain regions were using the reference frame of the route to accomplish the task of fragmentation, it could lead to interesting results in how the brain would compensate for either the hippocampus or parietal cortex to encode the necessary abstract spatial information.

In conclusion, we have both behavioral and neural data that are evident of the rats having learned the abstract spatial rule of fragmentation. Only will further investigation of this study improve our understanding of the relationships between the hippocampus and parietal cortex as the animals move through these mentally fragmented spaces.

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Figures

Figure 1

Dimensions of the T-maze

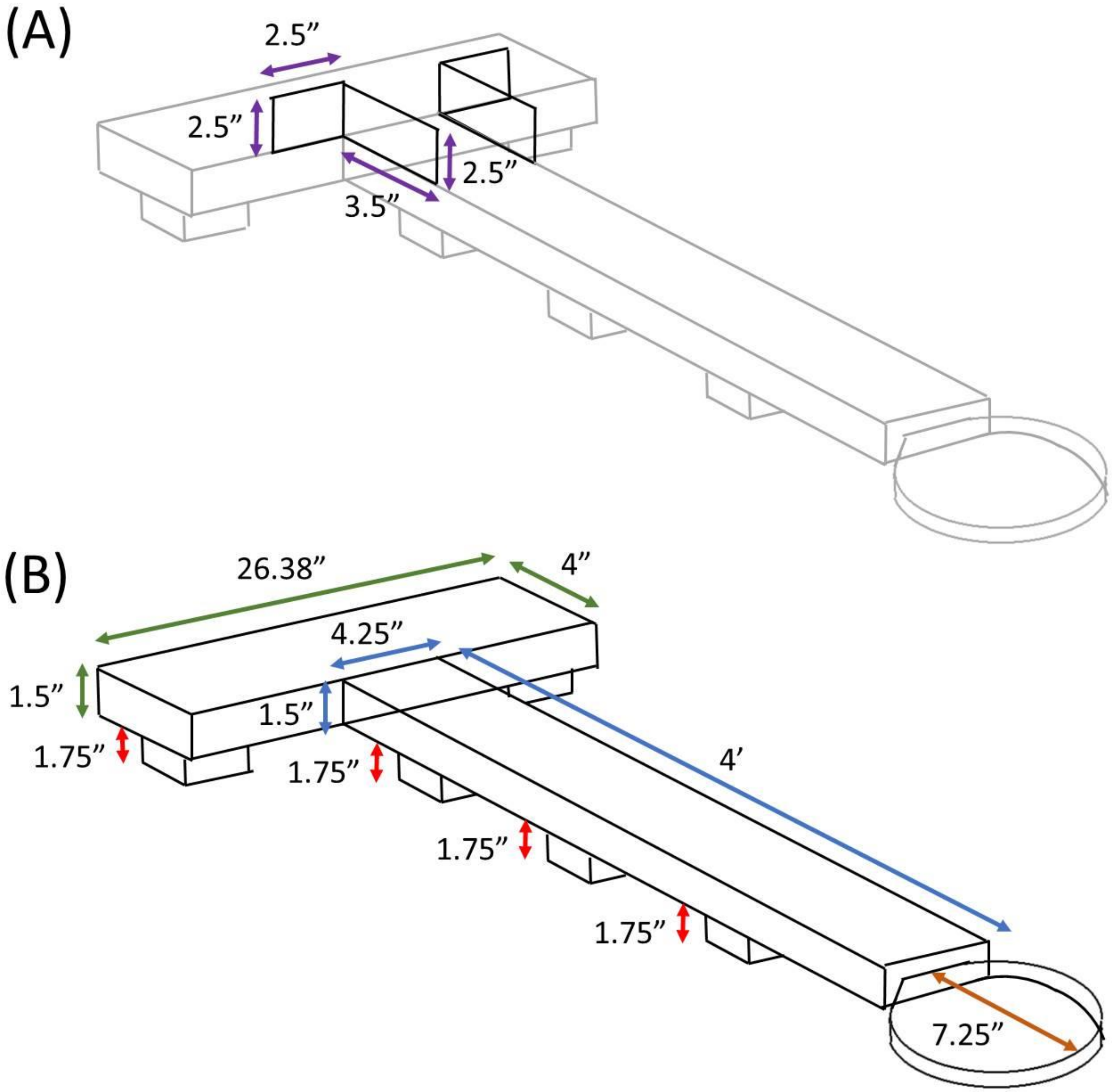


Figure 2

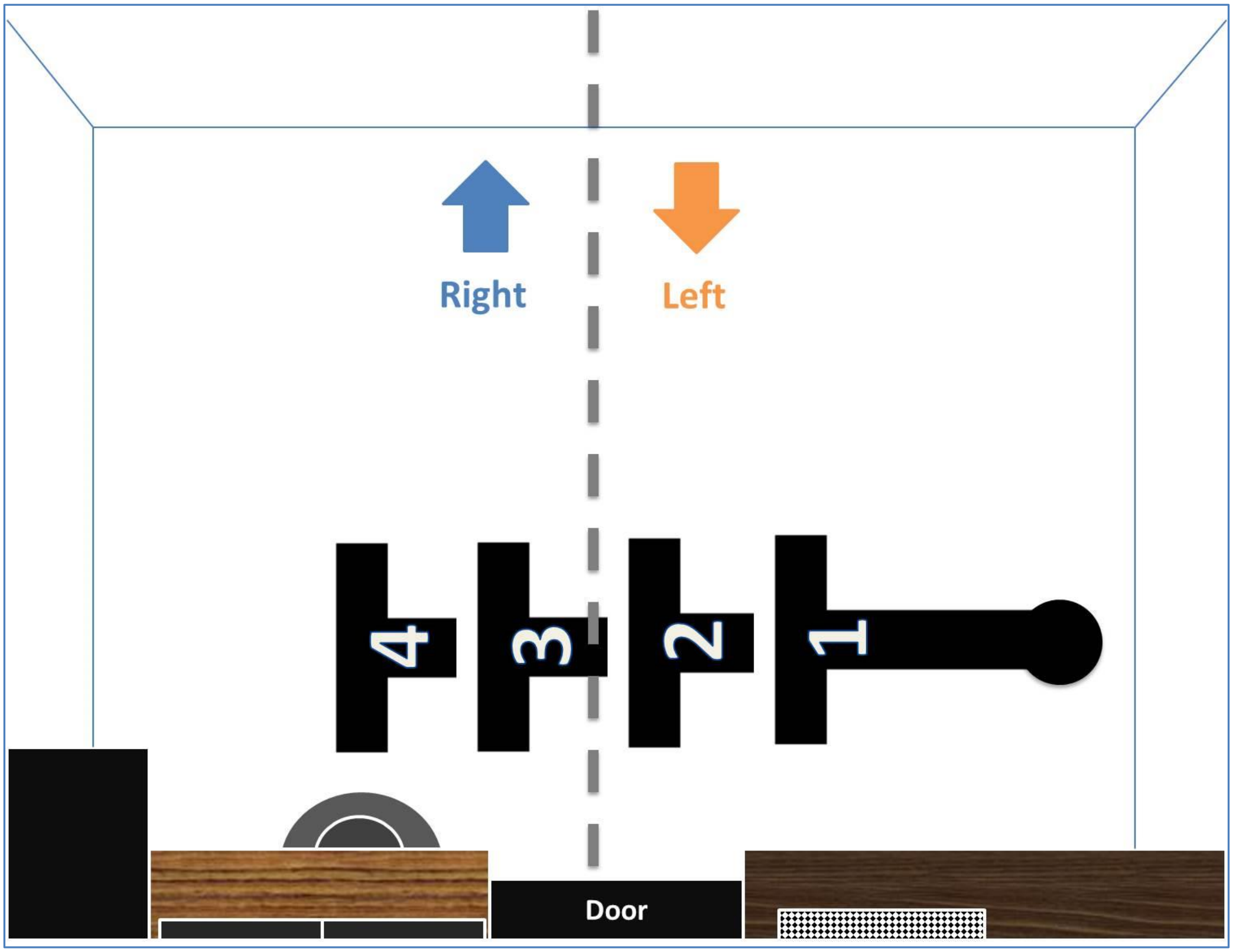


Figure 3

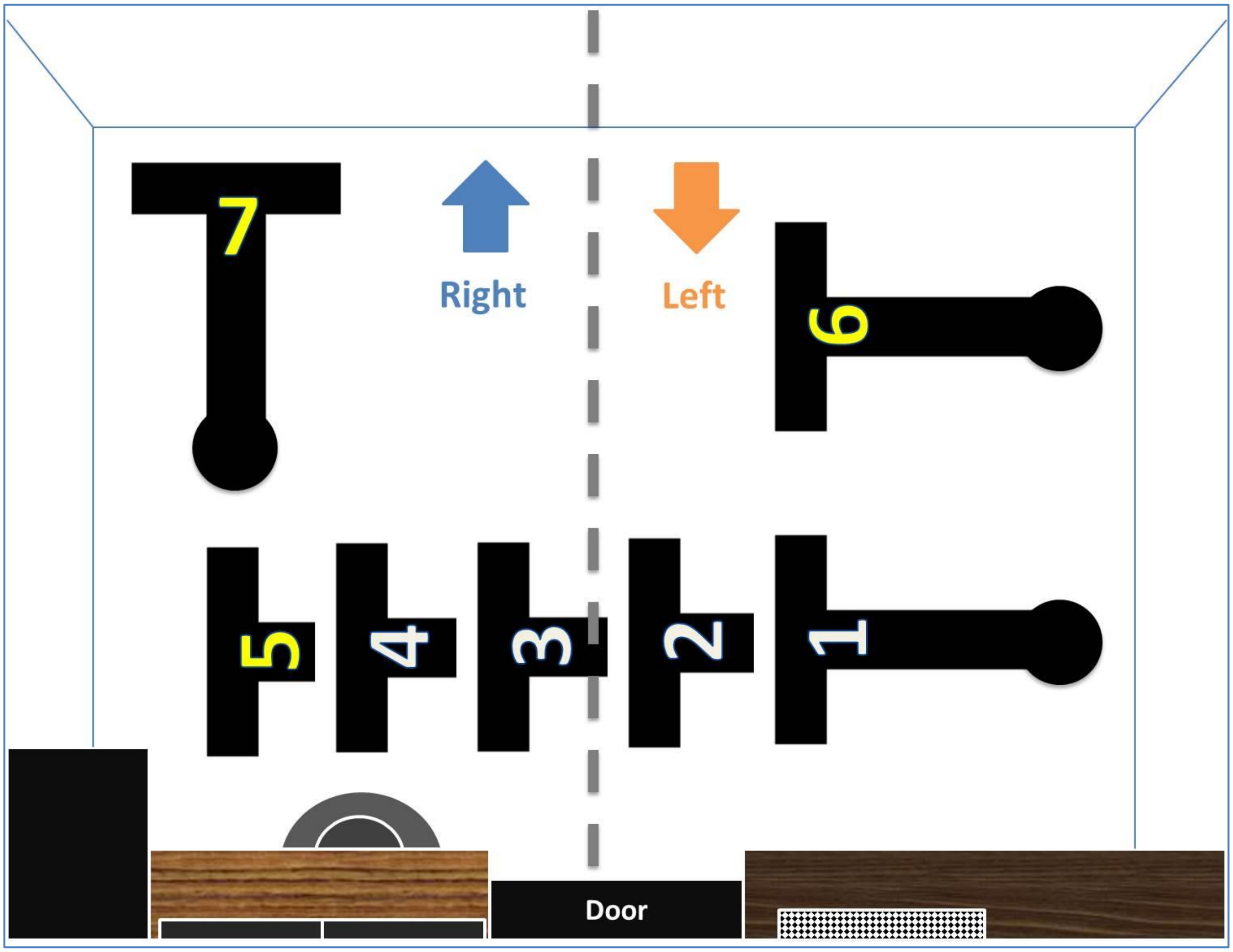


