Temporal Representations in Odor Context-Specific Hippocampal Sequences

Yasmine Ayman
yra2102@columbia.edu
Columbia University, New York

Sophia Kolak
sdk2147@columbia.edu
Columbia University, New York

Derek Wacks
djw2157@columbia.edu
Columbia University, New York

ABSTRACT

Spatiotemporal cognition relies on overlapping and interacting systems that converge in the hippocampus. Although movement through space and time is thought to be coupled, spatial and temporal coding can be coded differentially in the hippocampus depending on the behavioral demands. Along with place cells that encode spatial features of the environment, "time cells" fire at specific time points and durations thereby organizing our experiences temporally as well as spatially. One open question is how exactly the hippocampus organizes the combination of spatial and temporal aspects of memory, and how each of these features influence the interpretation of sensory stimuli when learning tasks. In this study, we examine activity recorded from CA1 neurons during a virtual navigation task, where identical odors are presented at different points along a four meter track. It has been shown that place cells and internal path integrators are calibrated by these odor landmarks. Here we examine the role that the time cell network plays in temporally structuring the experience of running on a 4m track. We compare time cell and place cell activity patterns during the task with and without odor, as well as during the reward period. We also assess the role of learning on time cell representations by examining the time cell patterns in no odor trials before and after learning.

1 INTRODUCTION

When you ride the New York City subway, you have an internal sense of how long it will take you to reach your destination. If you board the subway at 116th street intending to ride to 168th street, and you make this trip often, you already have an understanding of how long it will take. This internal representation of the journey exists with or without using subway stops in between as progress milestones. Subway stops along the way (like 125th street and 157th street, for example) help you gauge the remaining duration of the trip. Furthermore, you have a notion of how long it takes to get to these stops.

The idea that your memories are temporally organized is a fundamental organizing dimension of episodic memory. Research on the temporal dimension of memory, as well as the interplay between spatial and temporal aspects of memory, has gained traction over the past several years. For example, neurophysiological studies have shown that many hippocampal pyramidal neurons fire at specific moments aligned to conditioned responses. The hippocampus has also been shown to play a key role in discriminating close differences in elapsed time. Thus, the hippocampus appears to do much more than form a cognitive spatial map, it also appears to form a cognitive temporal map.

Despite this promising preliminary research, the mechanisms underlying the cognitive temporal map are largely unknown. In the context of spatial representations in the hippocampus, place cells are thought to play a key role in firing when an animal occupies a particular location in space. In an analogous manner, time cells have been found to fire briefly in sequence between periods of salient events or in relation to time elapsed along a distance traveled [3]. Furthermore, studies have indicated that time cell sequences predict the spatial trajectory that is taken by a rat after a delay period. This finding suggests that time cells fire in accordance with planning a future path [4].

Importantly, time cells and place cells are composed of the same neurons [1]. This conclusion is supported by the finding that time cell activation is dependent on the animal's location. With rats performing a spatial alternation task in which they run on a treadmill between alterations, time-specific firing was identified when the rats were running (where there were no salient spatial features). However, place-specific firing was identified when the rats crossed other segments of the maze. The same population of simultaneously recorded cells included both time cells and place cells. Intriguingly, some of the same neurons fired when the rat was on the treadmill and when the rat was in specific locations along the maze [3].

Although spatial and temporal features are thought to be fully integrated within the CA1 neuronal network, it remains unclear how the hippocampus organizes the combination of spatial and temporal aspects of memory, and how each of these features influence the interpretation of sensory stimuli when learning tasks.

A recent study found that that odor cues serve as landmarks to guide navigation, and that the convergence of these sensory landmarks with internal path integration generates a cognitive spatial map in the hippocampus [2]. The task consisted of mice running a virtual linear distance of four meters during which they were presented with odors at one meter

and three meters along the four meter track. Large-scale neural ensemble activity in the CA1 of the hippocampus was then collected to see whether odor landmarks at multiple locations affect navigation. The place cell density and sparsity patterns indicated a process through which two distinct networks, place cells and internal path integrators, are calibrated by an external event, in this case an odor.

