Hippocampal spatial and sequential representations of event structure scaffold precise episodic temporal order memory

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Article

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Abstract

The hippocampus plays an important role in representing spatial locations and sequences and for transforming representations via pattern separation and completion. How these representational structures and operations support memory for the temporal order of random items is still poorly understood. We addressed this question by leveraging the method of loci (MOL), a powerful mnemonic strategy for temporal order memory that particularly recruits hippocampus-dependent computations of spatial locations and associations. Applying representational similarity analysis to fMRI activation patterns revealed that hippocampal subfields contained representations of both temporal context and multiple features of sequence structure, including location identity, distance, and sequence boundaries. Critically, the hippocampal CA1 and CA23DG exhibited spatial and sequential pattern separation, respectively, enabling the encoding of multiple items in the same location and reducing swap errors across adjacent locations. Our results suggest that the hippocampus can flexibly reconfigure multiplexed event structure representations to support accurate temporal order memory.

Introduction

Episodic memory is inherently structured according to the temporal order of experiences ¹. As the core brain structure for spatial navigation and episodic memory, the hippocampus and surrounding areas of the medial temporal lobe (MTL) have been consistently implicated in spatial and temporal coding in rodents and humans ^{4, 5, 6}. In particular, the hippocampal-entorhinal system supports representations of temporally ordered events via sequential activation of spatially tuned cells ⁷, suggesting that space and time can be unified into a common internal coding scheme.

Recent rodent research has revealed two types of temporal order representation in the hippocampal-entorhinal system: The representations of stable event sequence that is increased in well-practiced structured events, and the temporal flow that is formed automatically with one-shot learning ⁸. Human functional imaging studies have implicated the hippocampus and the anterior-lateral entorhinal cortex (alEC) in the representation of event structure ^{9, 10}, and recall of temporal order of real-life events from a movie ¹⁵. Although temporal context binding has been consistently implicated in temporal order judgment ^{14, 16, 17, 18}, little is known regarding how the learnt event structure can be used as a scaffold to support effective temporal order memory.

This question is critical for the understanding of human temporal order memory, because people are generally bad at one-shot, episodic-like temporal order memory for random events, perhaps because the contextual drifts may be rather nonlinear and unstable. By contrast, learnt event structures may be used to dramatically boost performance. In fact, ancient Greek and Roman orators had long realized that linking novel material to sequential locations along a familiar and well-organized route can significantly improve temporal order memory ¹⁹. Since then, this mnemonic strategy, called method of loci (MOL) or

memory palace, has been commonly used by memory experts and athletes²⁰. Several studies have shown that the MOL primarily engages spatial and associative processes in the MTL^{20,21,22}.

Leveraging the ancient mnemonic strategy of MOL with representational similarity analysis of fMRI data, our study for the first time revealed multiplexed representations of event structure and temporal context in human hippocampus. More critically, the event structure representations in the hippocampus exhibited spatial and sequential pattern separation, suggesting that the hippocampus may flexibly reconfigure event representations to optimize temporal order memory.

Results

MOL quickly improved temporal order memory performance

Twenty-nine college students (10 males) participated in this experiment (Fig. 1A). On Day 1, subjects were tested for baseline performance, and then given a 2-hour video lecture on the MOL strategy including presentation of a world map with 10 landmarks (see Methods) (Fig. 1B). Participants then practiced the MOL in three consecutive sessions (Day 2 to Day 4) before the scanning session (Day 5). During the baseline test, the two later practice sessions, and the fMRI scanning, subjects performed two encoding-retrieval runs, while they only finished one run on Day 2. In each run, subjects studied a list of 30 words, and after a 6-min delay (filled with a working memory task), they recalled the temporal order of these words (Fig. 1C). To reduce interference or practice effect, new words were used in each run across the whole experiment.

