The Role of the Subiculum in Mapping Hidden Reward Structure

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Abstract

Although neurons in the subiculum region map out various kinds of travelling trajectories and neurons in the posterior parietal cortex encode spatial position within a route for pathways that the animals have never seen, whether the subiculum encodes spatial relationships for spatial structures that are never seen remains unknown. We trained rats to search for three honeynet Cheerios buried at corners of an equilateral triangle while recording from the posterior parietal cortex, the subiculum, and the hippocampal CA1 region. From behavioral data, the rats seem to understand and to learn the concept of an equilateral triangle, which is implied from the spatial relation among the three food reward sites. This finding indicates that this behavioral training method is successful, and the next step is to determine whether subiculum activity reflects the structure of a triangle.

Introduction

The awareness of one's present location and orienting oneself to other places in space is necessary and important for both humans and animals. Navigating space requires remembering all the past locations and locating oneself. During food foraging and location navigating, external environment influences the spatial mapping and provides information on the animal’s movement. Two structures of the brain are thought to be particular important when we study the spatial cognition in both human and rodents, the hippocampus (HPC) and its closely related structures, the subiculum (SUB) and the retrosplenial cortex (RSC), and the posterior parietal cortex (PPC). It is necessary to understand the different frames of reference when we are studying the role of the hippocampus and parietal cortex in spatial navigation. Frames of reference help human and rodents to locate themselves and to locate other objects. There are two main categories of frames of reference – allocentric frame of reference and egocentric frame of reference. The PPC is egocentric and HPC is allocentric in terms of frames of reference, where allocentric refers to the position of something relative to the environment and egocentric refers to the position of something relative to a person’s sensory organs (Nitz, 2006; Nitz et al., 2017). HPC performs many operations. One of these operations is mapping out location in the environment in an allocentric frame of reference, especially in CA1 region. In contrast, PPC encodes egocentric information. RSC and SUB lay in between HPC and PC, they mediate and transfer information about the animal’s current location and where it is relative to the environment. RSC has head direction cells in it that are associated with place and orientation (Kesner et al., 2008). RSC and SUB are both egocentric and allocentric, they are in charge of route specific encoding and momentary location encoding.

In the context of spatial mapping, place cells in the hippocampal CA1 region and route mapping in PPC is different. In order to investigate if subiculum contributes to the learning and the understanding of the geometric layout of food rewards, we designed an experiment that let two laboratory rats search and find three cheerios buried in the shape of a equilateral triangle. This present study aims to find the evidence of SUB maps spatial structures in routes by analyzing the form of neural activity that map out equilateral triangles. We built microdrives to record three brain regions in the rats' brains – left SUB, right SUB, and hippocampal CA1 region. If the rat learns the triangle and the subiculum can encode the spatial structure that is never seen and never part of a route, subiculum plays a crucial role in mapping hidden reward structure.

The subiculum (SUB) receives input mostly from the hippocampus (HPC) and provides output to many other brain areas that are involved in learning and memory functions, such as reward learning and working memory. The SUB is in charge of navigation-related functions, for example route planning, locating the reward, and analyzing the spatial information on aversive stimuli. The activity in the SUB indicates the momentary location of the animal in space, which is important for spatial navigation (Sharp 1994). One of the most important roles of SUB and retrosplenial cortex (RSC) is mapping structure of the environment – a kind of place. "Boundary vector cells" in the subiculum use external environment cues, such as boundaries, in order for an animal to locate itself in space. More specifically, boundary vector cells fire when the animal is at a specific allocentric reference and fire more intensely as the animal moves toward the target (Stewart et al., 2013).

The triangle is an abstract shape and concept that is never indicated to the rat and the trajectory to get all three cheerios is never showed to the rat in order to integrate a path. Collett et al. (1986) revealed that the rats already planned the trajectory before they run the task and used the spatial relationship between landmark and the target to navigate them to find the food reward. The findings of this study leave the question that whether the animal stored the given geometrical property explicitly or they computed the relations based on the representation information. Gothard et al. (1996) believed that the rats used the spatial relationship between the landmark and the target to navigating themselves while the they are performing the task instead of using other cues, such as sensory information or visual information of the goals.