Along with difficulty decoupling temporal and spatial features of the environment, the interaction between the temporal representations and sensory stimuli in learning is also unclear. This is due, in part, to the fact that the dynamics governing sensory and internal representations have predominantly been studied separately. This begs the question whether different representations are encoded with similar reliability, long-term stability and adaptability or whether they have different dynamics.

In order to tackle this question, a study looked at the process through which hippocampal networks combine sensory inputs along with their shifting temporal relationships [5]. It compared the multi-modal representations after learning a behavioral context. The mice learned how to perform an olfactory delayed non-match-to-sample task where two odors were presented with a 5 second delay. The mice were trained to release a water reward if the two odors did not match and refrain from licking if they did. Activity from pyramidal cells in the CA1 of head-fixed mice was recorded over multiple days during and after the mice had learned the task, as well as when the trail structure was altered. Their results demonstrated distinct representational trial structure intermixed within CA1 neural networks – both a stable sensory code and a dynamic temporal code. The distinct codes reflect both the fixed elements of the external world, in this case odor, and the transient temporal relationship between these fixed elements. What results is a map of sequential experiences in memory space [5].

Drawing on research conducted by Fischler et al. [2] in their examination of the role of olfactory landmarks and path integration in the hippocampus, this study seeks to examine the unique relationship between time and sensation. We examine how the hippocampus multiplexes spatial and temporal representations in the context of a virtual spatial navigation task with olfactory landmarks. We begin by identifying time cells and place cells in the hippocampus and examining their activity in both no-odor and odor trials. We then look at whether cells code for time as the animal is receiving the reward. Finally, we examine the number of place and time cells before and after the mouse has learned the task to see if the learning impacts the number of time and place cells. Our findings will reveal a new dimension to the formation of cognitive maps in the hippocampus in response to sensory landmarks such as odor.

2 METHODS

Our analysis focuses on the behavior and calcium imaging data of one mouse. We wrote several python programs to undertake data analysis. For more details please refer to our git hub repository: [1]

Behavior Training

The mouse was habituated to head fixation on the spherical treadmill for several days before being put on water restriction. Following 3 days on water restriction, the mouse was trained to walk a certain linear distance to receive water rewards. The distance was gradually increased from 0.5 meter to 4 meter in 1 meter increments when the mouse was able to complete >60 trials in one 20 minute session. After 3 consecutive days of training on the 4 meter track where the muse completed >80 trials in a single 20 minute session for 3 consecutive days. At this point data was collected.

Reward Delivery

The mouse was rewarded with 2-4 ul drops of water. As soon as the mouse reached the 4m point on the virtual track, the first reward is triggered. The reward delivery after the first reward is controlled by the mouse, where each subsequent reward is triggered by two licks. After reaching the 4m point, the mice can continue triggering rewards for a period of up to 4 seconds or 1 meter, after which the rewards will cease. The cessation of the reward signals the start of the next trial.

Trial Structure

In this paper, three of the 6 days of the experimental timeline, namely day 0, day 4 and day 5, were chosen for analysis (Fig. 1). After the training phase, day 0 referred to trials in which there were no odors delivered and the mice simply had to run for 4m before reaching the reward. Day 1-4 included odor landmarks at 1m and 3m along the 4m track consisting of a pseudo-random alternation of limonene and pinene, after which the reward was again delivered at 4m. On individual trials, the same odor was presented at 1m and 3m. On day 5, half of the tracks had no odor and half of the tracks had pinene at the 1m and 3m points. A reward was again delivered at the 4m mark.

Calcium Imaging

A miniature microscope and the genetically encoded fluorescent Ca2+ indicator GCamp6f was used to image somatic Ca2+ activity of CA1 pyramidal neurons per session. Imaging data was collected at a frame rate of 20Hz. The calcium imaging movies were preprocessed using the Mosaic software package Inscopic and individual neurons were isolated

[1] https://github.com/sophiakolak/6998-what-is-time

using the CNMF-E algorithm [2] . The resulting calcium signals were then deconvolved using the Oasis algorithm [3] and the inferred activity was used as the data for our analysis.