Behavioral analysis showed that memory performance improved dramatically across days ($F(3.43, 95.97) = 60.04$, $P < 0.0001$, $\eta^2 = 0.50$) (Fig. S1A), demonstrating that MOL can quickly improve temporal order memory. During the fMRI scanning, the overall accuracy was at 49.9% (Fig. 2A). Post-hoc t-tests (Tukey HSD) revealed that although the accuracy during scanning was slightly lower than during the last practice session (61.9%, $t(28) = -4.04$, $P = 0.003$, Cohen's $d = 0.75$), probably due to the restricted learning environment and noise in the scanner, it was significantly higher than during baseline (19.5%, $t(28) = 8.41$, $P < 0.001$, Cohen's $d = 1.56$) and the first practice session (28.5%, $t(28) = 6.12$, $P < 0.001$, Cohen's $d = 1.14$).

We further examined the behavioral pattern to ensure that subjects were actually using MOL. First, if they were relying on the locations to encode the temporal order of the studied items, we would predict recency and primacy effects based on the 10 locations. In contrast, if subjects were encoding the items serially without the MOL, we would predict that the recency and primacy effects would be based on the overall list of 30 items. Second, if the subjects used MOL and three words were encoded for each location, we would expect their accuracy to decrease from the first set of 10 words to the second and third sets because of an increasing load at each location (i.e., the fan effect)²³. Finally, we would also expect

significant within-location swap errors (i.e., confusion of items that were 10 or 20 positions apart). The fan effect and within-location swap errors should not occur if subjects did not use MOL.

To test these hypotheses, we conducted ANOVAs on the behavioral performance during the fMRI scanning as a function of run (subjects finished two runs of 30 words), set of words (1 to 3), and serial position (Initial: locations 1 to 4; Middle: locations 5 to 7; Final: locations 8 to 10). Since we did not find a significant main effect of run ($F(1, 28) = 2.14$, $P = 0.155$) or interactions with run ($F_s < 3.12$, $P_s > 0.05$), the two runs were combined for the following analyses (Fig. 2B). Consistent with our hypotheses, a two-way ANOVA with MOL position and serial position as repeated measure revealed significant main effects of set ($F(1.99, 55.61) = 24.82$, $P < 0.0001$, $\eta^2 = 0.14$) (Fig. 2C) and serial position ($F(1.91, 53.43) = 7.84$, $P = 0.001$, $\eta^2 = 0.05$) (Fig. 2D), but importantly, no set by serial position interaction ($F(3.23, 90.42) = 0.30$, $P = 0.842$). Planned post-hoc t-tests indicated that memory performance decreased from set 1 to set 3 ($t_s > 2.74$, $P_s < 0.05$, Cohen's $d_s > 0.53$), showing a clear fan effect (Fig. 2C). In addition, there were significant recency (final vs. middle, $t(28) = 2.82$, $P = 0.004$, Cohen's $d = 0.52$) and primacy effects (initial vs. middle, $t(28) = 3.81$, $P < 0.001$, Cohen's $d = 0.71$) (Fig. 2D). By examining the pattern of errors, we found that subjects were most likely to confuse items between adjacent locations (237 trials, 13.62% of total trials), followed by within-location swap errors (123 trials, 7.07% of total trials) (Fig. 2E). These results strongly suggest that subjects indeed relied on the 10 locations to encode the words.

A different pattern of results was found for the behavioral data during the baseline test. Specifically, we found a significant set by serial position interaction ($F(3.27, 91.47) = 11.11$, $P < 0.0001$, $\eta^2 = 0.11$), indicating only a primacy effect in set 1 (initial vs. middle/final, $t_s > 5.21$, $P_s < 0.001$), but not in sets 2 and 3 ($t_s < 1.78$, $P_s > 0.05$) (Fig. S2C). No significant within-location swap error was found (Fig. S2D). These results suggest that before practice the 30 words were encoded into a single list, rather than based on the 10 locations. Across the baseline and three training sessions, the location-based pattern gradually emerged (Fig. S2 to S5), showing increased ratios of within-location swap errors to all errors (Fig. S1B). Together, our behavioral data suggest that subjects effectively learnt to use the MOL to encode the word order, which not only improved the overall performance but also changed the behavioral patterns.

