Complementary Task Structure Representations in Hippocampus and Orbitofrontal Cortex during an Odor Sequence Task

Highlights
- Neural encoding of an odor sequence in both HPC and OFC is shaped by task demands
- HPC emphasizes early states where explicit information must be encoded in memory
- OFC emphasizes later states where hidden information must be recalled for behavior
- Thus, HPC and OFC can play complementary roles in representing task states

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In Brief
Analyzing neural representations of an odor sequence, Zhou et al. show that HPC emphasizes early positions where demands to encode explicit information in memory are high, while OFC emphasizes later positions where hidden information must be recalled. These findings show that HPC and OFC can play complementary roles in representing task states.
Complementary Task Structure Representations in Hippocampus and Orbitofrontal Cortex during an Odor Sequence Task

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SUMMARY

Both hippocampus (HPC) and orbitofrontal cortex (OFC) have been shown to be critical for behavioral tasks that require use of an internal model or cognitive map, composed of the states and the relationships between them, which define the current environment or task at hand. One general idea is that the HPC provides the cognitive map, which is then transformed by OFC to emphasize information of relevance to current goals. Our previous analysis of ensemble activity in OFC in rats performing an odor sequence task revealed a rich representation of behaviorally relevant task structure, consistent with this proposal. Here, we compared those data to recordings from single units in area CA1 of the HPC of rats performing the same task. Contrary to expectations that HPC ensembles would represent detailed, even incidental information defining the full task space, we found that HPC ensembles—like those in OFC—failed to distinguish states when it was not behaviorally necessary. However, hippocampal ensembles were better than those in OFC at distinguishing task states in which prospective memory was necessary for future performance. These results suggest that, in familiar environments, the HPC and OFC may play complementary roles, with the OFC maintaining the subjects’ current position on the cognitive map or state space, supported by HPC when memory demands are high.

RESULTS

We recorded from the HPC in 9 rats trained to perform the odor sequence task previously used to record in the OFC (Figures 1A and 1B) [8]. Briefly, the task consisted of a sequence of “go/no-go” odor discrimination steps. In each step in the sequence (i.e., trial), one of 16 different odor cues was presented, and the rat had to decide whether to “go” to a nearby fluid well within 2 s to obtain a sucrose reward or withhold responding to avoid a...
prolonged inter-trial interval (ITI). The 16 odor cues were presented in predictable sequences. There were two major sequences (S1 and S2), each of which nested two subsequences (a and b). Each sequence consisted of six different odors or positions (P1–P6). Odors at the first two positions (P1 and P2) were unique for each subsequence (8 odors; two for each of the 4 subsequences), while odors at each of the last four positions (P3–P6) were common across the two major sequences (8 remaining odors). Thus, S1a and S1b shared one set of odors at these positions, and S2a and S2b shared another set. All but two of the odors had a fixed association with reward or non-reward, so for most of the trial types or positions, correct behavior did not require information about the sequence. However, for the odors at P4 and P5 in S2, the significance of the odor differed for the two subsequences S2a and S2b. This required the rats to maintain information across several trials to perform correctly.

A bundle of 16 stereotrodes (32 channels) was implanted in the dorsal HPC after the rats were extensively trained. After recovery from surgery, single-unit activity was recorded from CA1 as rats performed the task (Figure 1C; n = 2,056 neurons). Rats were highly proficient on the task during recording, performing accurately on the discrimination at all positions, including P4 and P5 in S2, where performance required memory.
The Precision of Hippocampal Encoding of Task States Was Dampered by Proximal Reward and Task Irrelevance

To compare task structure representations, we constructed pseudo-ensembles of CA1 single units and tested for representation of the 24 positions in the 4 sequences, using data from epochs corresponding to each of the 6 different events in a trial (“light,” “poke,” “odor,” “unpoke,” “choice,” and “outcome”). Like ensembles constructive of OFC neurons, HPC ensembles contained significant information about sequence positions shown in confusion matrices (Figure 2A), particularly during the periods surrounding odor sampling that were key to correct responding. In both areas, the patterns evident in the confusion matrices developed gradually as ensemble size increased and
did not depend on recordings from any particular subject (Figure S4), suggesting that the observed effects reflect information distributed across relatively large portions of the population. This is consistent with the single-unit analysis presented above and also with the varied patterns evident in individual single units (Figures S1 and S2; see also Figure S3 in [8] for OFC single units).