Time and Place cell identification

Temporal Binning

We made several design choices while drafting our methodology. First, it was necessary to reset the time values for data in all subsequent trials following the first trial, zeroing after every trial ended. We chose to only analyze data from the first 30 trials from our two initial data sets. This was due to the fact that the mice became satiated after licking the reward over numerous trials, which lead to skewed data in subsequent trials (irregularly long trial durations as a result of the mouse moving at slow speeds, or the mouse navigating past a set reward on the virtual track, for example).

Next, we set all neural firing indicators in the deconvolved spike time data to a binary (0,1), allowing us to calculate the firing frequency of any particular neuron more easily. Second copies of the underlying data frame were kept intact to analyze neural firing activity intensity further in our methodology. To bin the data took several steps. We calculated the duration of the shortest trial, using this as a baseline to derive the total number of bins we would utilize. For "Day 0" trials, we found the shortest duration to be 8,702 +/- 1 ms corresponding to trial 29. For "Day 4" trials, we found the shortest duration to be 6,901 +/- 1 ms corresponding to trial 4. We then used a binning size of 200ms, derived from relevant literature [2]. Bins for each trial were filled with corresponding data points that fell into each time-range associated with each bin. An example time bin has 4-5 imaging frames, with 13 columns of behavioral data and either 255 or 459 columns of neuron firing data, depending on the data set. We used the same integer number of bins with the same 200ms bin size across all trials. This allows us to perform the shuffle test before further analysis.

Spatial Binning

The technique used for spatial binning was similar to that of temporal binning. We isolated the data in the underlying data frame corresponding to the pre-reward period, before the mouse reached 4000cm on the virtual track. We then binned together imaging frames that fell within with 100cm intervals to obtain 40 bins that encompassed all data within every individual trail pre-reward period.

Shuffle Test

A shuffle test [6] is a statistical method for determining the likelihood that an observed correlation is due to randomness. In a shuffle test, the dependent variable is randomly permuted (shuffled) a number of times, and the correlation between variables is then measured successively. At the end of the method, the chance that the observed relationship was due to randomness can then be determined, and if it is below the desired p value (in this case, $p = .05$), then the null hypothesis that the correlation was due to randomness can be rejected.

Our data included the firing activity of many neurons who were not necessary “time cells", and so we used a shuffle test for significance in order to isolate the cells within the experimental data whose firing was associated with temporal moments of the behavior task. After creating temporal bins (as described in the previous subsection), and following the methodology of Fischler et al. [2], we measured the mutual information score between each time interval and the neuronal activity for each time bin. We then performed 100 random shuffles of the neuron spiking data. After each shuffle, we measured the mutual information score between the neuronal activity and the arbitrary time interval. Using a significance level of .95, (p=.05), we successfully isolated "time cells" from the other neurons in our data.

With this infrastructure in place, we were able to compare the correlation between temporally binned cells and neuronal activity against those binned spatially. This allows us to somewhat decouple spatial and temporal features within the CA1 neuronal network, although a full decoupling is not possible given the mouse’s constant motion.

3 RESULTS

Anticipatory Licking Behavior

To assess the mouse’s performance in relation to internal time representations, we examined the number of licks corresponding to both distance and time on the map in both the day 0 and day 4 plots (see Figure 1). We view licking prior to the 4000 mark, where the reward is released, as a sign of anticipatory licking.

The anticipatory lick results provided insight into the mouse’s path integration capabilities with and without utilizing olfactory landmarks. As shown in figures 2 and 3, without olfactory landmarks the mouse consistently loses its internal representations of space and time after 2m or 5,000ms. This effect is drastically reduced when odors are introduced as olfactory landmarks, as shown in the plateaus in Figures 4 and 5.

Time and Place Cell Identification

After measuring the relationship between time passed and anticipatory licking, we identified time cells and place cells from the deconvolved calcium signals to see how many CA1 pyramidal cells fired in relation to the mouse’s position in time and how many cells fired in relation to the animal’s position in space. The results are displayed in Figures 8, 9 and 11. The activity of the time cells in the no odor and odor trials is further displayed in Figure 10.