One way to learn how the rats understand the spatial relation among the corners of an equilateral triangle is to consider the rats' learning progress in the form of "schema". The Morris water navigation task is widely studied in spatial learning and memory in the field of behavioral neuroscience. In this task, a rat is placed in a pool of opaque water and must locate an invisible hidden platform in order to escape the water. There is no local cue of the platform's location, meaning that rat needs to locate this invisible platform without using any auditory, visual, or olfactory cues. In this case, spatial memory is crucial for accurate navigation (Morris et al., 1981). It is suggested that the rat also developed a schema during the period of swimming to the platform in the pool. Tse et al. (2007) reveals that the animal remembered that the relationship between the environment and the reward and used it to perform the task. Same as Tse's founding, the platform location in Morris water tank never change in the experiment, the rat learned to locate the platform position relative to the environment.

Csicsvari et al. (2019) trained rats to learn a daily hidden reward structure. They found that the grid cells distort according to the constantly changing representations of the goal. Different than the past finding, the data shows that grid cells, in hippocampus, encode goal information locality, instead of the global and universal environment. Therefore, the hippocampal CA1 region is grid sensitive and constantly adapting and adjusting based on the changing rewards and goals. Giocomo et al. (2019) also found the distortion of the grid pattern which are resulted in the various rotation across animals. The grid cell distortion is less environment affected and its spacing is more concentrated in a remembered condition than a random condition. So, we do not know if the SUB neurons encode various arrangement of a geometry shape or not.

In Nitz et al. (2017), SUB might be involved with coding and understanding structural figures of environment when the structure was not directly observed, nor the trajectory. Some subiculum neurons robustly encode the axis of travel, and so are called axis-tuned neurons. These neurons fire very differently for different kinds of route; they fire strongly in the vertical axis and not so much in the horizontal axis, when even the same track is rotated. So, SUB neurons do not fire for the same part of the track, but the same kind of the track. PPC also encodes structural features, the animal's position along a route of a particular shape no matter where that route is in the world. In Nitz (2006), neurons in PPC is body-oriented and egocentric, the animal uses itself as a reference when it performs the task while neurons in HPC is event- or scene-based and allocentric, meaning that the animal uses the experiment room or the background as a reference in order to navigate itself. The animal's posterior parietal cortical neurons plan the route in both individual and multiple navigational perspectives despite the size and spatial configuration of these two perspectives. PPC neurons use "route" as a frame of reference. But, in this experiment, the rat does experience the shape, or the structure, of the route by moving continuously through it.

These previous researches show that the SUB neurons encode certain kinds of spatial and structural relationships in rats' navigation and the PPC neurons map out the paths that the rats have never seen. We want to take away the visual experience and the navigational experience of an equilateral triangle and see if the rat can still learn it, if it does, whether the SUB neurons whether the subiculum encoded the spatial structure of that triangle.

Methodology

Subjects

Two adult, male Sprague Dawley rats were used in this experiment with IACUC protocols and the UCSD Animal Care Program. They were provided water and food that maintain their body weight to 85% of its baseline.

Materials

A dim light room is used in this experiment. There are various visual cues in the room, such as a computer, a table, a track, and different drawings on each side of the walls, in order to let the rats understand where it is at while performing the tasks. A square arena is used in this experiment, which is measured 122cm by 122cm. The arena is put on four bricks on a table that is 100 cm above ground. The rat is put into a pot on another table that was located 40 cm next to the arena. This distance allows the experimenter to transfer the rat manageably within a short period of time while holding the recording cables. Camera locates on top of the arena in order to record the whole process of the experiment. Beddings are put on top of the arena evenly and the surface of the bedding is kept flat. Three honey nut cheerios are hidden deeply in the bedding by using an equilateral triangle shaped model, on three vertices of the it. A star shaped red flag is glued on a stick to make a visual cue for the rat and located randomly on one of the vertices randomly.

Behavioral Training

Animals are trained to dig and find all three cheerios. The experimenter shines light on top of all three vertices, which allow the camera to capture the triangle on video tape. After removing the model from the arena, the experimenter then put the rat on the arena, allowing it to find the cheerios freely. When it is having trouble finding any of the cheerios after 30 secs since it finds the last cheerio, the experimenter shows it where the cheerio is. After finding all three cheerios, the rat is put back into the pot in order to prepare for the next trial.