However, contrary to the hypothesis that HPC would represent sequence information with higher fidelity than OFC—and with less bias for behavioral relevance, internal goals, or reward—HPC ensemble activity at different positions (P1–P6) performed generally no better and often worse than OFC ensembles at correctly identifying the rat’s specific state (i.e., one of 24; Figure 2B). Indeed, rather than being less sensitive to behavioral relevance, HPC ensembles seemed, if anything, more sensitive to this factor in what they represented (Figures 3 and 4).

The influence of behavioral relevance on the encoding of positions by HPC ensembles was particularly evident in two aspects of the results. One was in how the HPC ensembles represented P3–P6 in S1, where the rats did not need to distinguish subsequences (Figure 3). OFC performed at chance at distinguishing these positions (Figure 3B), providing an effective floor against which to see a better representation of incidental sensory associations in HPC. However, HPC ensembles also performed at chance at decoding these states; like OFC, they were much better at distinguishing these positions in S2, where
doing so was relevant to the rats’ behavior (Figure 3A). The second area where the impact of behavioral relevance was evident was in the strong effect of current reward on encoding by the HPC ensembles (Figure 4). HPC ensembles showed much better state discrimination between non-rewarded positions than between rewarded positions during both poke and odor time (Figure 4A). OFC ensembles also showed similar trends but with smaller effect sizes (Figure 4B).

HPC Activity Distinguishes Different Sequences at P2 and P3 Better Than OFC

To further investigate the differences between HPC and OFC, we next looked at the representation of P3 in sequence 2a versus 2b, where the rats were required to maintain the memory of past odors without discriminative sensory cues in order to know what to do in the following position (P4). Distinguishing P3 in sequence 2a versus 2b was significantly better for HPC ensembles as compared to OFC ensembles during the poke time (Figures 5A and 5B). Ensemble performance at poke time was particularly intriguing because, during this period, there was no confound from bottom-up sensory-driven neural responses as may occur during the odor period. Thus, any information encoded during the poke time must be supported purely by the sequence information at all positions. We therefore asked whether encoding by HPC ensembles was better than by OFC ensembles at all 24 positions at poke time. This analysis identified 8 positions or states that were significantly better represented by ensembles from HPC than from OFC (Figure 5C). These 8 states all occurred at P2 and P3—critical positions in each sequence where the rats transitioned from the odor-unique positions, where external information was provided regarding sequence, to the odor-common arms, where tracking sequence required memory for prior events.

Complementary Emphases on State Representations by HPC and OFC

To further test whether this transition from the unique to the common arms captured a complementary pattern of encoding in HPC and OFC, we sorted the 24 states based on how well each was represented by the two areas (Figures 6A and 6B) at the poke time. Task states best represented by each area were largely non-overlapping, with HPC better representing states around the transition and OFC better representing states in the common arms after the transition.