Figure 4

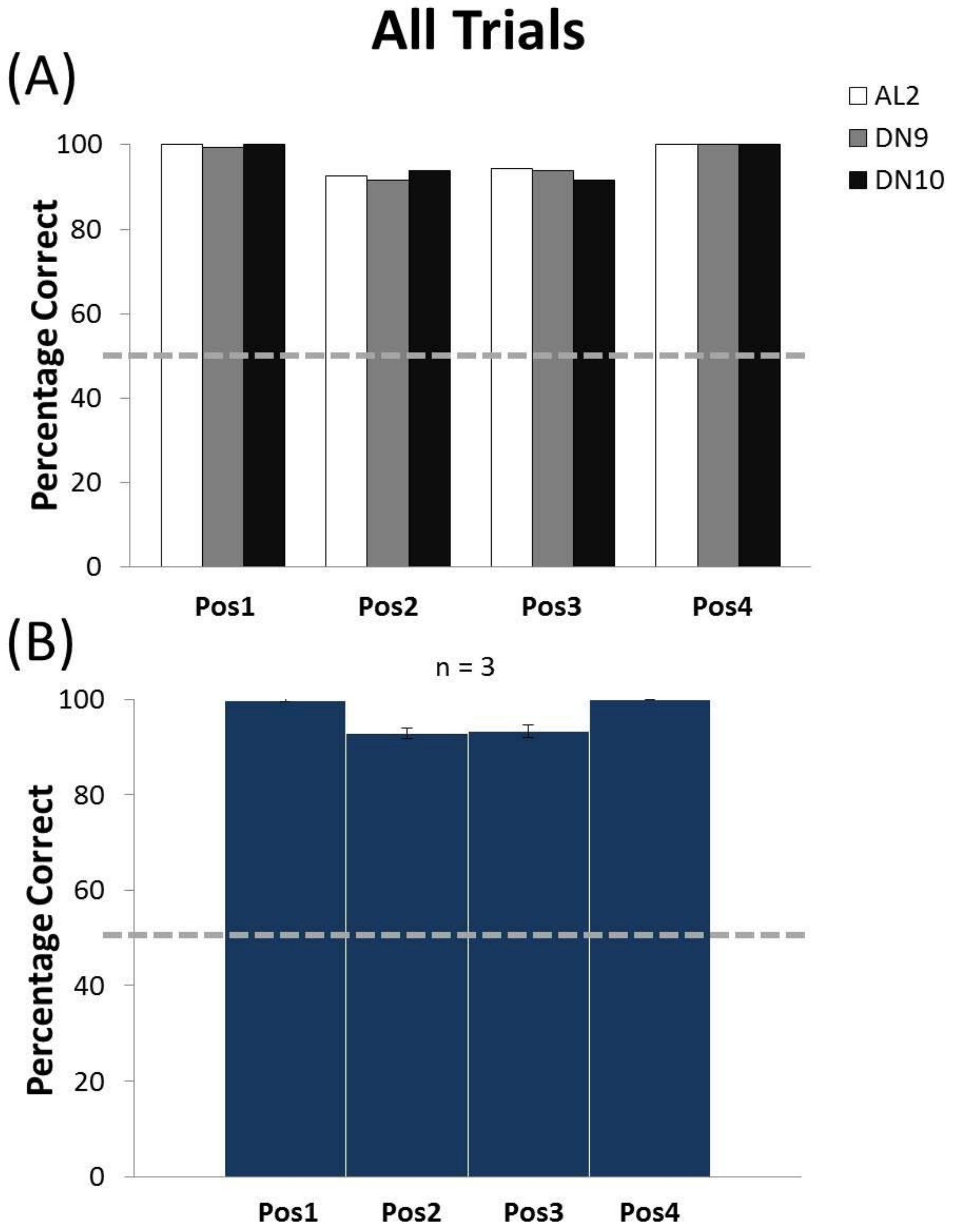

All Trials
(A)
☐ AL2
▨ DN9
■ DN10
100
80
60
40
20
0
Percentage Correct
Pos1
Pos2
Pos3
Pos4
(B)
n = 3
100
80
60
40
20
0
Percentage Correct
Pos1
Pos2
Pos3
Pos4

Figure 5

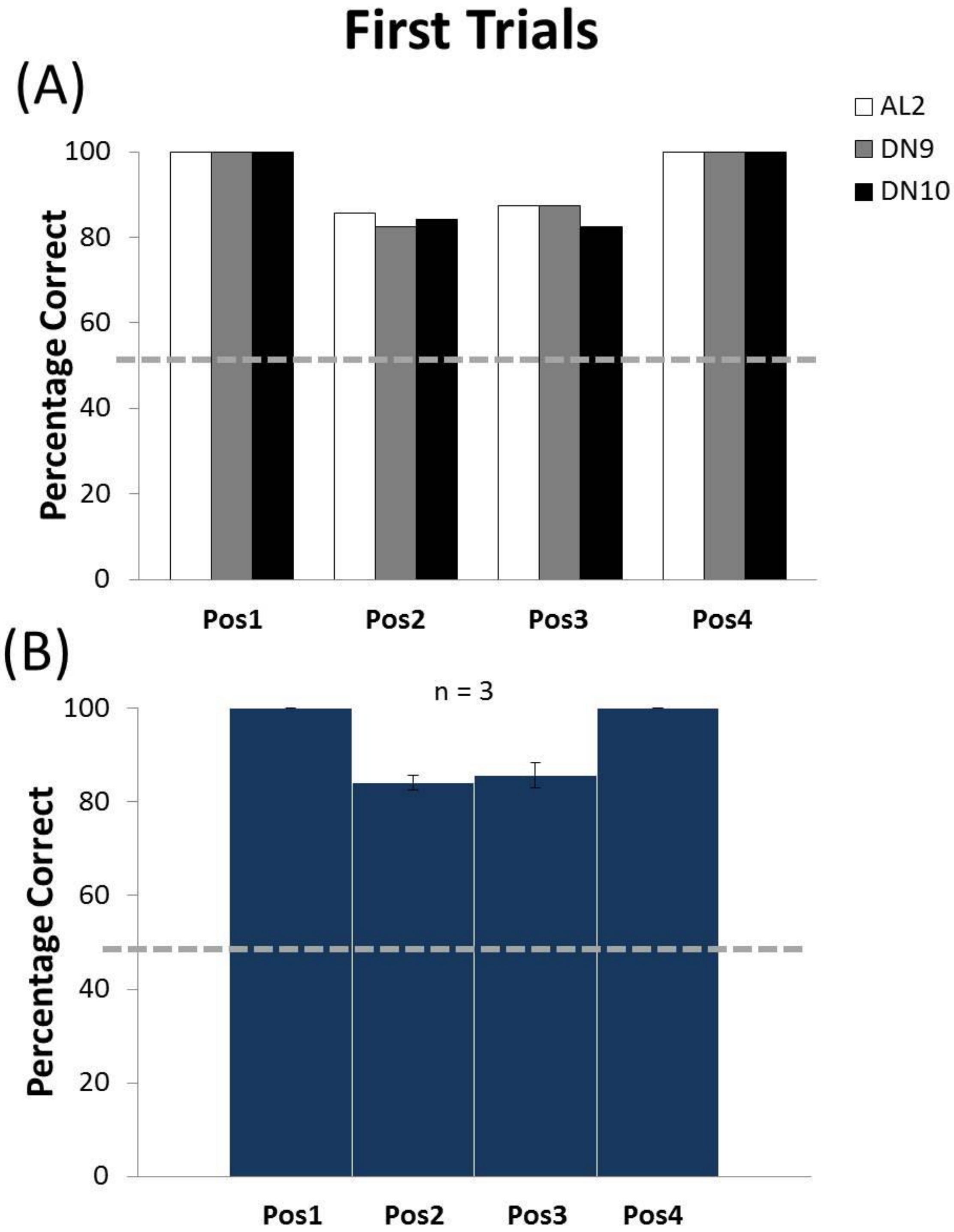

First Trials
(A)
☐ AL2
▨ DN9
■ DN10
Percentage Correct
100
80
60
40
20
0
Pos1
Pos2
Pos3
Pos4
(B)
n = 3
Percentage Correct
100
80
60
40
20
0
Pos1
Pos2
Pos3
Pos4