[2]https://elifesciences.org/articles/28728

[3]https://www.oasis-brains.org/

Time Cells during the Reward Phase

We next asked whether there were time cells in the reward phase. Fischler et al. stopped their analysis of place cells after the 4m mark. We wanted to see whether the mouse was encoding time or place while it was licking the reward. As mentioned in the methods section, the reward period ended after either 4 seconds or 1m of self-triggered licking, whichever endpoint came first. We ran the shuffle test on all the temporally binned reward periods in the day 0 trials and found that only 2 cells fired at a statistically significant rate and could hence be labeled time cells (Figure 9). This suggests that there are fewer time cells firing during this period. Unfortunately we could not spatially bin the reward period accurately, but we predict that the place cell number would be similarly low.

Time Cells before and after learning

To investigate the effects of learning on time and place cell number, we compared the total number of cells classified as time and place cells in the day 0 and day 5 no odor trials. As day 5 consisted of pinene trials interleaved with no odor trials, we only selected the no odor trials for our analysis. This allows us to compare cell firing in a no odor trial prior to olfactory landmarks with cell firing with no odor following 4 days of olfactory landmarks. We found that the percentage of time cells goes from 2.7 % in day 0 to 15.87 % in day 5 (Figure 12). This supports the hypothesis that odor landmarks strengthen our internal representation of time.

Pearson Correlation Coefficient

Day	Variables	Correlation	Figure
Day 0	Licks vs dist.	0.990	(Figure 3)
Day 0	Licks vs time	0.981	(Fig 2)
Day 4	Licks vs dist.	0.971	(Fig 5)
Day 4	Licks vs time	0.983	(Fig 4)

This table shows Pearson's correlation coefficient for days 0 and 4 data, across the first 30 analyzed trials. The slightly higher coefficients for day 0 show the linear relationship of the data corresponding to the mouse licking in anticipation of the reward at a steady rate after 2000cm (where the mouse frequently lost a sense of place and time). This higher values are in contrast to day 4's lower values which were a result of the plateau, as shown in figures 5 and 4 where the mouse did not lick until passing the second odor cue at 3000cm.

4 DISCUSSION

Fischler et al found that olfactory landmarks converge with path integration to allow mice to construct spatial maps that support navigation over distances far greater than what path integration alone can navigate. This was corroborated by our results in Figures 4 and 5, where the greater number of plateaus indicates that the mice are able to more accurately gauge how long it will take, in terms of both distance and time, before they reach the reward. Given the differences in speed, as well as the difficulty in discriminating between the effects of time or space, we do not believe we can ascertain whether the mice start anticipating the reward after a certain duration of time or a certain distance.

A pertinent challenge in this research was decoupling time and distance, since time necessarily increases as distance does in these experiments. In order to decouple these factors as much as possible, we correlated neuronal and behavioral data with distance and time (respectively). As shown in figure 3, we find that both time and distance are highly correlated with behavioral data, supporting the theory that the CA1 region is critical for path integration. Future experiments could more strongly isolate these two variables by studying activity in the CA1 region when the mouse is not moving.

Our results from the time and place cell identification reveal that there are cells that fulfill our criteria in each of the different trial structures, though we are not sure whether these represent the complete set of time or place cells from the data. There were noticeably more time and place cells in the day 4 trials where there were odor cues, suggesting that additional cells are firing for the location of these landmarks, which in turn assist the mouse in navigating the track. Intriguingly, there was a significant increase in the % of cells that were labeled as time cells in the no odor day 5 trials, which suggests that the 4 days of learning with the olfactory landmarks prompts more cells in the hippocampus to fire in relation to time, and hence organize the memory of those trials temporally.