We next asked whether this pattern was also evident in the ability of the ensembles to distinguish positions (P2–P5) across each sequence pair (S1a versus S1b and S2a versus S2b; Figure 6C). We defined P2 and P3 as the “transition” phase and positions P4 and P5 as the “after-transition” phase of the sequence. Consistent with data shown in Figure 3, both HPC and OFC did poorly at distinguishing positions in S1a versus S1b once the odors and outcomes were the same (P3–P5), confirming that neither region maintained information that was not required for the behavioral performance (Figure 6C, left panel). By contrast, in each of the other comparisons, the HPC ensembles performed significantly better at discriminating positions at the transition than did ensembles composed of OFC neurons. After the transition, this pattern was largely reversed, with OFC ensembles providing better decoding than HPC ensembles at P4 and P5 as long as the value was the same (Figure 6D; S2a versus either S1a or S1b). When the value of these positions differed in the comparison (Figure 6C, right panel; S2a versus S2b), both regions performed well after the transition. However, for HPC, this performance is deceptive, since it is based not on true representation of the two states at each position but rather on the relatively uniform representation of all the rewarded positions in both sequences (see dendrograms; Figure 4). In the comparisons, when the reward availability was
the same, HPC showed the best decoding during the transition but decreased its ability in distinguishing sequences after the transition, while OFC showed the opposite pattern (Figure 6D).

DISCUSSION

The current study sought to test the hypothesis that the HPC represents detailed, sometimes incidental, information about the cognitive map of a task, while such information is tailored in the OFC to highlight distinctions more directly relevant to behavioral goals [4, 5, 24, 30]. In the odor sequence task used here, this hypothesis predicts that the identity of the 24 task states should be better encoded in activity recorded in the HPC as compared to the OFC. In particular, we expected that HPC ensembles would perform as well as those from OFC at distinguishing behaviorally relevant states and much better at distinguishing states—such as those in the common arm of S1—whose discrimination was unimportant for performing the task correctly. However, our data showed that the task representations in both OFC and HPC were closely tied to behavioral demands; neither maintained sequence information that was not required for behavior (Figure 3), and the HPC ensembles actually performed more poorly than those in OFC at distinguishing different rewarded states (Figure 4).
the two areas under the same specific conditions. This leaves open the possibility that, earlier in learning, the HPC-OFC system might operate differently. However, the current results suggest that, once an environment is familiar, neither region maintains much information about the structure of the task that is incidental to task performance.

What then distinguishes the cognitive maps in OFC and HPC in familiar environments? One striking difference between the HPC and OFC was that the HPC encoding was highly biased toward the non-rewarded task states [31]. This did not seem to simply reflect non-reward, however, since the non-rewarded positions at P5 (1a5–, 1b5–, and 2a5–) were poorly represented by the HPC (Figure 6D). Instead, this finding seems to be better explained by the proximity of these states to entry into the common arms of the sequences. This transition point is where information must be encoded in episodic memory, so that later states in the common arm can be properly distinguished. The bias in HPC to strongly represent these positions, and the relatively poor encoding of rewarded positions, is reminiscent of evidence that the HPC represents the start of journeys through a mapped environment [32–34] and is also similar to distinctions between HPC and prefrontal representations seen in prior work on context [35–40]. For example, theta oscillations in the HPC have been shown to precede those in the medial prefrontal cortex (mPFC) when subjects first enter a unique context, while mPFC theta activity precedes that in the HPC later in the trial when decisions are made within this context [41]. In our task, the unique arms may be thought of as setting the context for determining the rules or map to apply in the common arms.

For the most part, discriminating between sequences on the common arms had to be based on latent or hidden information, available only in memory. In this regard, it is interesting that, once in the common arms, the HPC generalized across states with similar external information, whereas OFC became better at discriminating these hidden task states. This suggests that one distinction between the cognitive maps represented in the two areas may be the degree to which inference or encoding of latent information underlies what is represented. While this may be interpreted as a bias in OFC for task-relevant information, the seeming task relevance of OFC representations may simply reflect the importance of latent states in determining goals and behavior in such tasks. By providing a task in which sections were identical except for the necessity for inferring latent causes, our results highlight this difference.