Figure 6

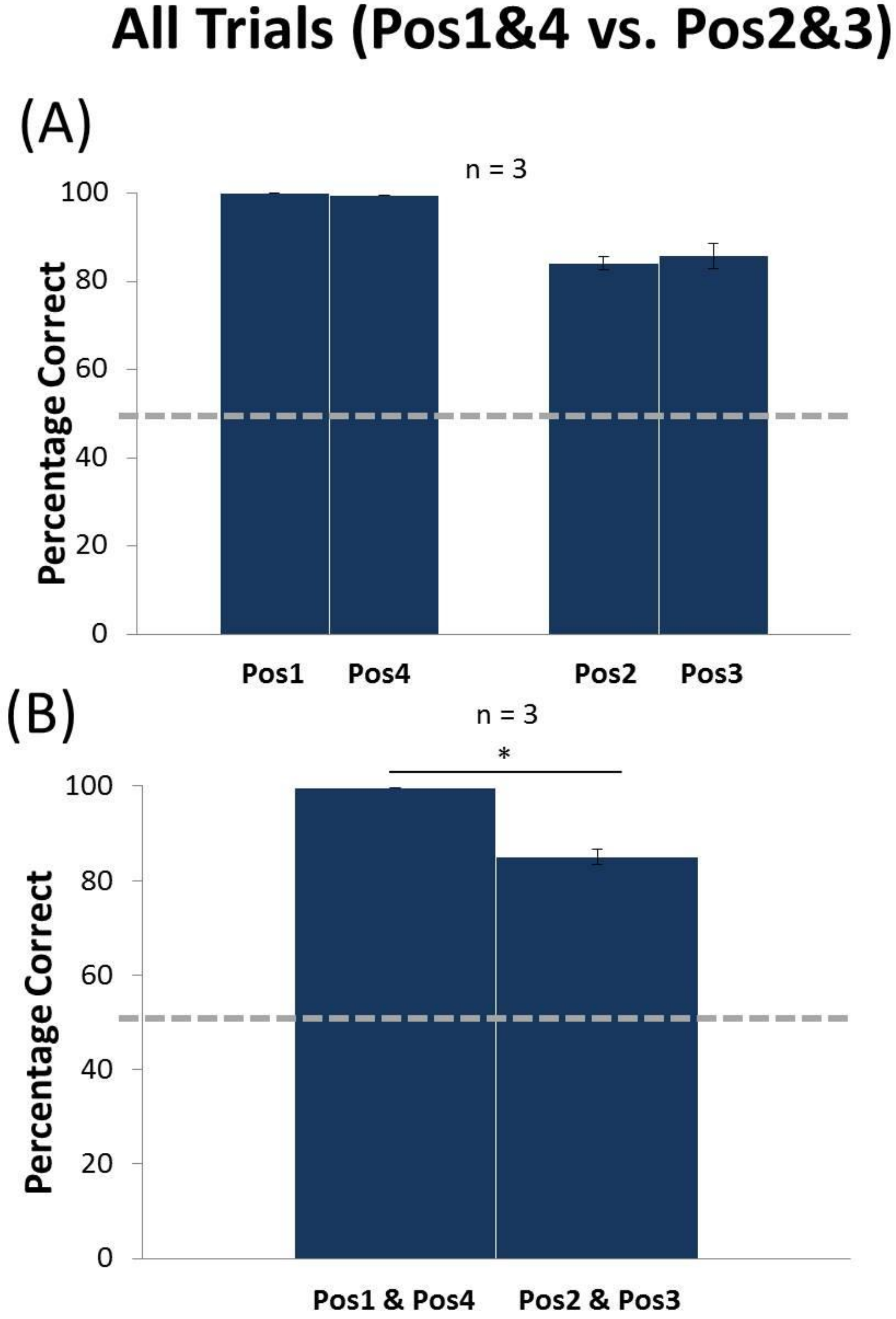
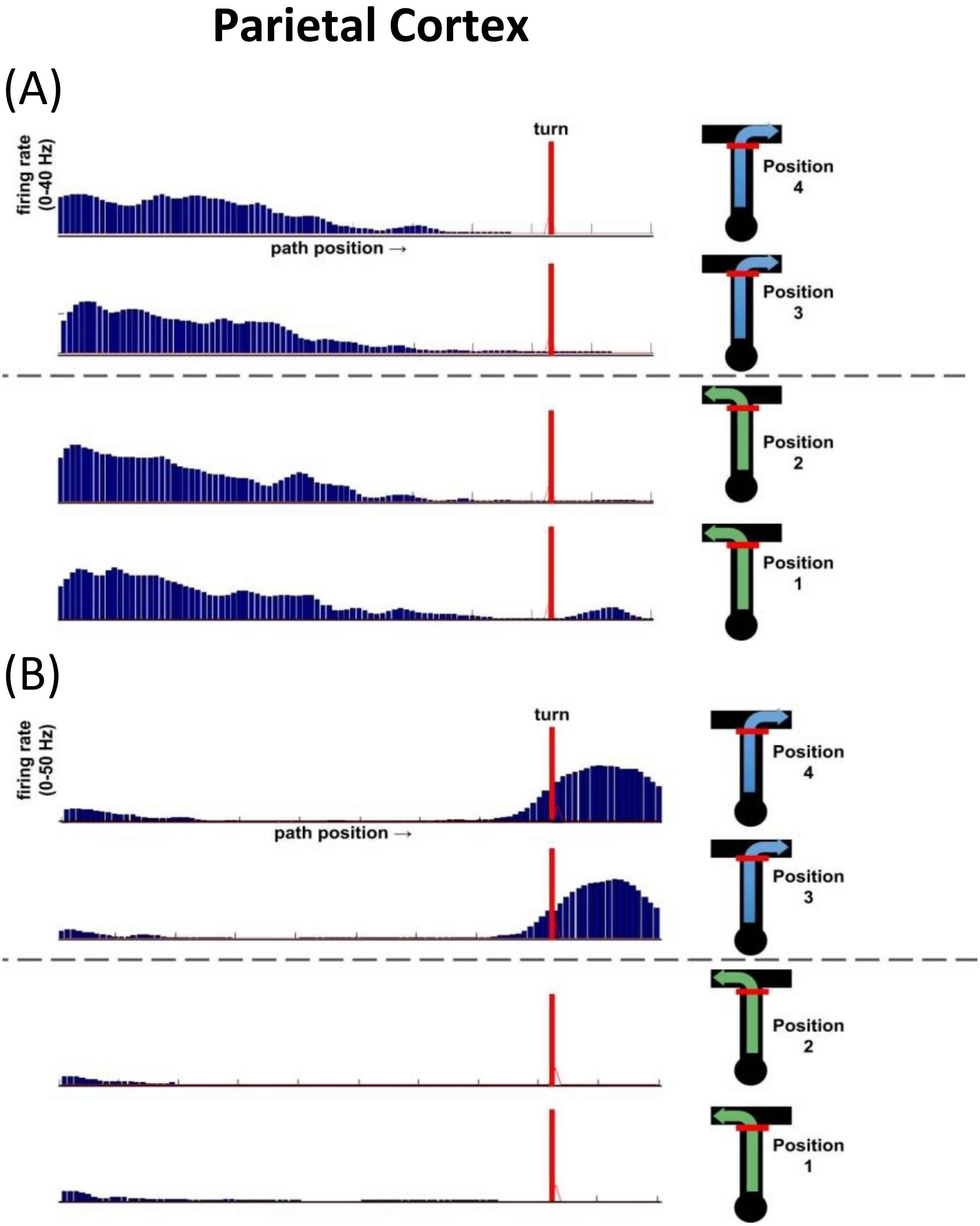

