Hippocampal Ensembles Represent Sequential Relationships Among an Extended Sequence of Nonspatial Events

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Abstract

The hippocampus plays a significant role in temporal organization of events, such as remembering the past and predicting the future. A substantial amount of research suggests this function holds across species. Despite this knowledge, the more intricate mechanisms at play remain poorly understood. In this study, we aim to apprehend the relationship between the hippocampus and events taking place over an interval of time (temporal events) that do not relate to the subjects' position in space (nonspatial events). We do this by analyzing data from an experiment that presented a series of odors to rodents and recorded their neuronal activity during the time of stimulus presentation. We implement standard statistical tests, then fit a multinomial logistic regression model to decode neuron ensemble activity per odor presentation. The model suggests that there is a strong association between the hippocampus and non-spatial temporal events.

Background Information and Scientific Goals

In the experiment, rats were initially trained to poke their nose into the smelling port to identify whether the one of five odors was in sequence or out of sequence; when correctly determined, a water reward was given. Once rats reached a baseline level of performance, trials were recorded. Using devices called tetrodes implanted into rats' hippocampi, we are able to measure electrical activity for individual neurons in the area.

We are working to understand how memory is stored for non-spatial sequential events. Our goals involve finding an association among individual neurons and being able to decode odor presentation based on ensembles. Using this information, we can observe if there is any direct relationship between certain neurons in the hippocampus and non-spatial memory.

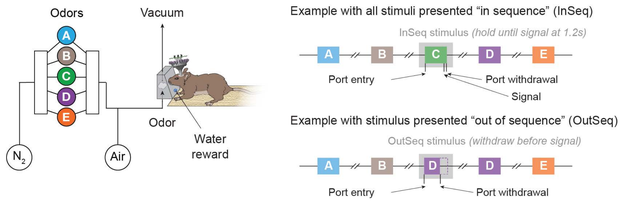


Figure 1. Odors are presented on the left with the rat's nose in the port. The right side shows a representation of an in-sequence trial on the top and an example of out of sequence on the bottom. The rats have 1.2 seconds to withdraw their nose if the odor is out of sequence.

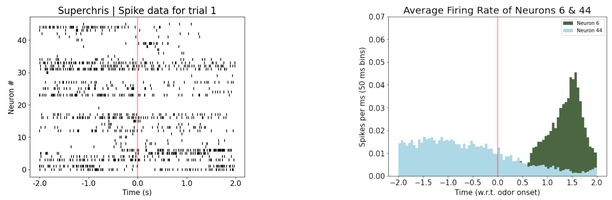
Data Structure and Variables

Our data was provided by Fortin Lab and included 3 data sets:

1. **Trial Data** - Characteristics of each given trial: odor presented, in sequence or out of sequence and whether the rat correctly identified the sequence.
2. **Spike Data** - Spikes per ms for a given rat, trial number, time (10ms bins), and neuron number. A spike is any non-zero (*binary*) signal recorded by the fitted tetrodes.
3. **LFP Data** - Magnitude of local field potential (a continuous reading of the electric potential in the space around neurons) for a given rat, time, and channel number. LFP signals contrast with spike data in that it reflects regional activity as opposed to individual neurons, is *non-binary* and oscillates in theta cycles, which we will later show to be pivotal to modeling.

Our analysis is on the rat "Superchris" and our spike data is filtered into 50ms bins.

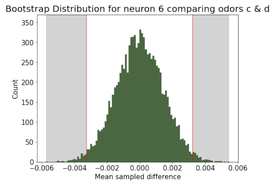
Exploratory Data Analysis



(a) Raster Plot (b) Peri-Stimulus Time Histogram

Figure 2. Fig. (a) depicts the spikes (black columns) for all neurons over time. The stacked PSTHs in Fig. (b) show the stark contrast in average firing rate over time between the two neurons. In both figures, the vertical red line denotes the odor onset at time = 0s.

Before attempting to fit models to decode, a statistically corroborated association between individual neurons and the olfactory sense must first be determined. To do so, we compare the firing rates both between neurons and between odors of a given neuron. We quantify this difference using **bootstrapping** and ***t*-tests** to determine if a statistically significant difference is present.