Whether the superior performance of the OFC at representing the hidden or latent information represents a true difference between this area and HPC is less clear from our data, and we would view that as unlikely. Both areas have been clearly and repeatedly implicated in both representing and using such information [26–29, 42–44], so the important question to us seems to be not which one does it better or more strongly but rather how they cooperate in this general representational function and under what circumstances one or the other is dominant. In this regard, the current results provide a rare chance to directly compare when and how strongly hidden information is represented in the two areas under the same specific conditions. Additional work will be required to suss out what about the current task drives the observed dichotomy, but at least two possibilities spring to mind. One, discussed earlier, is that it may reflect the relatively large amount of training given to these rats. The HPC may play a different role in supporting the representation of latent information earlier in learning, which then is minimized or unnecessary in advanced stages of training. A second possibility is that the representation of latent information in the HPC was rendered less prominent by the position of this information near the end of the sequences. Perhaps if there was a need to represent this information at the start of the sequences, then it would be better represented in HPC.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

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**SUPPLEMENTAL INFORMATION**

Supplemental Information can be found online at https://doi.org/10.1016/j.cub.2019.08.040.

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**AUTHOR CONTRIBUTIONS**

J.Z. and G.S. designed the experiments, with advice and input from Y.N.; J.Z. and M.M.-C. collected and analyzed the data, with advice and technical assistance from A.M.W. and M.P.H.G.; and J.Z. and G.S. wrote the manuscript with input from the other authors.

**DECLARATION OF INTERESTS**

The authors declare no competing interests.

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**REFERENCES**

**STAR METHODS**

**KEY RESOURCES TABLE**

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**LEAD CONTACT AND MATERIALS AVAILABILITY**

This study did not generate any unique reagents, however further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Geoffrey Schoenbaum (geoffrey.schoenbaum@nih.gov).

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

Male Long-Evans rats (Charles River, 175 – 200 g, ~3-month-old) were individually housed on a 12-h light/dark cycle with ad libitum access to food in an animal facility, which was accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Rats were deprived of water before they performed the task then had free access to water for 10 min in their home cages after each session. All behavioral testing was conducted at the NIDA-IRP. Animal care and experimental procedures complied with US National Institutes of Health (NIH) guidelines and were approved by National Institutes on Drug Abuse Intramural Research Program (NIDA-IRP) Animal Care and Use Committee (ACUC).

**METHOD DETAILS**

**Behavioral Task**

Rats were trained in aluminum boxes (~18” on a side) with an odor port and a fluid well for odor and sucrose solution delivery, respectively. Odor and fluid delivery were enabled by solenoids that were controlled by a custom-written C++ program. Odor port and fluid well entries were monitored through infrared beam sensors. The availability of each trial was signaled by the illumination of two house-lights in the front wall above the odor panel. Rats initiated the trial by poking into the odor port within 5 s after light onset. If a trial was initiated successfully, an odor would be delivered to the odor port after a 500-ms delay. Odor delivery continued until the rats withdrew their noses from the odor port. The trial was aborted (indicated by light off) if the rat left the odor port in less than 500 ms. Otherwise, at the time of leaving the odor port, rats had a 2 s time window to respond at the fluid well. On trials with rewards, responding at the fluid well in time led to the delivery of a sucrose solution (10% w/v; 50 μL) after a delay of 400 – 1500 ms. After the rats consumed the sucrose solution and left the well, the house lights were turned off, indicating the end of the trial and the beginning of the ITI. If the rat did not respond within the 2 s window, the house lights were turned off at the end of the 2 s period. If the rat responded at the fluid well within the 2 s time window on a non-reward trial, an exceptionally rare event in recording, the house lights were extinguished, an indication of the end of a trial, and no reward was delivered. A 4 s ITI followed correct “Go” or “No-Go” trials, and an 8 s ITI followed trials on which the rat made an error.

On each trial, one of 16 odors were delivered. The 16 odors were organized into two pairs of sequences (S1a, S1b, S2a, and S2b) as below. Reward and non-reward trials were labeled as positive (+) and negative (−), respectively, following the odor numbers.