Hippocampal contributions to temporal order memory

The above behavioral evidence suggests that subjects indeed used the MOL strategy to aid memory encoding. We then turned to the fMRI data to examine how employment of this strategy affects activity levels and stimulus-specific representations in hippocampal subfields. The hippocampus and surrounding medial temporal lobe areas were segmented into 5 regions, including CA1, CA23DG, anterior lateral entorhinal cortex (alEC), posterior medial entorhinal cortex (pmEC), and parahippocampal cortex (PHC) (Fig. 3A; Methods). Univariate analyses revealed marginally significant subsequent memory effects (SME) in CA23DG ($t(28) = 2.35$, $P = 0.026$, corrected $P = 0.074$, Cohen's $d = 0.44$) and PHC ($t(28) = 2.29$, $P = 0.030$, corrected $P = 0.074$, Cohen's $d = 0.43$), with subsequently remembered items showing greater activity than subsequently forgotten items (Fig. 3B). Whole-brain analysis revealed a significant SME in

the left parahippocampal gyrus, the left frontal medial cortex, and the left orbital frontal cortex (FWE-corrected for multiple comparisons), consistent with previous observations²⁴ (Fig. S6A; Table S1).

During retrieval, we found that the CA1 ($t(28) = 2.50$, $P = 0.018$, corrected $P = 0.046$, Cohen's $d = 0.46$) and CA23DG ($t(28) = 2.84$, $P = 0.008$, corrected $P = 0.042$, Cohen's $d = 0.53$) were more active during remembered than forgotten items (Fig. 3C). Whole brain analysis revealed several additional brain regions for this contrast, including the bilateral supramarginal gyrus (SMG), bilateral superior parietal lobule (SPL), bilateral lateral occipital cortex (LOC), and bilateral frontal pole (FP) (Fig. S6B; Table S2).

Hippocampal representations of structured location sequences

Having shown the involvement of hippocampus in temporal order memory, we further examined whether specific hippocampal representations supported temporal order memory. In the MOL strategy, the spatial location was not presented to the participants during either memory encoding or retrieval. However, its well-learned structured sequence of locations (i.e., mental route) could be reactivated and linked with the to-be-learned words during encoding, and again reinstated during retrieval. To probe the neurocognitive representation of this sequential location structure, we examined how hippocampal representational similarity was modulated by location identity, the distance between locations, and the boundaries of the sequence.

Hippocampal spatial pattern separation

To examine the representation of spatial locations, we compared the pattern similarity of items sharing the same location across two runs (i.e., same-location pairs) with those encoding adjacent but different locations (i.e., near-distance pairs, ordinal distance = 1) (Fig. 4A). This analysis could be performed during both encoding and retrieval (according to the temporal distance during encoding). Notably, this cross-run pattern similarity should not be affected by intrinsic autocorrelations of the BOLD signal. We found that the PHC showed greater pattern similarity for same-location pairs than near-distance pairs during encoding ($t(28) = 3.42$, $P = 0.002$, corrected $P = 0.010$, Cohen's $d = 0.64$), while a reverse pattern was found during retrieval ($t(24) = -2.61$, $P = 0.015$, corrected $P = 0.039$, Cohen's $d = 0.52$; four subjects were excluded from this analysis due to fewer than 10 trials in any condition, see Table S3) (Fig. 4B). The same effect of pattern separation during retrieval was found in CA1 ($t(24) = 2.81$, $P = 0.010$, corrected $P = 0.039$, Cohen's $d = 0.56$), but not in CA23DG ($t(24) = 0.372$, $P = 0.713$) (Fig. 4C), and there was no significant region (CA1 vs. CA23DG) by location identity interaction ($F(1,24) = 2.011$, $P = 0.169$). Whole-brain searchlight analysis did not reveal any representation of location identity elsewhere in the brain.