All Trials (Pos1&4 vs. Pos2&3)
(A)
n = 3
Percentage Correct
100
80
60
40
20
0
Pos1
Pos4
Pos2
Pos3
(B)
n = 3
*
Percentage Correct
100
80
60
40
20
0
Pos1 & Pos4
Pos2 & Pos3

Figure 7


Parietal Cortex
(A)
turn
firing rate (0-40 Hz)
path position →
Position 4
Position 3
Position 2
Position 1
(B)
turn
firing rate (0-50 Hz)
path position →
Position 4
Position 3
Position 2
Position 1

(C)

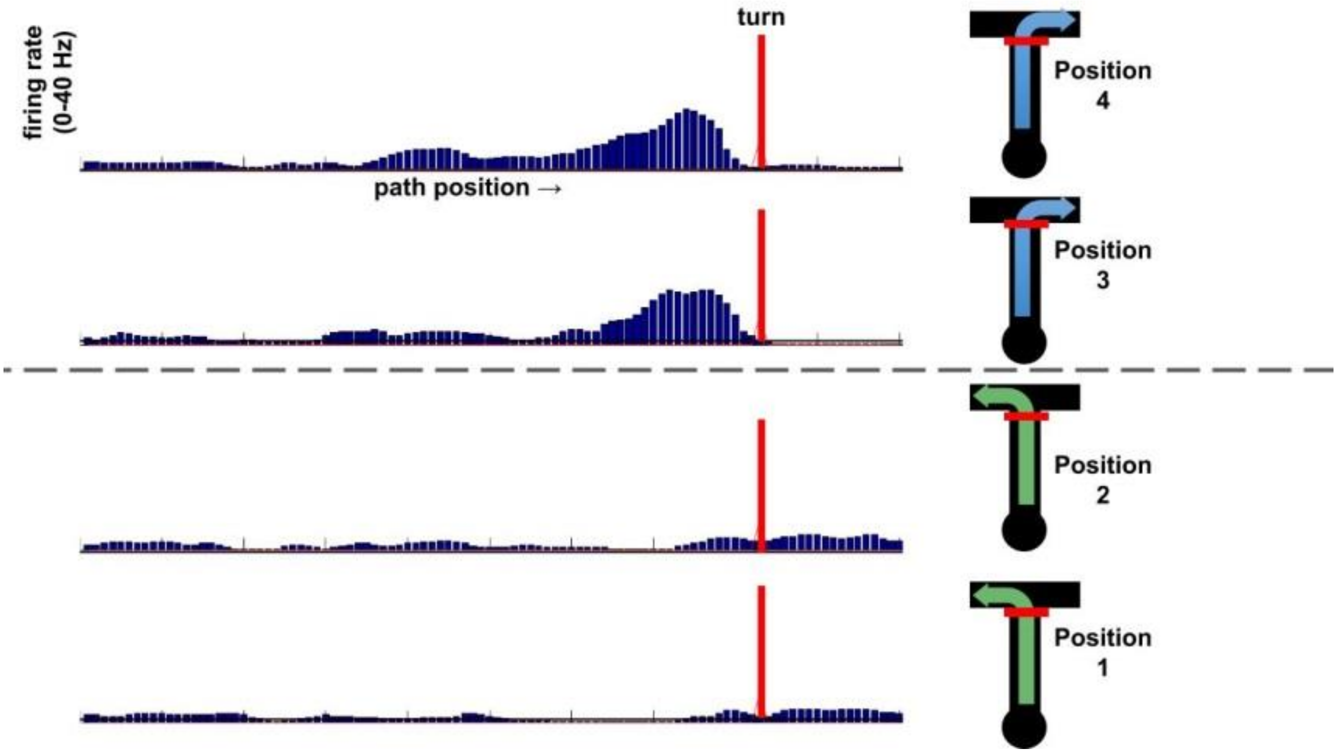
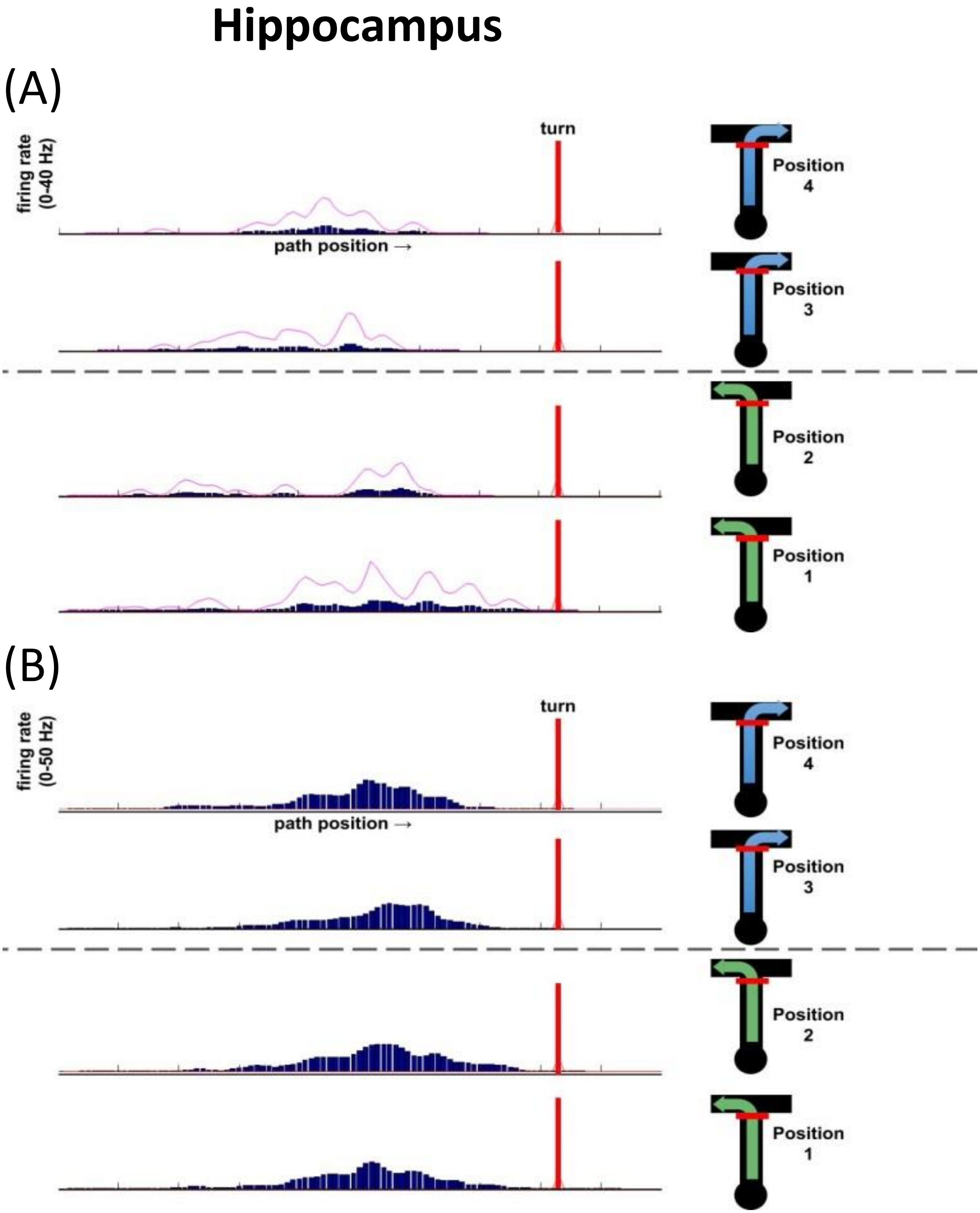