In this study, we have successfully identified time cells and place cells in the population of CA1 pyramidal neurons during variations of a virtual navigation task. Our results align with the findings in Fischler at al. in demonstrating that olfactory sensory cues are crucial for spatial navigation across longer distances and that both internal path integration and external sensory landmarks are two mutually dependent sources of the cognitive spatial map. However, we go a step further in positing that in addition to increased place cell firing at the different olfactory landmarks, there may also be time cell firing that contributes to the improved performance of mice in the virtual navigation task. Despite the limited nature of our results, we believe we have shown that the temporal organization of memories in the hippocampus may also play a salient role and provides an exciting new direction for this line of research.

Future directions for this project will further examine the role of time and place cells in the behavioral paradigm at the level of individual cells, as well as a population of cells. For example, one could look at whether time cells confer a unique identity to identical odor landmarks presented in

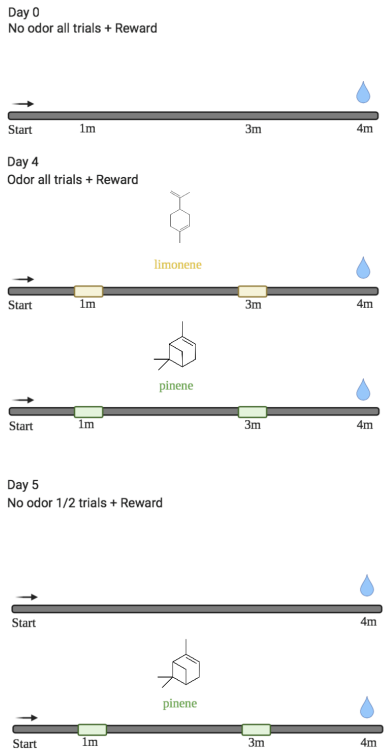

Figure 1: Virtual Navigation Task Set Up

sequence, similar to how Fischler et al found that the odors presented within a given trial at 1m and 3m had unique place cell representations despite being identical odors. Another interesting future direction would be to look at decoding the population of time cells to see either what odor a given representation of time cells corresponds to or what point in time it corresponds to. This could be done via Support Vector Machines or Bayesian decoders respectively. Further, as place cells active at one location in the task were found to influence the number of place cells at subsequent locations, one could look at whether time cells display the same property.

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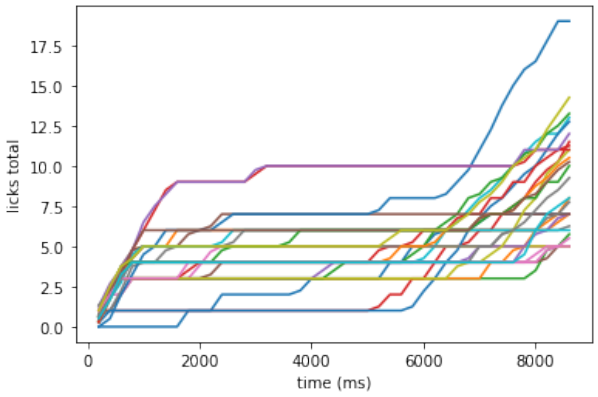

Figure 2: Anticipatory licking before the reward as a function of time in "Day 0" trials. Individual trials are represented by unique colors.

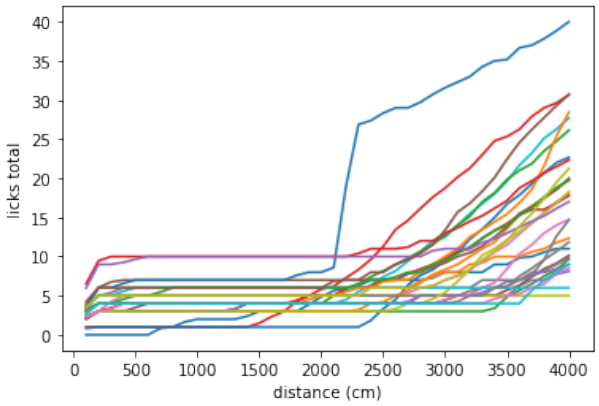

Figure 3: Anticipatory licking before the reward as a function of place in "Day 0" trials. Individual trials are represented by unique colors.