Given the similar pattern separation effect in CA1 and PHC, we further examined the relationship between representational patterns in CA1 and PHC. Representational connectivity analysis (see Methods; Fig. S7) revealed that the CA1 pattern was positively correlated with the PHC pattern during both encoding ($r(28) = 0.036$, $t(28) = 3.77$, $P < 0.001$) and retrieval ($r(24) = 0.076$, $t(24) = 4.00$, $P < 0.001$). Interestingly, the CA1 encoding pattern was marginally negatively correlated with the CA1 retrieval pattern ($r(24) = -0.030$, $t(24)$

= -1.81, $P = 0.083$). These results suggest that the CA1 representation of spatial location could be flexibly modulated via pattern separation processes, which could then modulate the PHC representations (even though the direction of the effects of course cannot be inferred).

The spatial pattern separation could help to differentiate the temporal order of items encoded at the same location during memory retrieval. If the CA1 and PHC pattern separation indeed aided temporal order judgment, we would predict the degree of pattern separation (near-distance minus same-location pairs pattern similarity) in these regions to be negatively correlated with the numbers of within-loci swaps. Robust regression revealed a marginally significant effect in the PHC ($t(27) = -1.98$, $P = 0.058$).

Hippocampal sequential pattern separation

Second, we examined how neural pattern similarity was modulated by the distance between two locations in the well-trained sequence^{9,10}. Note that distance here refers to the ordinal distance between two locations (ranging from 1 to 9) rather than their Euclidean or geodesic distance in the real world. We compared the pattern similarity of words that were encoded at near distance (i.e., ordinal distance = 1), middle distance (i.e., $2 \leq \text{ordinal distance} \leq 3$) and far distance (i.e., ordinal distance > 3) (Fig. 4A). We found that the CA23DG region showed a main effect of location distance during encoding ($F(1.80, 50.49) = 5.40$, $P = 0.009$, corrected $P = 0.047$, $\eta^2 = 0.03$). Post-hoc t-tests (Tukey HSD) showed higher pattern similarity for far-distance pairs than near- ($t(28) = 2.93$, $P = 0.018$, Cohen's $d = 0.54$) and middle-distance pairs ($t(28) = 3.30$, $P = 0.007$, Cohen's $d = 0.61$), but no difference between near- and middle-distance pairs ($t(28) = 0.07$, $P = 0.998$) (Fig. 4D). No significant location distance effect was found in CA1 ($F(1.92, 53.88) = 0.718$, $P = 0.487$) or in other MTL subfields ($P_s > .29$). The region (CA1 vs. CA23DG) by location distance interaction was marginally significant ($F(1.64, 45.84) = 3.20$, $P = 0.059$). A reverse pattern was found in neocortical regions during encoding, with greater neural pattern similarity for near-distance pairs than far-distance pairs in the right occipital pole (OP), bilateral LOC, left SMG, and left FP (Fig. S8; Table S4). No significant effect of location distance was found during retrieval.

Hippocampal sequence boundary effects during encoding

In addition to location identity and distance, the location structure also contains sequence boundaries. Unlike previous studies where the boundary was introduced by background context or different sequences^{11,14}, the boundary in the current study was introduced by the repetition of location sequence. That is, when the 11th word was encoded, subjects would return to the first location, which would break the sequence contiguity. As a result, for a given temporal distance (e.g., 2), we can construct both within-boundary pairs (e.g., location 8 - location 10) and cross-boundary pairs (e.g., location 9 - location 1).

To compare within vs. cross-boundary pairs of matching distances, we analyzed temporal distances of 4 to 7 ordinal positions during encoding (Fig. 5A; Table S5). Following a previous study¹⁴, we predicted that the similarity of neural representations would be higher for within-boundary pairs than cross-boundary pairs. Indeed, this was found in both CA1 ($t(28) = 3.70$, $P < 0.001$, corrected $P = 0.002$, Cohen's $d = 0.69$) and CA23DG ($t(28) = 4.37$, $P < 0.001$, corrected $P < 0.001$, Cohen's $d = 0.81$) (Fig. 5B). This effect

was specific to the hippocampus and did not occur in EC or PHC, or in any other brain region in the whole-brain analysis.