The rat runs 36 trials per day, which contains all possibilities of the positions of the hidden triangle. The arena is divided into 9 sections (see Figure 1). Taking a closer look to each section, there are 4 possibilities for the triangle arrangements, allowing same side of the triangle model paralleling with all 4 sides of the squared arena (see Figure 2). These randomized arrangements are programmed by using MATLAB to ease out the human bias.

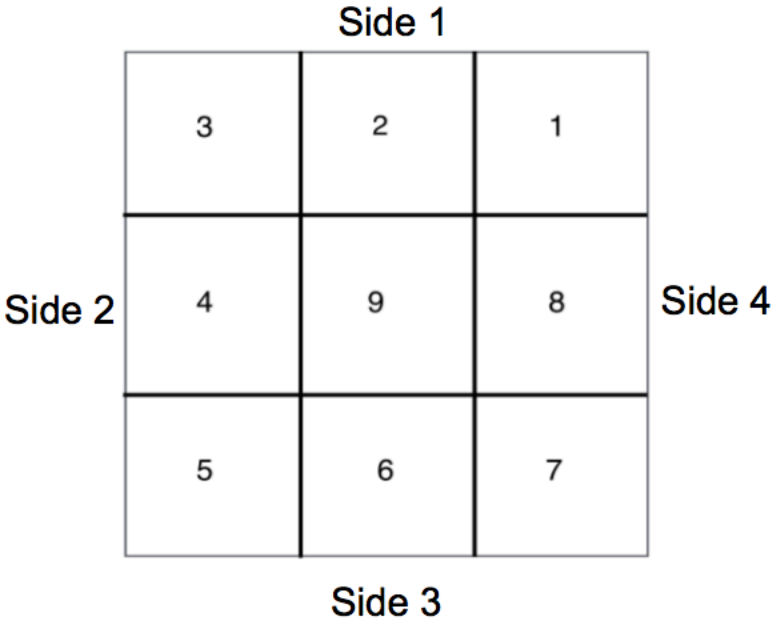

Figure 1: This is the arena area divided into 9 sections and 4 sides.

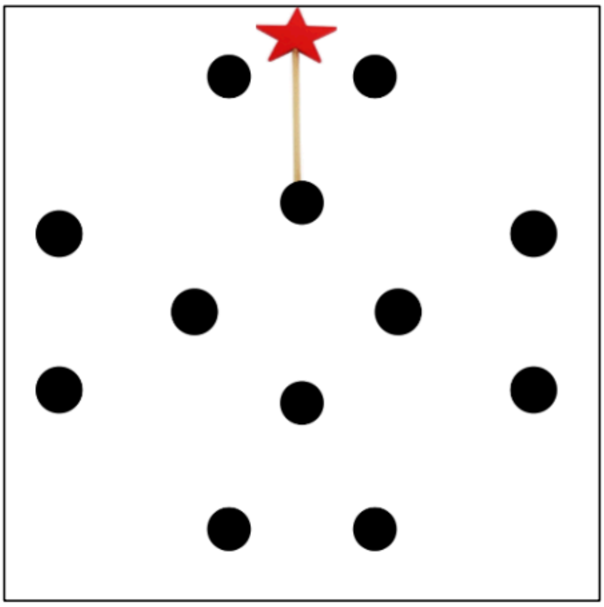

Figure 2: This is one section of the arena with four triangle arrangements and a flag as a landmark.

Recordings and Surgery

For the purpose of recording neurons, we construct microdrives that allow us to record with microwires in CA1 and subiculum. Electrodes are made into microdrives in order to get the local field potentials and single unit action potentials in the rats' brains while they are performing the task. Both rats are surgically implanted with microdrives in the brain regions on left subiculum, right subiculum, and right hippocampus/posterior parietal cortex. During the recording period, the electrodes are turned down 1/4 or 1/8 of the screw each time based on the activity of the neurons between recordings. This turning process collects cells in each brain area. The electrodes move to CA1 region and subiculum in the hippocampus.