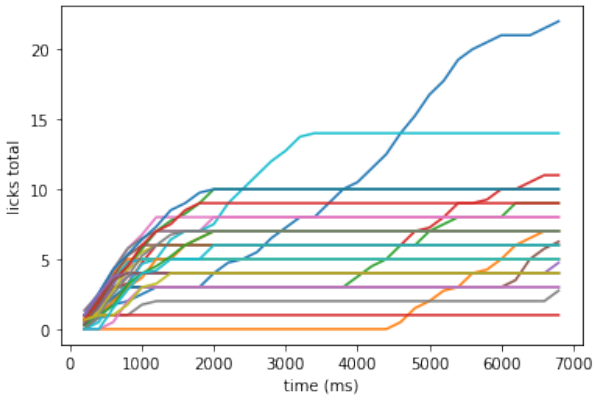

Figure 4: Anticipatory licking before the reward as a function of place in "Day 4" trials. Individual trials are represented by unique colors.

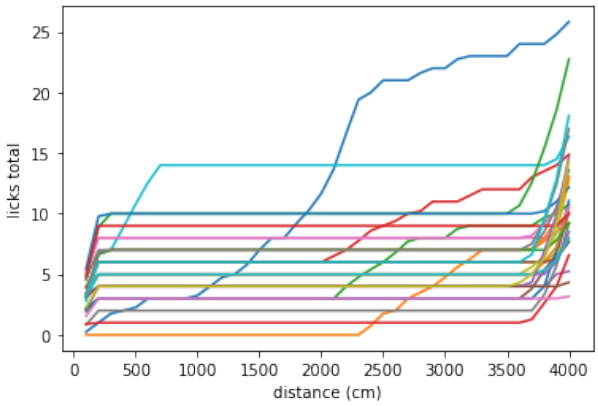

Figure 5: Anticipatory licking before the reward as a function of time in "Day 4" trials. Individual trials are represented by unique colors.