Hippocampal temporal context reinstatement during retrieval

The above analyses reveal that hippocampal representational patterns are modified according to the well-trained structured location sequence, exhibiting spatial and temporal pattern separation to aid temporal order memory. In the following analysis, we further examined whether representations in hippocampal subfields also support another type of temporal order, i.e., the episodic-like temporal context that was formed through one-shot learning and should be specific to a given event sequence. Due to the autocorrelation of fMRI BOLD signal, we could not directly compare the representational similarity of temporally adjacent pairs with more distant pairs. Instead, we examined the reinstatement of temporal context during retrieval (Fig. 6). In particular, for a given temporal distance during retrieval (TDr, ranging from 1 to 29), we grouped the pairs, according to their temporal distance during encoding (TDe), into Short ($TDe \leq 3$) and Long ($4 \leq TDe \leq 6$) conditions (Fig. 6A). This grouping was motivated by the small number of trials of each individual distance and by previous findings that effects of temporal contexts decayed quickly beyond 3 items¹². We restricted the analysis to correct trials and $TDr \leq 20$, as there were very few pairs for $TDr > 20$ (Table S6). We predicted that the brain regions containing representations of temporal context should show higher pattern similarity for short-distance pairs than long-distance pairs. Consistent with this prediction, we found this pattern in CA1 ($t(23) = 3.17$, $P = 0.004$, corrected $P = 0.021$, Cohen's $d = 0.65$; four subjects were excluded due to fewer than 10 trials in any condition, and one subject was excluded as an outlier, i.e., 2.5 SDs above the mean, see Table S7) (Fig. 6B). No temporal context reinstatement was found in any other brain region.

Control Analysis: The Hippocampus Did Not Represent Word Semantics

In all the above analysis, we only considered the representation of the event structure and the temporal context, but not the representations of the word, as previous studies did not reveal strong item representations in the hippocampus. To further examine this issue, we conducted latent semantic analysis to generate the semantic similarity matrix of the words, using a well-trained Chinese word embedding model, Directional Skip-Gram²⁷ (See Methods). Correlating semantic similarity with the neural representational similarity (Fig. S9A) did not reveal significant semantic representation in any hippocampal subfield ($P_s > 0.082$) (Fig. S9B). In addition, all the above results remained unchanged after controlling the semantic similarity. Interestingly, we found significant semantic representations during encoding in the vmPFC ($r(28) = 0.017$, $P = 0.031$), and a marginally significant effect in the SPL ($r(28) = 0.020$, $P = 0.068$).

Discussion

Inspired by the ancient mnemonics of MOL, the current study revealed a novel neural mechanism that supports precise temporal order memory of random events. In addition to hippocampal temporal context binding, the current study found that hippocampal event structure representations could be exploited to improve the accuracy of temporal order memory. These representations were shaped by two types of pattern separation processes acting on spatial locations and sequential sequences, which were associated with CA1 and CA23DG, respectively. These results emphasize the multiplexed and flexible nature of hippocampal representations in the service of precise temporal order memory.

CA1 temporal context reinstatement and temporal order memory

The current study revealed clear evidence of temporal context reinstatement in the hippocampal CA1 area, with greater pattern similarity for item pairs studied at closer as compared to more distant temporal intervals. According to the temporal context or temporal drift model^{16,17}, an episodic element is 'tagged' to the random and slowly changing neuronal background activity that is present at the time of encoding²⁸. This temporal context is then reinstated during recall and provides information about the temporal distance by assessing the degree of disparity between the reinstated and the present neuronal background activity¹². Supporting the role of hippocampal temporal context in temporal order judgment, it has been shown that (a) lower hippocampal pattern similarity (i.e., higher representational distinctiveness) was associated with more accurate temporal order judgments¹⁸; (b) changes in EC pattern similarity during encoding of a narrative were correlated with later duration estimates between events²⁹; and (c) manipulation of context shifts by changing background images increased subjective feelings of temporal distance¹⁴. Our results add to this literature by showing hippocampal temporal context representations when participants were asked to rely on existing sequence structure, suggesting that temporal context binding might be automatic.