After the rat is recovered from the surgery, we retrain them in order to get them used to the task and perform well for the recordings. After retraining for two weeks, recordings occur once per day for each rat. The experimenter brings the rats to the recording room and connects their three connecters with a software called Plexon SortClient. The experimenter then attaches two LED lights to the rat's head after the experimenter connecting the three connecters. The LED lights tracks its head position and body movements.

To record the performance of the rat, we collect the behavioral data on the order of digging the "flag" cheerios, which cheerio or cheerios it did not get, if it digs for the cheerios or not. Each day we run a probe trial to check if the rat will search in a logical way when there isn't any reward at all.

Training Stages

There are three stages of the rat's learning. First, rat forages freely for randomly placed cheerios that are in triangle shape in relation to each other. This gets it used to the new environment and the smell of the bedding. After the rat cannot find the rest of cheerios for a considerable period of time, the experimenter points and shows the rat the position of the food reward.

After about 30 days' training, the rat was able to find all three cheerios 70% of the time. We discovered that the majority of 30% of the time that the rat missed the trial was it missed the last cheerio. The experimenter then started second training stage. We trained the rat to think and learn the two possibilities after finding the first two cheerios. The experimenter repeated the two possible positions for the last cheerio. He or she kept two cheerios in a line of the triangle and the third cheerio could be either on the top or on the bottom of the line, forming a triangle. This method helped the rat to rule out the impossible locations of the triangle and to develop a strategy for finding the last cheerios. We then found that the rat spent longer time on the arena before it is digging for the first cheerio. We created a landmark flag for the rat as a visual cue for the possible locations of the cheerios. The third training stage began by putting the landmark on the vertex of the equilateral triangle. We put the rat on the arena and it went to the landmark flag at majority of the trials and dug for the cheerios successfully.

The experimenter records the detailed behaviors for every trial and make notes on the trials for further analysis. We make different types of statues for the rat's behavior in order to make the behavior recording easier and faster. If the rat successfully finds all three cheerios in a short period of time without running out of the circle range that includes the two triangle possibilities, we put a "checkmark" on the behavior recording sheet. If the rat didn't get all three cheerios, we mark the cheerio that it gets before that on the sheet, for example, if the rat did not get the last cheerio but successfully found the first two quickly and with range, we mark "x2"; if the rat did not get the second cheerio and the last cheerio, we mark "x12". We create four keyboard settings to capture the significant moments of each trial. These keyboard settings are "start", "dig", "end", and "give up". "Start" and "end" are used to mark the whole trial and avoid considering the next and previous trial during analyzation. Each trial usually contains three "dig", each is associated with the three cheerios, we mark the "dig" as soon as we see the rat is digging for the cheerio in the right position. When the rat spends too much time on the arena and wondering around without digging or searching for cheerios, we click "give up" keyboard. If the experimenter makes a mistake, he or she makes a note on the recording sheet about that mistake for the further analyzation to rule out the false input.

Rate maps

The neural data was acquired through "Plexon Offline Sorter" to identify the single units recorded in the animals. When the single units have been identified and the behavioral scoring was complete, the two sets of data were combined to create rate maps, which showed the amount of firing of each neuron each time the rat occupied a position on the track.

Results

The subjects, two lab rats, successfully learned the concept of a geometry shape, a triangle. After about 4 months and 60 training days, they were able to find all three Cheerios in a fast pace for about 82% of the time. During behavioral training period, there was a gradually increase in this successful behavior. During recording period, the rat performed significantly well by finding all three cheerios in a fast pace for 80% of the time. For example, we recorded 36 trials per rat every day, the rat gets about 25 to 28 trials right for many recordings.

In the trials that the animal got all three cheerios, it did not go directly from one to another in a triangle trajectory. It went to the flag for its first digging position at 87.36% of the time. After eating the first cheerio, it spent time on the landmark location, looked around the area, and compared the other two vertices of the triangle. It went to the second cheerio position and dug for the cheerio, then it ran to the third cheerio quickly. This scenario occurred 81.77% of the trials, which was significantly higher above the probability of simply searching for cheerios at random. This data could also be calculated into each recording – the rat gets all three cheerios accurately and efficiently for 29.43 trials in all 36 trials. This was a promising evidence to support that the rats understand the triangle shape with the concept of the spatial relationship between its vertices.