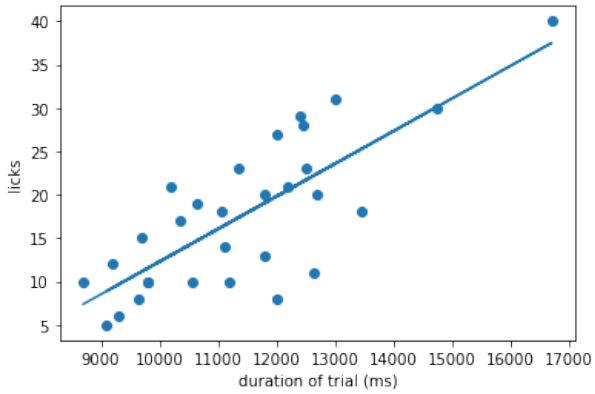

Figure 6: Trial duration versus total lick count in "Day 0" trials

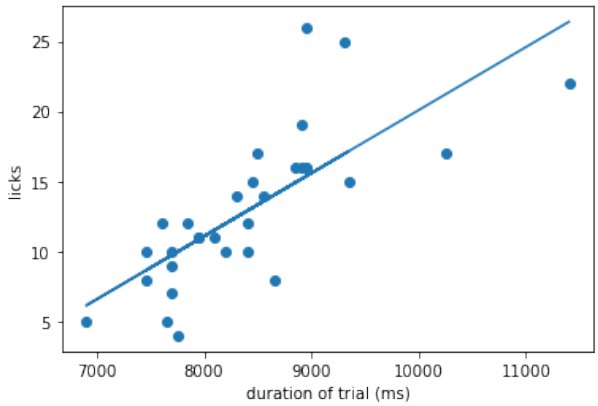

Figure 7: Trial duration versus total lick count in "Day 4" trials

Figure 8: Time cells on Day 5, no odor after training

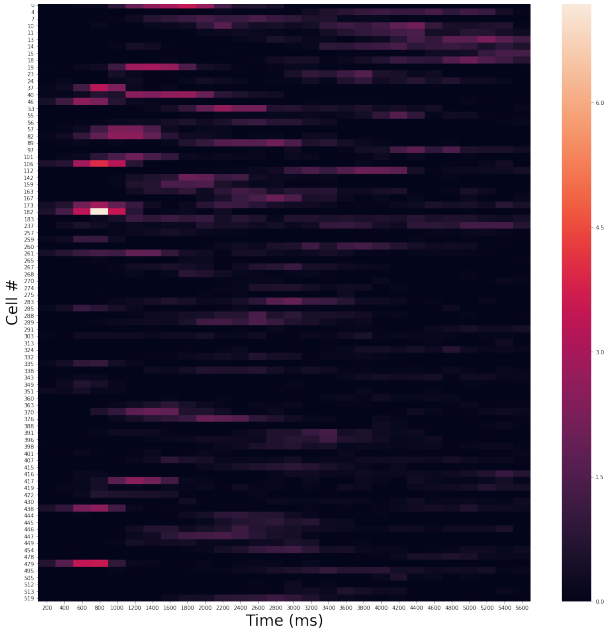

Figure 9: Time cell identification during reward period

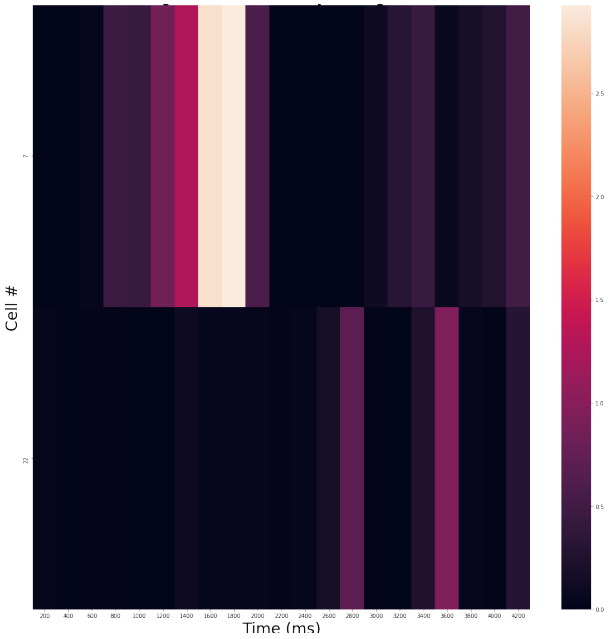