- S1a: 0+ 1- 4- 5+ 6- 7+
- S1a: 2+ 3- 4- 5+ 6- 7+
- S2a: 8+ 9- 12- 13+ 14- 15+
- S2b: 10+ 11- 12- 13+ 14- 15+

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Before training with any odors, rats were first shaped to nose poke at the odor port and then respond at the well for a reward. Then, they were trained to discriminate an odor pair (one rewarded and one non-rewarded odor) from sequence 1a or 1b. Sessions consisted of a maximum of 480 trials. After rats reached a high criterion in performance (> 90% correct ratio), additional odor pairs were added until the rats were able to perform well in a session containing sequences 1a and 1b. After learning sequences 1a and 1b, rats were trained to discriminate between odors 13/14 from sequence 2, including several reversals of the valence of the pair. After the third reversal, additional odor pairs were added from sequence 2 if the rats were able to maintain accurate performance (> 75% correct) on each trial type. Once sequence 2 had been fully introduced in this manner, the rats began sessions containing both pairs of sequences (S1a, S1b, S2a, and S2b).

In this final training phase, each sequence was repeated for 20 times to make 480 trials in total. Sequences 1a and 1b were always followed by 2a or 2b with roughly equal probability (0.55 and 0.45, respectively). Sequence 2a and 2b were always followed by 1a or 1b also with slightly more dissymmetry in probability (0.67 and 0.37, respectively). The overall sequence was repeated from start to finish in each session.

1b 2a 1a 2a 1a 2b 1b 2b 1b 2a 1b 2a 1a 2b 1a 2a 1a 2b 1a 2b 1b 2a 1b 2b 1a 2a 1a 2b 1a 2b 1b 2a 1b 2b 1a 2a 1a 2b 1a 2b 1a 2a 1a 2b 1a 2b 1a 2a 1a 2b 1a 2b 1a 2a 1a 2b 1a 2b 1a 2a

Before electrode implantation, animals were kept training on the full sequence for at least three weeks until they were able to perform well (> 75% correct) on every trial type.

Surgical Procedures
Rats were implanted with a drivable bundle of 16 stereotrodes made from nickel-chromium wires (17.8 μm in bare diameter; AM Systems, WA) that targeted the left hippocampal CA1 (AP: −3.5 mm; ML: 2 mm). Wire bundles were housed in a cannula and cut with a pair of fine surgical scissors to extend 1.5 – 2 mm beyond the end of the cannula inside the brain. The tips of wires were initially placed at 1 mm ventral from the brain surface. After surgery, rats were given Cephalexin (15 mg/kg) orally twice a day for two weeks to prevent any infection.

Single-Unit Recording
Electrophysiological signals were recorded with the Plexon Multichannel Acquisition Processor (MAP) systems (Plexon, Dallas, TX). The initial signals collected by the electrodes were sequentially amplified through a headstage (20 ×), a differential preamplifier (50 ×), and a final acquisition processor (1 – 32 ×). A low pass filter (300 Hz) was used to record field potentials, while a band-pass filter (250 – 8,000 Hz) was used to isolate spike activities. For spike recordings, a threshold was set manually for each active channel to capture unsorted spikes. Timestamps for behavioral events were sent to the Plexon system, synchronized and recorded alongside the neural activity. Spikes were sorted to identify single units offline using Offline Sorter (Plexon, Dallas, TX) with a template matching algorithm. Sorted files were exported as NeuroExplorer (Nex Technologies, Colorado Springs, CO) formatted files to extract unit and behavioral event timestamps, which were then exported as MATLAB (MathWorks, Natick, MA) files for further analysis.

After two-week recovery from the surgery, the electrodes were slowly moved down (~10 μm each time) over ~2 weeks to search for strong theta and sharp-wave signals in the local field potentials, indicators of the hippocampal CA1 area. The electrodes were considered to be in the hippocampal CA1 area when both the field potential signatures (theta and sharp-wave signals) and typical pyramidal neurons (wide waveforms and low baseline firing rates) appeared. Recording sessions continued daily until pyramidal activity disappeared. The electrodes were moved down by ~40 μm every two weeks or by ~20 μm due to the lack of pyramidal activity. At the end of testing, rats were euthanized by an overdose of isoflurane. The final positions of electrodes were marked by passing a small constant current through the wires, and the brains were processed with Nissl staining for histological examination.