In the rest 18.23% of the recording trials, the rat failed to find all three cheerios in various types of behaviors. Most of these trials were the rat could not find the last cheerio, which occurred 11.65% of all the recording trials. In this kind of trial (“x2” trial), it found the second one relatively quick after digging the first cheerio. After eating the second cheerio, it looked back to the first cheerio position and starts to run around the edges of the arena. The “goes off” behavior took about half of the trial time. When the rat returned, it figured out the location of the third by looking at the previous ones. There was another scenario for this kind of trial. After digging for the second cheerio, the rat went to the other possible location for the third cheerio, sniffed and realized it was the wrong position, then immediately directly went to the right position. This behavior implied that the rat already knew the two possible locations for the last cheerio before it dug for the last one. In 4.48% of the time, the rat dug for the first cheerio and goes off, then went back to the first cheerio position, smelled and dug again, then found the second and the third cheerio at a fast pace. In 2.10% of the time, the rat did not find the second and the last one. In this scenario, we operated “give up” and ended the trial.

To test the rats’ understanding of the spatial relationship between the equilateral triangle vertices, we designed a probe trial for each recording session. In the probe trial, we only placed the flag as a landmark on the arena without burring an actual cheerio. At about 90% of the time, the rat went directly to the flag and digs, circled around the flag to search for multiple possible positions, then dug for the second possible location. After failed to find the first cheerio, sometimes, the rat only searched for the possible locations without digging for the food reward. The animals’ performance implied two findings. First, it ruled out the possibility that the smell of the cheerios being a factor for finding the food reward successfully. The rat still dug for the flag position even there was no food under it. It used the flag as a landmark and a visual cue for the rest of the two cheerios. Secondly, the rat still dug for the other possible locations indicated that it understood and learned the spatial relationship between the vertices of the triangle. This also implied that the rat knows the concept of a triangle no matter where the equilateral triangle mode located.

We collected the tracking data at the animal’s locations for a full set of 36 trials (figure 3). However, from this perspective the trajectory looked disorganized. We recognized the tracking data into the consideration of the flag and discovered something different. We transferred the map so that the flag location is concentrated in the center (figure 4). The rat spends a lot of time at the locations surrounding the flag. We then further analyzed the data into the perspective of a triangle. We lined up all the triangles according to the first, second, and third locations and we see that the animal spends most of its time in a 20 cm space surrounding the three locations (figure 5).

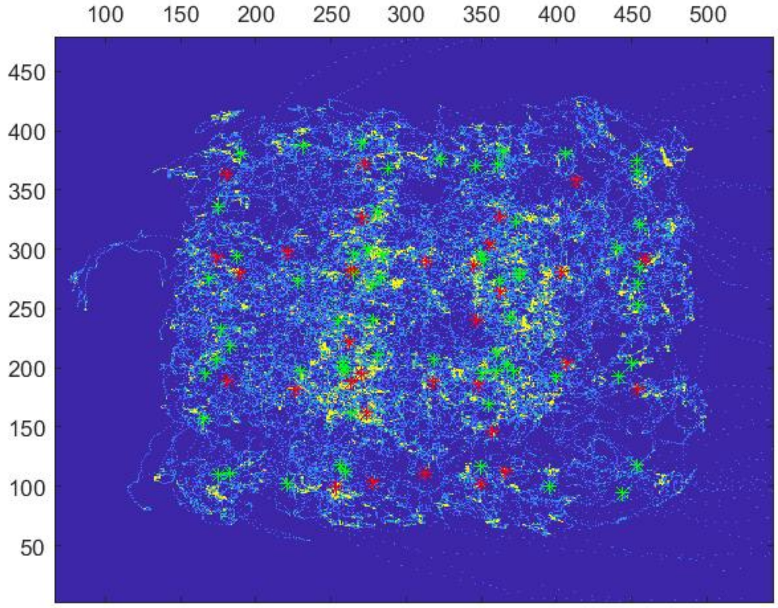

Figure 3: This is a camera map that contains tracking data for all 36 trials. In figure 3, 4, 5, the red dots are the flag locations, which are also the first flashes. The green dots are the flashes for the other 2 vertices. The yellow trace is the animal activity tracking lines.