turn
firing rate (0-40 Hz)
path position →
Position 4
Position 3
Position 2
Position 1

Figure 8


Hippocampus
(A)
firing rate (0-40 Hz)
turn
path position →
Position 4
Position 3
Position 2
Position 1
(B)
firing rate (0-50 Hz)
turn
path position →
Position 4
Position 3
Position 2
Position 1

(C)

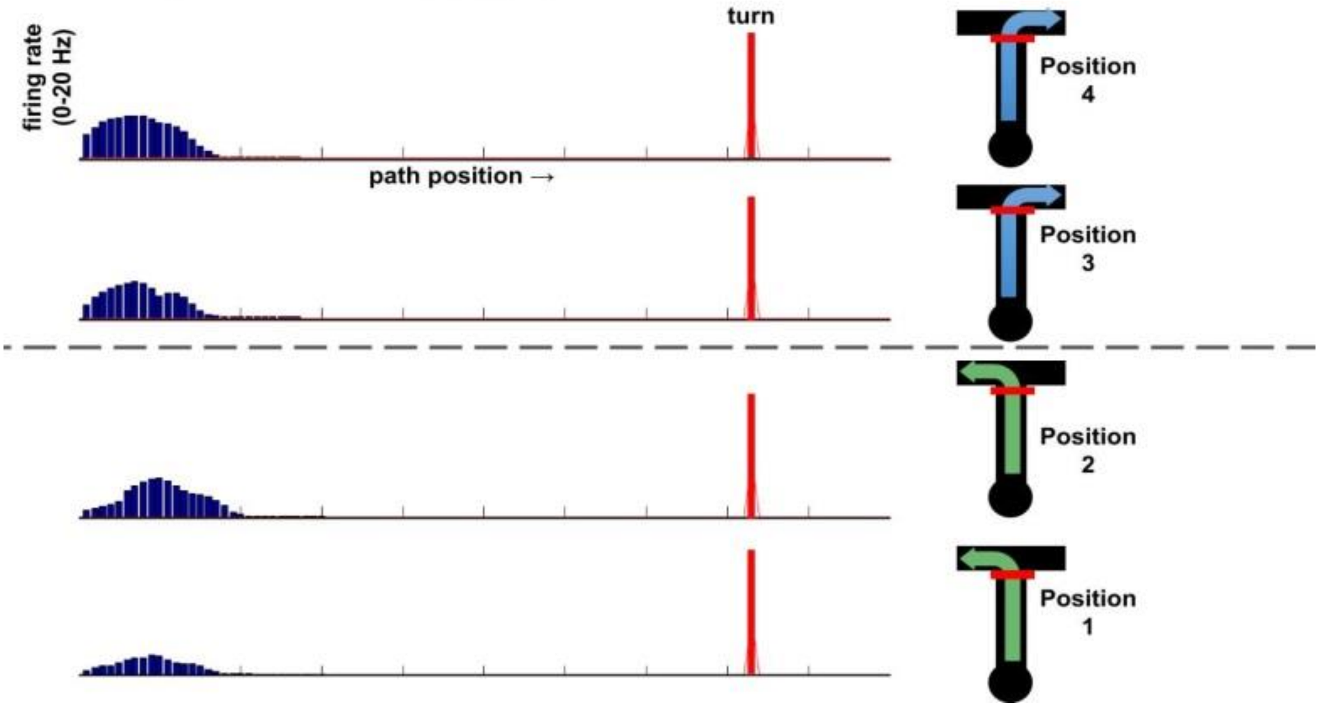

turn
firing rate (0-20 Hz)
path position →
Position 4
Position 3
Position 2
Position 1