Hippocampal representation of well-trained event structure

In addition to episodic temporal context, the hippocampal-entorhinal system can explicitly represent spatial sequences. First, the hippocampal-entorhinal system contains place cells³⁰ and grid cells³¹ that provide two complementary representational metrics of spatial locations and distances, respectively. Second, the hippocampus exhibits specific neural mechanisms such as cross-frequency coupling of high-frequency bursts of activity to the phase of low frequency oscillations, which may support the representation and pairwise binding of event sequences^{32,33,34}. Third, rodent studies analyzed activity while animals traveled along well-trained spatio-temporal sequences and found that CA1 "time cells" showed context-specific activities at unique time points of an experience^{35,36}. A recent study revealed both types of temporal order representations, i.e., encoding both temporal flow and trial structure, by the same aIEC neurons⁸. Interestingly, in well-trained structured experiences, the encoding of temporal flow across trials was reduced, whereas the encoding of trial structure was increased. Human neuroimaging studies have also found that both hippocampus⁹ and aIEC¹⁰ can represent well-studied event sequences

or event maps. The hippocampus exhibits increased activity during events that violate an expected sequence^{37,38}, shows increased within-sequence similarity and decreased between-sequence similarity³⁹, as well as greater pattern similarity between adjacent items in well-learned object sequences than random sequences¹¹. Finally, the hippocampal representation is sensitive to sequence boundaries^{11,14}.

Our results significantly extend these observations. First, the current study revealed all three types of event sequence representations in one single study, including spatial location representations, location distance representations, and sequence boundary representations. Together with the representation of temporal context, our study for the first time revealed the rich multiplexing of spatial, temporal and sequential representations in human hippocampus. Second, unlike previous studies, the location sequence in the current study was not presented during encoding, suggesting these representations could be reinstated via conscious memory retrieval, resembling the spontaneous sequence replay during awake resting state and sleep in rodents^{40,41} and humans^{42,43}. Third, while representations of location distance were restricted to CA23DG, we found significant temporal boundary effects in both CA1 and CA23DG. This is consistent with the proposal that both CA1 and CA3 are involved when memories for events must be held over long intervals⁴⁴. Finally, several recent studies have implicated the aIEC in temporal order memory^{8,10,15}, by representing the temporal event structure. The current study mainly found sequence representations in CA1 and CA23DG. One obvious feature of the current study is that the temporal event structure was used as a scaffold for temporal order memory of words, with an emphasis on the binding of items into a structured context. Consistently, both human imaging and rodent studies have also implicated the hippocampus in context coding⁴⁵. Future studies should further examine the role of aIEC and hippocampal subfields in event structure representations and context binding.

Spatial and sequential pattern separation and temporal order memory

Strikingly, we found clear evidence for both spatial and sequential pattern separation. Spatial pattern separation effects were found in CA1 during retrieval, revealing a pattern that is different from that in PHC during encoding. In the current study, each location was associated with three words, and consequently there were significant within-location swap errors. To achieve a high accuracy, the representations thus needed to be reconfigured in order to enable the encoding of multiple items into the same location and yet keep them separate. This requirement is similar to effects of spatial pattern separation during path disambiguation in both rodent and human. For example, hippocampal representations of overlapping routes⁴⁶ or object sequences¹¹ become more distinct than non-overlapping routes/sequences. A previous rodent study also revealed that hippocampal neurons encoded different episodes in a task of overlapping sequences of odors⁴⁷.

In addition to spatial pattern separation, we observed significant pattern separation for sequences in CA23DG, a region that has been implicated in temporal pattern separation before⁴⁸. Unlike the previous studies which found greater pattern similarity for temporally closer items in both aIEC and hippocampus

^{9, 10}, representations of more adjacent representations were more distinct in CA23DG. This pattern was different from that observed in neocortical regions, and was also different from the temporal context representation in CA1, which showed higher similarity for temporally adjacent than for more distant items. Together with the spatial pattern separation, our results suggest that hippocampal representations can be flexibly configured in order to support temporal order memory.