QUANTIFICATION AND STATISTICAL ANALYSIS
The number of rats and the number of neurons were not predetermined by any statistical methods but are comparable to those reported in previous publications from our lab. All data were analyzed using MATLAB (MathWorks, Natick, MA).

Task Events and Peri-event Spike Train Analysis
Each trial was divided into 6 epochs associated with different task events: “light,” “poke,” “odor,” “unpoke,” “choice,” and “outcome.” On rewarded trials, the time of well-entry was labeled as “choice.” The “outcome” was at the time of reward delivery. On unrewarded trials, the end of the 2 s window for responding was labeled as “choice” and a time point 1.5 s after the “choice” as the “outcome.” Behavioral performance was quantified as the percent of trials on which the rats responded correctly, their reaction time from the odor port to the fluid well, and the latency with which they initiated a trial after light onset. The data analysis only included sessions in which the percent correct was above 75% for every trial type. Error trials were removed for all the analyses. The spike train for each isolated single unit was aligned to the onset of each task event. Pre-event time was set to be 200 ms, and post-event time 600 ms. Spike number was counted with a bin = 100 ms. A Gaussian kernel (σ = 50 ms) was used to smooth the spike train on each trial.
Classification Analyses
We used a linear discriminant analysis (LDA) algorithm (MATLAB function: fitcdiscr) to classify 24 trial types or states for each one of six task events. Neurons recorded from different sessions were aligned to form pseudo-ensembles. Firing rates during a period of 100 – 600 ms after each task event were used for classification of 24 states. Each trial was seen as an observation that contained firing rates from 2,056 neurons from the HPC or 1,078 neurons from the OFC (480 trials in total but only 360 trials were used in this study as a result of the removal of error trials; 360 trials = 24 trial types × 15 trials). The classification accuracy was assessed by leave-one-out cross-validation. Specifically, one trial from each trial type was left out for future testing, and all the other trials were used for the training. Principal component analysis (PCA) was used for feature extraction and dimension reduction in the training set. The classifier was trained on the principal components (PCs) necessary to explain 80% variance (HPC: 103.7 ± 0.5 PCs; OFC: 151.2 ± 0.4 PCs). The same PCA transformation from the training set was applied to the test set. Trial order for each neuron was shuffled within the same trial type. The trial-order shuffling was repeated for 100 times. For each time of trial-order shuffling, the leave-one-out cross-validation was repeated for 500 times to estimate the decoding accuracy. The mean decoding accuracy for each trial type as shown in the confusion matrix was the mean across 100 runs (corresponding to 100 times of trial-order shuffling). The statistical significance of the mean decoding accuracy was determined by the 95% confidence interval estimated by running the same decoding process with label-shuffled data. For the binary classification of subsequences at each time point (bin size: 100 ms) or after task event (time period: 100 - 600 ms), the processes were the same except only the first three PCs were used, and the trial-order shuffling was repeated for 200 times.

Hierarchical Clustering Analyses
The hierarchical agglomerative clustering was performed on data that was projected onto the LDA space. Each trial was organized as a vector with firing rates of neurons as dimensions. The original data was first transformed to PCA space with 80% variance retained, then transformed to LDA space supervised by 24 trial-type labels. A dissimilarity matrix was computed by measuring the Mahalanobis distance between each pair of trial-type means. Based on the dissimilarity matrix, an agglomerative hierarchical cluster tree was generated with the unweighted average distance method. The clustering results were plotted as dendrograms.

DATA AND CODE AVAILABILITY
The dataset and MATLAB scripts used in this study will be made available upon request by the lead contact, Geoffrey Schoenbaum (geoffrey.schoenbaum@nih.gov).