Figure 9

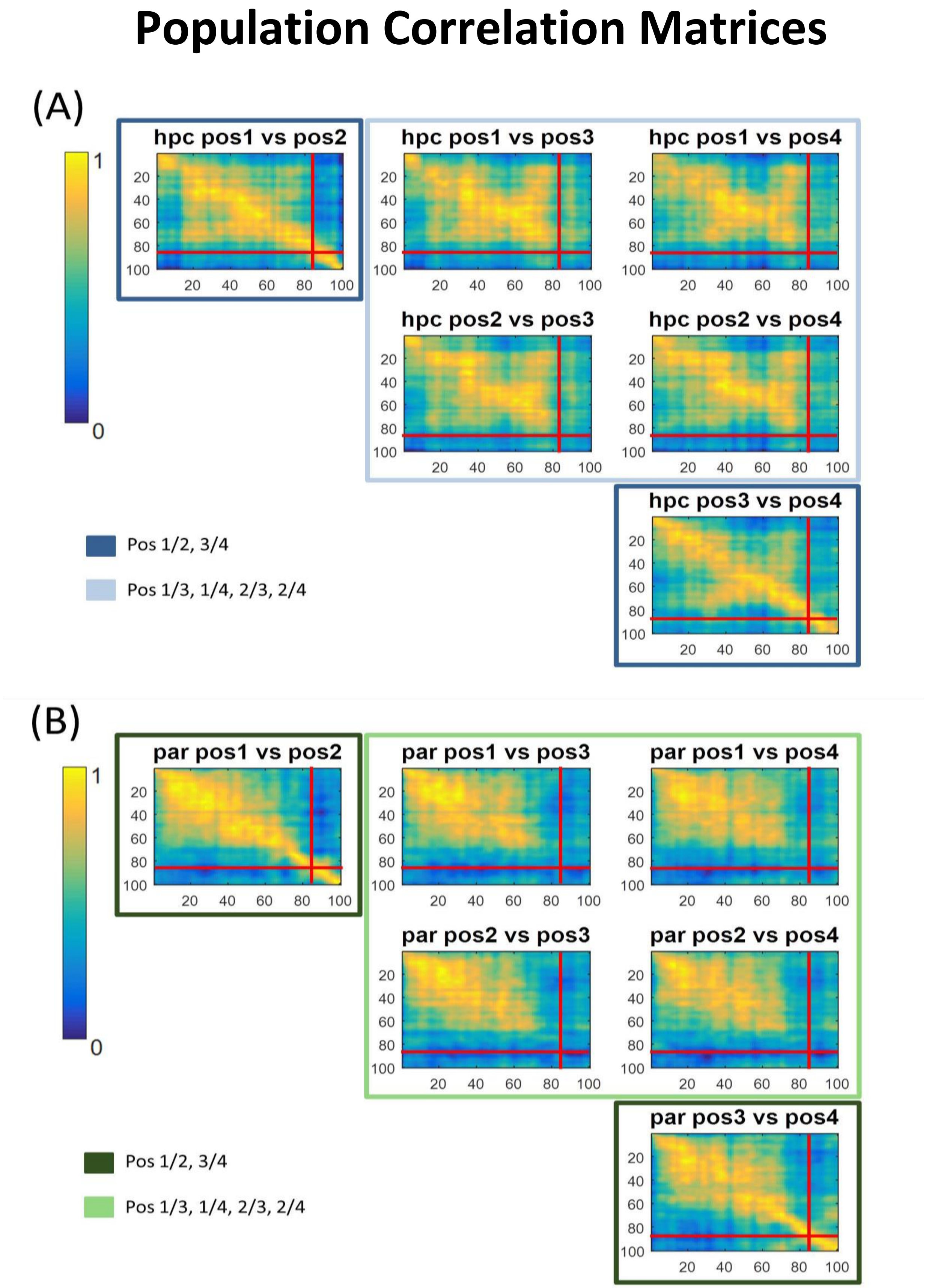

Population Correlation Matrices
(A)
hpc pos1 vs pos2
hpc pos1 vs pos3
hpc pos1 vs pos4
hpc pos2 vs pos3
hpc pos2 vs pos4
hpc pos3 vs pos4
20
40
60
80
100
1
0
Pos 1/2, 3/4
Pos 1/3, 1/4, 2/3, 2/4
(B)
par pos1 vs pos2
par pos1 vs pos3
par pos1 vs pos4
par pos2 vs pos3
par pos2 vs pos4
par pos3 vs pos4
Pos 1/2, 3/4
Pos 1/3, 1/4, 2/3, 2/4

Figure 10

Comparison of the Correlations between the Hippocampus and Parietal Cortex

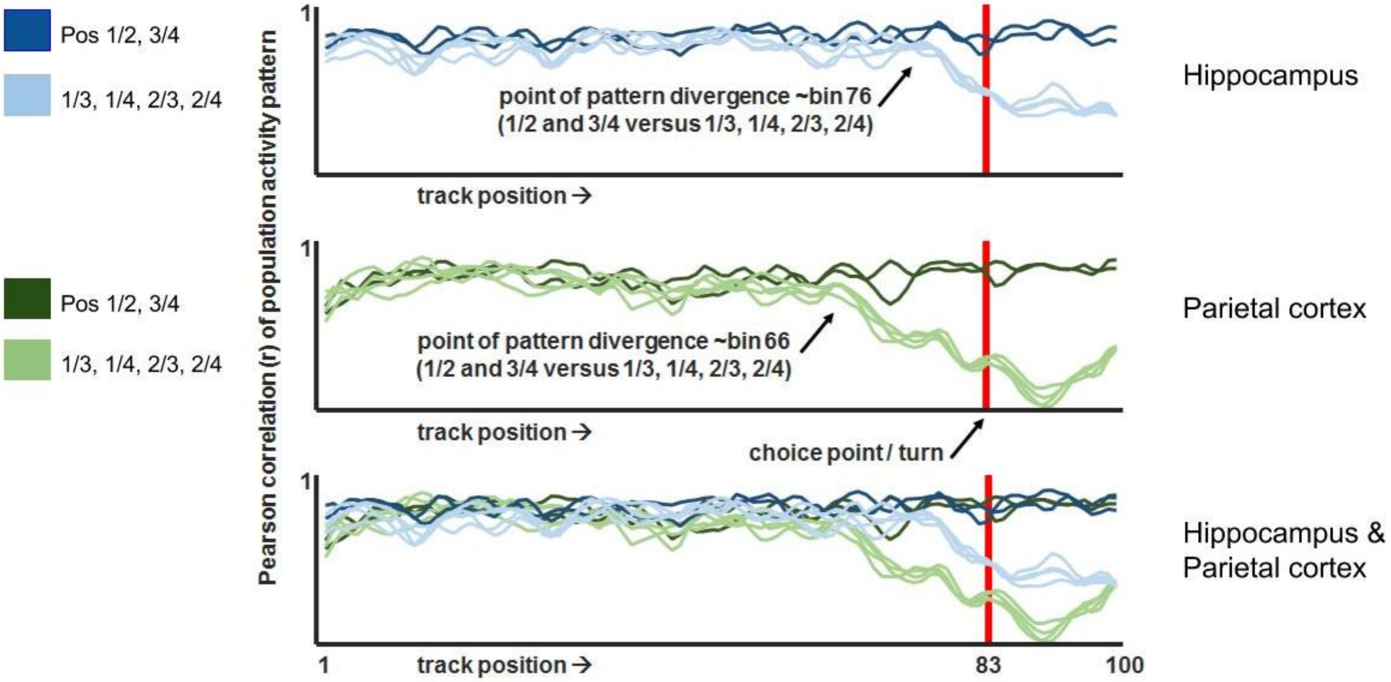


Figure Captions

Figure 1. The dimensions of the entire T-maze are shown, which include (A) the L-shaped blockers or barriers, and (B) the stem, side, and platform of the maze.

Figure 2. The layout of the dimly lit room where the animals were trained and recorded. The cues on the walls included the door, desks, computer, and other various objects that allowed for the animals to use as a frame of reference. The four different positions (Pos1, Pos2, Pos3, and Pos4) are shown where they were placed in the room and how distant they were from the other positions. The boundary line is represented by the fragmentation rule and is neither visible nor physically there. The arrows indicate the implemented rule, where Pos1 and Pos2 on one side of the fragmented room required the animals to make a left turn, and Pos3 and Pos4, which are on the other side of the fragment, required the animals to make a right turn.

Figure 3. The layout of the dimly lit room where the animals were trained and recorded with the introduction of three new positions: Pos5, Pos6, and Pos7, which are highlighted in yellow. The original four positions are also shown to visually demonstrate the arrangement and distances between all seven positions. The visual cues on the walls included the door, desks, computer, and other various objects that allowed for the animals to use as a frame of reference. The boundary line is represented by the fragmentation rule and is neither visible nor physically there. The arrows indicate the implemented rule, where Pos1, Pos2, and Pos6 on one side of the fragmented room required the animals to make a left turn, and Pos3, Pos4, Pos5, and Pos7, which are on the other side of the fragment, required the animals to make a right turn.