(a) Bootstrap Distribution

Neuron 6	Bootstrap	*T*-Test
A & B	≈ 0	≈ 0
A & C	.603	.058
A & D	.005	.002
B & C	≈ 0	≈ 0
B & D	≈ 0	≈ 0
C & D	.027	.002

(b) P-Value Table

Figure 3. Fig. (a) shows the bootstrapped distribution of randomly sampled odor c and odor d trial mean differences for neuron 6, with the grey region depicting values equal to or more extreme than the observed difference. The *p*-value quantifies the probability of lying in this region, which is listed in Fig. (b) for both tests.

With the Bonferroni corrected significance level set at .00018, we see the emboldened values are significant enough to **conclude a difference in mean firing rate after odor onset** for this neuron. Approximately half of the observed neurons met this stringent criterion.

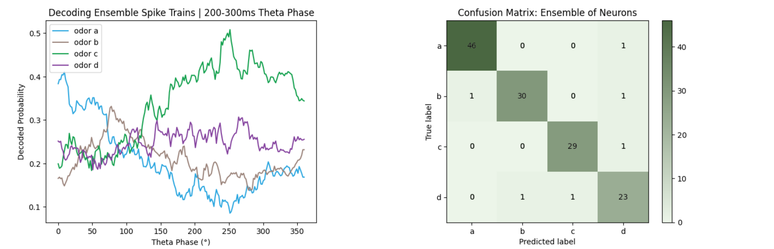
Modeling

Decoding neural activities across theta phases plays a critical role in cognitive neuroscience. The theta rhythm, oscillating at 4-12 Hz, is vital for memory and spatial navigation. Analyzing how the rat's brain processes and distinguishes odors at different phases of the theta cycle provides valuable insights into the neural representation of odors and their sequential patterns.

We employ the **Multinomial Logistic Regression (MLR)**, a powerful statistical model that allows us to classify and predict odor labels, a discrete outcome, from the firing rate of *individual* and an *ensemble of neurons*. We implement a standard machine-learning pipeline, encompassing data cleaning, preprocessing, cross-validation, model training, and performance evaluation.

We also employ a **Recurrent Neural Network (RNN)** to predict whether a rat was participating in an in-sequence or out-sequence trial, based the trial spike data. The RNN is well-suited for this analysis as it considers the sequential nature of the data when making predictions.

Results



(a) Decoded Probabilities Graph (b) Confusion Matrix

Figure 4. In Fig. (a), we observe the variability of neuronal preference for odor a and c for the 200-300ms theta phase while Fig. (b) illustrates the model's label predictions with an accuracy score of **95.52%**.

	Precision	Recall	F1-Score	# Trials
Out-Sequence	1.00	0.56	0.71	9
In-Sequence	0.90	1.00	0.95	35
Macro avg	0.95	0.78	0.83	44

Table 1. Classification report for Superchris, odor d

Using the MLR model, we decode probabilities for the neuronal ensemble at distinct theta phases, quantifying the rat's ability to remember odors in-sequence during different points of the cycle.

The RNN demonstrates high accuracy in classifying trial types for rats, correctly identifying precision and recall **83%** of the time on average; the hippocampus is indicative of trial type.

Our findings suggest **the recorded neurons in the CA1 region of the hippocampus encode odors in sequence**, and thus discernible differences in sequential non-spatial stimuli.

Future Work

We would like to explore models that more directly implement LFP and spike data together in order to discern any potential patterns that may arise through this more holistic view. We would also like to experiment with Convolutional Neural Networks for predicting trial type.

Acknowledgments

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References

[1] Babak Shahbaba, Lingge Li, Forest Agostinelli, Mansi Saraf, Keiland W. Cooper, Derenik Haghverdian, Gabriel A. Elias, Pierre Baldi, and Norbert J. Fortin. Hippocampal ensembles represent sequential relationships among an extended sequence of nonspatial events. *Nat Communications*, 13(787), 2022.