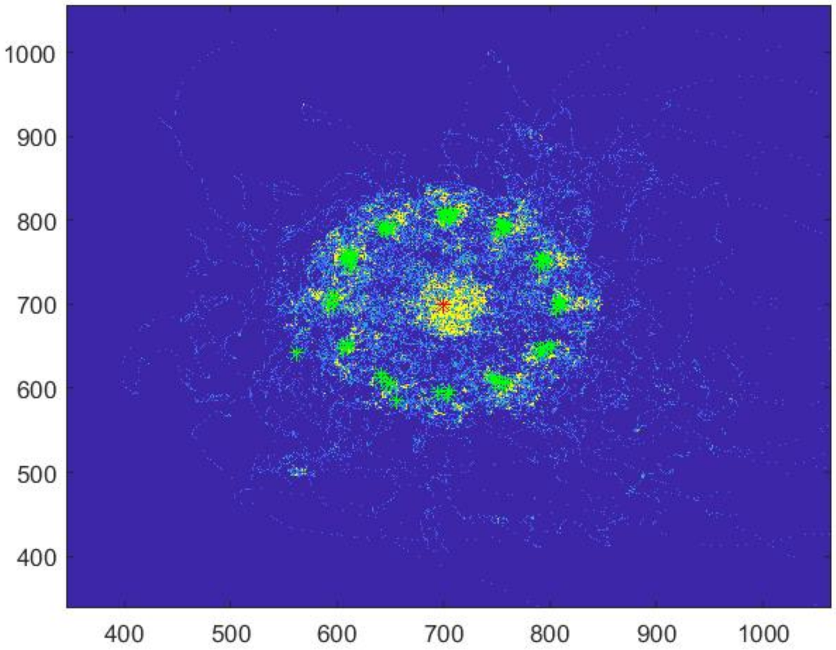

Figure 4: This is a transferred map that overlaps the flag positions.

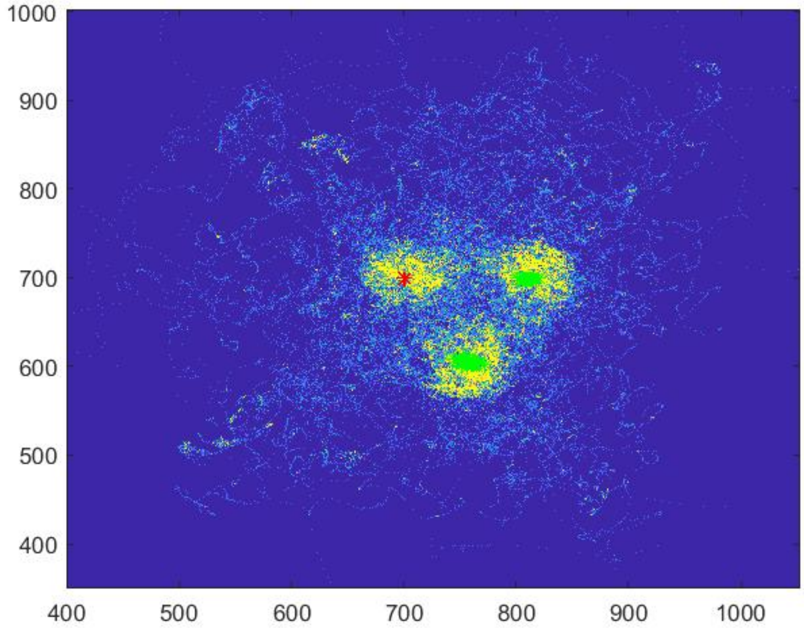

Figure 5: This is a transferred map that overlaps the triangles. In this figure, the second digging position is on the right side of the red dot while the third digging position is on the bottom.