The flexible employment and reconfiguration of hippocampal spatial and temporal representations in support of episodic memory has been frequently reported in the literature. For example, one study found spatial pattern separation but an opposite pattern for temporal representations ⁴⁹, whereas another study found greater pattern similarity for both spatially and temporally adjacent item pairs ⁹. Still, the CA23DG showed pattern separation effects when both spatial and temporal information was correctly retrieved ⁵⁰. Another study reported that CA1 showed greater pattern similarity for trials that shared the same episodic context as compared to those with different episodic contexts, whereas CA23DG showed the opposite pattern ⁵¹. Clearly, future studies are required to examine in greater detail when hippocampal representations match temporal and spatial distances and when they disentangle these distances via processes of pattern separation.

Integration of multiple representational formats supports temporal order memory

The current studies revealed rich spatial and temporal sequence coding in the hippocampus in a single memory task. A further question is how these representations are integrated to support episodic-like temporal order memory. According to the cognitive map theory, the spatial representational formats of the hippocampal formation can support flexible cognition and behavior, including episodic memory ^{52, 53}. Accordingly, it has been found that the same neurons represent information about both space and time ⁵⁴. This suggests that the two dimensions might be integrated into a common coding scheme of spatiotemporal proximity in the hippocampus, supporting the formation of hierarchical structures in a memory space ^{55, 56, 57}. Human fMRI studies found that the spatial and temporal aspects of autobiographical experiences are coded within the hippocampus across various scales of magnitude, up to one month in time and 30 km in space ¹³. After learning spatio-temporal trajectories in a large-scale virtual city, subject-specific neural similarity in the hippocampus scales with the remembered proximity of events in space and time ⁹. The joint coding of space and temporal context found in CA1 in the current study further suggests that spatial information can be combined with temporal contexts in order to support temporal order memory.

Beyond the representation of space and time, our study further suggests that the learnt structured event sequence may serve as a scaffold for the organization of temporal memory. In addition, a previous study showed that spatial and episodic information might be separately represented in different CA1 neurons, suggesting that in addition to place cells, other hippocampal neurons are involved to support episodic context coding ⁵⁸. Finally, although the MOL might not be commonly used, it represents just one

particularly prominent case of learnt structured knowledge – i.e., schemas – which have been consistently shown to be combined with learned material to facilitate episodic memory^{59,60}. This could be achieved via interactions between hippocampus and ventromedial prefrontal cortex^{61,62}. Interestingly, we also found significant representations of learnt words in the vmPFC during encoding. All these rich representations might be integrated with the ongoing hippocampal oscillation to form an integrated sequence of neuronal activities that shapes our temporal order memory⁷. Future studies should uncover the intricate interaction of externally driven and internally organized sequences (that are influenced by long-term learning) in shaping our experiences and memories. In particular, researchers could try to translate our paradigm into rodent or primate studies to uncover the neuronal mechanisms.

To summarize, using MOL to strongly engage the hippocampus, the current study uncovered rich and multiplexed spatial, temporal and structured sequence representations in the hippocampus. Through spatial and sequential pattern separation, the hippocampus generates robust and distinctive episodic contexts that are linked to the learnt material. This provides a novel neural mechanism by which humans can achieve precise temporal order memory.

Methods

Participants

Twenty-nine college students (10 males; ages 18–24 years, mean age = 20.3 years) participated in this experiment. All of them were healthy, had normal or corrected-to-normal vision and no history of psychiatric or neurological diseases. None of them had experience or practice with the method of loci (MOL) before the experiment. Two additional participants were also recruited but removed from final analysis (one did not finish the scan session and one had extremely low memory performance with less than 2% remembered trials). Written consent was obtained from each participant after a full explanation of the study procedure. The study was approved by the Institutional Review Boards at Beijing Normal University and the Center for MRI Research at Peking University.