Figure 4. The graphs show the percentage correct of the animals' performance for all trials at all four position. The position number is on the x-axis and the average percentage correct is on the y-axis. The grey dashed line indicates the performance at chance, which is at 50%. (A) When the animals were analyzed separately, the data shows that they were performing above chance. The code names for the three rats are represented by the different colored bars: white bars for AL2, grey bars for DN9, and black bars for DN10. (B) When we took the average of the three animals, the performance was still above chance. The blue bars represent the data averaged across the three animals. Error bars indicate the standard deviation of the animals' performance.

Figure 5. The graphs show the percentage correct of the animals' performance for the first trials only at all four positions. The position number is on the x-axis and the average percentage correct is on the y-axis. The grey dashed line indicates the performance at chance, which is at 50%. (A) When the animals were analyzed separately, the data shows that they were performing above chance. The code names for the three rats are represented by the different colored bars: white bars for AL2, grey bars for DN9, and black bars for DN10. (B) When we took the average of the three animals, the performance was still above chance. The blue bars represent the data averaged across the three animals. Error bars indicate the standard deviation of the animals' performance.

Figure 6. The graphs show the percentage correct of the animals' performance for all trials at all four positions. The position number is on the x-axis and the average percentage correct is on the y-axis. The grey dashed line indicates the performance at chance, which is at 50%. Performance for Pos1 and Pos4 was compared to Pos2 and Pos3. The blue bars represent the data averaged

across the three animals. (A) When Pos1, Pos2, Pos3, and Pos4 were graphed separately, the average percentage correct for Pos2 and Pos3 was slightly worse than that of Pos1 and Pos4, even though performance was above chance at all four positions. (B) The data from Pos1 and Pos4 were averaged together and were compared to the data from Pos2 and Pos3 averaged together. The animals overall had higher performance at Pos1 and Pos4 than at Pos2 and Pos3. Error bars indicate the standard deviation of the animals' performance. The asterisk indicates differential performance between Pos1 and Pos4, and Pos2 and Pos3, $p < 0.05$.

Figure 7. The single unit activity of the parietal cortex cells was consistent across cells. The x-axis represents the path position along the track (0-100 bins), and the y-axis represent the rate of the average firing activity for a specific point on the track. The blue bars represent the overall mean for all the trials for that point on the track, while the red bar represents the point on the track where the animal made a turn. (A) For this example of a parietal cortex neuron following the route space, the firing rate ranged from 0-40 Hz. Consistent with previous findings, this neuron in the parietal cortex fired at the same positions of the track even when the animal was moved to a different space (i.e., from Pos1 to Pos3). (B) This example of a parietal cortex neuron fired whenever a specific action was executed by the animal. The firing rate ranged from 0-50 Hz. This specific cell fired only when the rat turned right, as indicated by its activity after the turn at only Pos3 and Pos4. (C) The third example is the action planning cell which fired only before the choice point or turn. This cell has a firing rate ranging from 0-40 Hz. Because the overall mean of firing activity is high only prior to the turn and not at the point of the actual turn, it is believed that the animal was planning to make a right turn as it approached the choice point in a track position that required right turns, such as Pos3 and Pos4.

Figure 8. The single unit activity of the hippocampal place cells was consistent across cells. The x-axis represents the path position along the track (0-100 bins), and the y-axis represent the rate of the average firing activity for a specific point on the track. The blue bars represent the overall mean for all the trials for that point on the track, while the red bar represents the point on the track where the animal made a turn. (A) For this example of a first trial cell, the firing rate was 0-40 Hz. The magenta line represents the average firing activity for only the first trials for that point on the track, which fired at a higher rate than the overall mean. (B) This example of a place cell following the track space has a firing rate of 0-50 Hz. The overall mean firing rate occurred at the same positions of the track even when the animal moved to a different position within the environment (i.e., from Pos1 to Pos3). (C) As for the example of a place cell changing in the in-field firing rate, the firing rate on the y-axis ranged from 0-20 Hz. This neuron fired low to high as the animal moved from Pos1 to Pos4.

Figure 9. Population correlation matrices were created to analyze the activity of a population of cells. The six graphs represent the six different combinations we can have when we make comparisons for Pos1, Pos2, Pos3, and Pos4. For each graph, the position listed first is on the x-axis, and the position listed second is on the y-axis. The red line in all the graphs represent the point (bin 83) at which the animal made a turn on the track. The color bar represents the strength of the Pearson correlation between two positions, where the color blue at 0 means no correlation and the color yellow at 1 means a perfect or very high correlation. (A) For the hippocampus, the graphs highlighted by the dark blue color are comparisons of two positions within the same fragments (i.e., Pos1 vs. Pos2, Pos3 vs. Pos4), while the graphs highlighted by the light blue

color are comparisons of two positions from different fragments (i.e., Pos1 vs. Pos3, Pos1 vs. Pos4, Pos2 vs. Pos4, and Pos3 vs. Pos4). For the graphs comparing positions by the same fragment, there is high correlation on the same point of the route or track, indicated by the yellow pigmented, diagonal line. This means that the cells were mapping out the space of the track. The graphs comparing positions by different fragments show that the correlation drops earlier (before it reaches bin 83 at the red line). (B) For the parietal cortex, the graphs highlighted by the dark green color are comparisons of two positions within the same fragments, while the graphs highlighted by the light green color are comparisons of two positions from different fragments. For the graphs comparing positions by the same fragment, the patterns of the color maps are similar to that of the hippocampus. The correlation remains high on the same point of the route or track. The graphs comparing positions by different fragments show that the correlation drops much earlier.

Figure 10. The correlations of the diagonals of the matrices were plotted as a line for each of the position comparisons, which showed the Pearson correlation of population activity pattern as a function of where the animal was on the track. The x-axis is the point on the track (0-100 bins), and the y-axis is the same as the color map values, ranging from 0-1 where a higher number represents a higher correlation. For the hippocampus, the top graph shows the plotted diagonals of the correlations. The lines in the dark blue color are comparisons of two positions within the same fragments (i.e., Pos1 vs. Pos2, Pos3 vs. Pos4), while the lines in the light blue color are comparisons of two positions from different fragments (i.e., Pos1 vs. Pos3, Pos1 vs. Pos4, Pos2 vs. Pos4, and Pos3 vs. Pos4). The dark blue lines show that the correlation remained high for comparisons of positions that were within the same fragment while the light blue lines show that

the correlation dropped for the comparisons of the positions that were from different fragments just until before the turn, where the pattern began to diverge (at bin 76). For the parietal cortex, the second graph shows the plotted diagonals of the correlations. The lines in the dark green color are comparisons of two positions within the same fragments, while the lines in the light green color are comparisons of two positions from different fragments. The dark green lines show that the correlation remained high for comparisons of positions that were within the same fragment while the light green lines show that the correlation dropped for the comparisons of the positions that were from different fragments just until before the turn, where the pattern began to diverge (at bin 66). The third graph shows all the comparisons of the correlations of the diagonals for the hippocampus and parietal cortex.