After calculating and analyzing the behavioral data, we also analyzed the neural recording data to support the hypothesis of this study. We looked into the firing activity of SUB and HPC neurons in order to find how the brain encoded the information of a geometry concept – a triangle. We still have much more to do to characterize the behavior and we are now doing recordings of neural activity in PPC and SUB. We will continue to analyze more details of the animal's behavior in this task. A main question is to determine whether subiculum activity reflects the structure of a triangle.

We have many neural data and the analysis is complex and time-consuming. Below is an example of what we have done so far. There are two kinds of maps in our neural data, the firing rate map and the occupancy map. These two kinds of maps have two different measuring scales: the firing rate map reveals the number of spikes per unit time; the occupancy map reveals the number of times that the rat visits the space and the frequency of the rat's visiting to certain places. We zoomed in the maps from the perspective of the arena to the flag and to the triangle: the arena map is 122cm by 122cm in real life; the flag map is 49cm by 49cm, which is twice the length of the triangle mode that we utilize in the study; the triangle map is 36.75cm by 36.75cm, which is the exact length of one triangle mode (24.5cm) and two 6.125cm space surrounding it. In both kinds of the triangle maps, the flag and first digging position is on the left up, the second digging position is the right one, the third digging position is the bottom one.

As indicated in these figures (figure 6 & figure 7), as we are moving from the arena perspective to the flag perspective, then to the triangle perspective on any trial, we see that the distribution of neural activity gets tightened up and sharpened up. For neuron 2 in rat DN23 (indicated in figure 6), if we focus on the triangle locations, we see two location fire more intense than the other one – even when in the corresponding to the occupancy map, the rat spends more time on the location with less neural firing intensity. Furthermore, the three locations do not have the same firing rate. For another neuron, neuron 13 in rat DN23, only one location high firing activity even when the rat spends more time on this location and the other location. We attempt to say that the small part of the neural data implies that the subiculum neurons may discriminate the corners of the triangle by the order the rat digs.

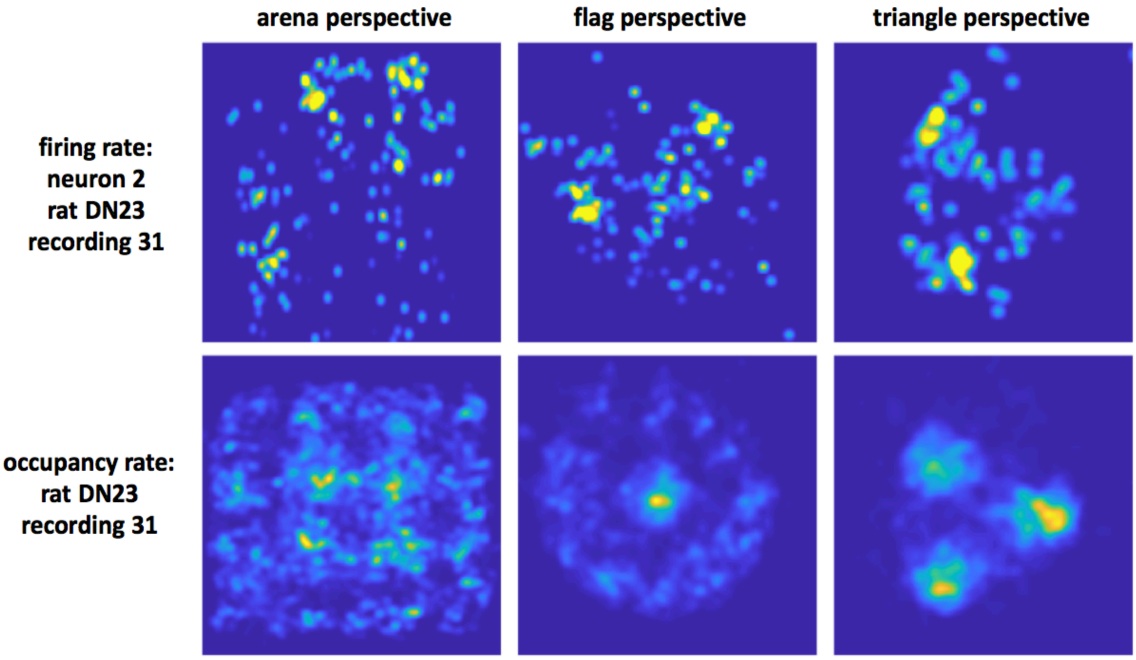

Figure 6: This figure shows the firing rate of one neuron and the occupancy rate of the whole recording. It contains the perspectives from the arena, to the flag, and to the triangle. For the firing rate map, the blue colored area indicates no firing rate while the yellow colored arena indicates the strongest firing rate among the activities. In the occupancy map, the blue areas are the areas that the rats did not travel or spent any time on; while the yellow areas are the areas that the rats spent the most time on and traveled in the highest frequency.