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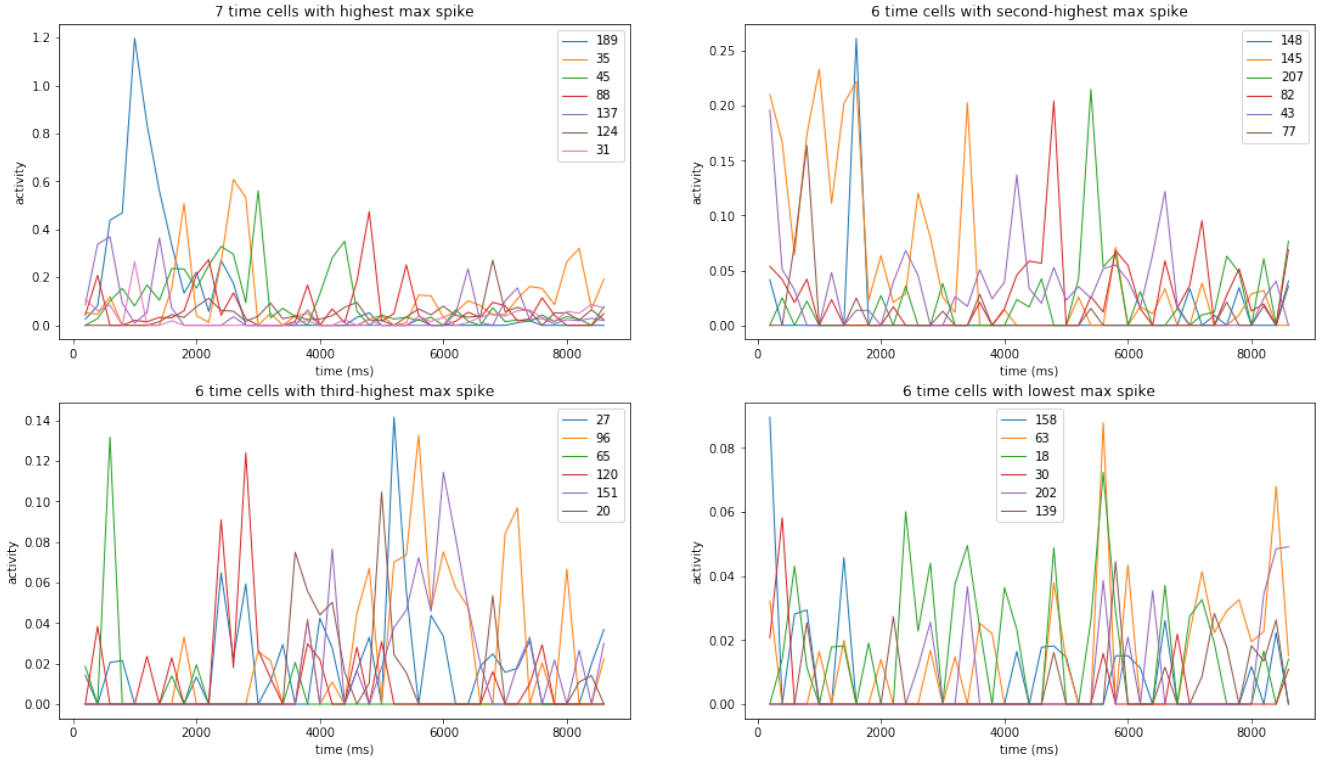
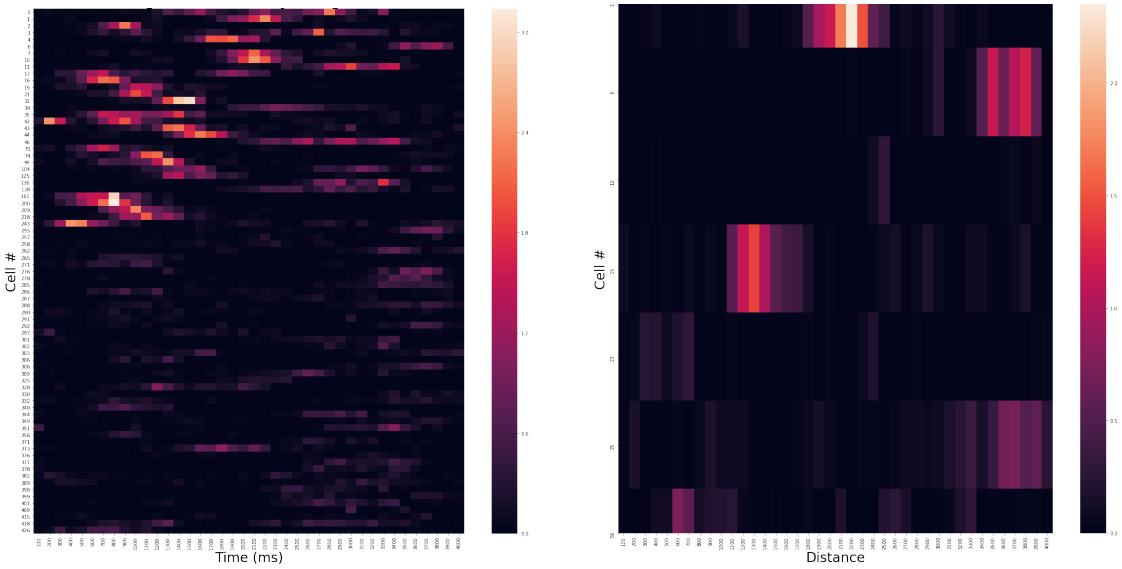
Figure 10: Time cell identification in no odor and odor trials





(a) Time cells on Day 4, odor 1 **(b) Place cells on Day 4, odor 1**

Figure 11: Comparison of time and place cells for day 4, odor 1

Figure 12: Number of time cells in non-odor (Day 0) and odor trials (Day 4)