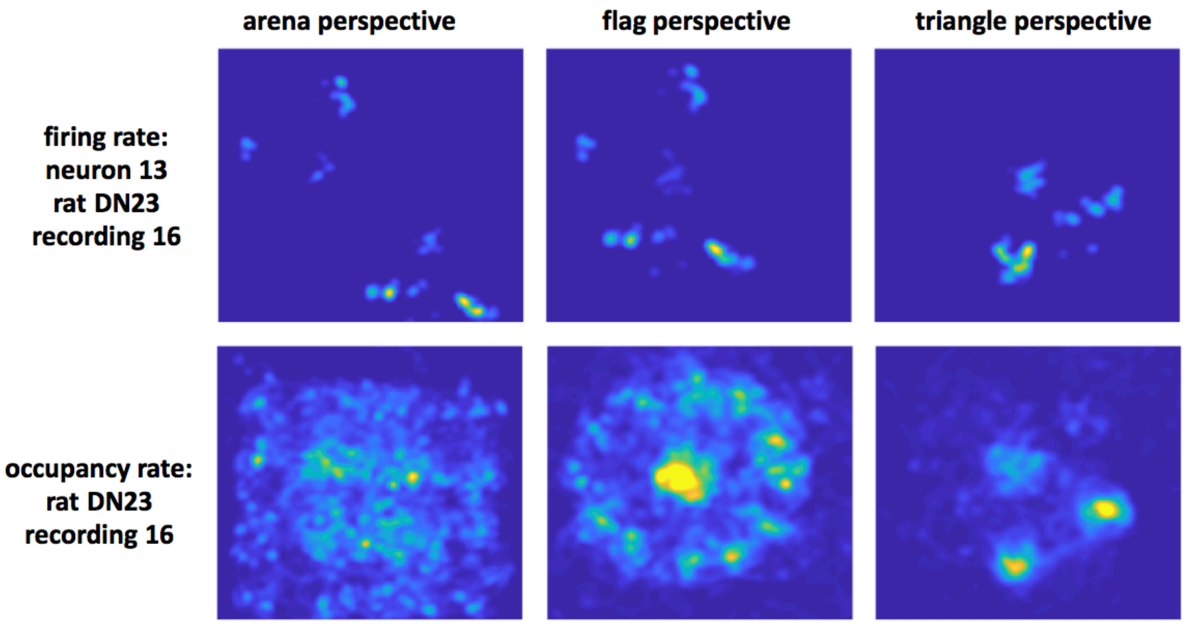

Figure 7: This figure shows the firing rate of another neuron and the occupancy rate of the whole recording.

Discussion & Conclusion

From our behavioral analyses, it is highly probable that both rats successfully learn the concept of a triangle based on a behavioral rule. This particular ability is first discovered by this project. It is a successful behavioral training task.

We found that certain cells in the subiculum use the frame of reference of the triangle instead of the arena or the experience room. We also found that as the animal approaching to the three reward locations, some subiculum neurons fires more intense in some particular locations than the other locations. As we transfer the perspective from the whole arena space to the perspective of the triangle, we found the firing activity of SUB neurons tightened up more and became more organized. However, we have many more analyses to do in order to understand if the SUB neurons encode the triangle.

There are some limitations of this study due to the time-consuming feature of this project. We did this study on only two subjects. In the future, we will add more rats to this study and record their neuron activities in order to get a reliable pattern while they are performing this behavioral task. In terms of confirming the animals have learned the spatial relation of a triangle, we need to track more kinds of triangle arrangement positions for each recording session.

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