Materials

The stimuli consisted of 270 two-character Chinese words that were randomly divided into 9 lists of 30 words each. Sixty words were used in baseline test (2 lists), 150 in practice sessions (5 lists) and 60 in the scanning session (2 lists). All words describe common objects (e.g., broom), animals (e.g., crow), fruits (e.g., lemon), or vegetables (e.g., onion). The same word lists were used in each session and the orders of the words were randomized across subjects.

Experimental procedure

Subjects completed five experimental sessions across five consecutive days (Fig. 1A). On Day 1, they finished two rounds of the temporal order task without using the MOL, which served as the baseline. Immediately after the task, they were trained with the MOL mnemonic by watching a two-hour video

developed by our lab (see below). On Day 2, they were asked to review the method and to apply it in the subsequent temporal order task. Only one round of the temporal order memory task was administered (Practice 1). On Days 3 and 4, they finished two rounds of the temporal order task each day (i.e., Practice 2 and Practice 3) to further improve their MOL skills. On Day 5, they completed two rounds of the temporal order task in the scanner.

Method of loci

The MOL uses visualizations of familiar spatial information to scaffold the memorization of a sequence of other information like words or objects. In short, it is carried out by mentally placing each to-be-remembered item into one specific familiar location, creating vivid mental associations between physical locations and the items. In the present experiment, the same locations (i.e., a world map with 10 landmarks) were introduced for all subjects to let them follow an identical memory route. During the two-hour video practice session, they were asked to remember the order of the 10 locations and learn to vividly associate the to-be-remembered words with the locations, e.g., “the Statue of Liberty-apple”. They were free to play back the videos and ask questions about the strategies. We also asked the subjects to explain the method in their own words to make sure they correctly understood. Before each practice session and the scanning session, subjects were asked to rehearse the main points of MOL and wrote down the names of the 10 locations in correct order. All subjects performed three practice sessions and reported they could properly use the strategy before scanning.

Temporal order task

Each temporal order task consisted of an encoding task and a retrieval task. The same slow event-related design (14 s for each trial) was used for the baseline test, the practice sessions, and the scanning session, and for both encoding and retrieval tasks. During encoding, a fixation cross was presented for 500 ms, followed by the two-character Chinese word for 2 s. Another fixation was presented for 4 s during which subjects associated the word with the correct location along the path. After that, they performed a perceptual judgment task for 7.5 s. A Gabor image tilting 45° to the left or the right was presented on the screen, and subjects were asked to identify the orientation of the Gabor by pressing one of two buttons as quickly and accurately as possible. The next Gabor started 100 ms after subjects responded. Subjects learnt 30 words in each encoding run, which lasted 7 min.

Subjects performed a 6-min working memory task between encoding and retrieval. During baseline test and fMRI scan, a spatial working memory task was used. Each trial started with a 2 s encoding phase, during which 3 to 6 dots were sequentially displayed for 250 ms on different positions of an (invisible) 5 × 5 checker board. After an 8 s delay, a probe was presented and subjects were asked to judge whether a dot had been presented in this location during encoding or not. A different working memory task (comprising of a 1-back task of one-digit numbers) was used in the three practice sessions so that subjects would not be over-trained on the spatial working memory task (which might confound the comparison of behavioral performance between baseline and fMRI scan).

During retrieval, each trial started with a fixation cross shown for 500 ms, followed by presentation of one of the studied words for 2 s. A scaled timeline was then presented on the screen for 4 s, and subjects were asked to indicate the exact temporal position of the word in the list (i.e., 1 to 30), by moving the red slider on the timeline with the “left” or “right” button. The slider would keep moving as long as the subjects held the button down. The initial position of the red slider was randomly selected, but was never the correct position for that word. The scale and slider would stay on screen for the whole 4 s period, and the last position of the slider was taken as the response. Subjects could press an “don’t know” button if they could not remember the word or its temporal order. Lack of responses were also categorized as “don’t know”. After the temporal order judgment, subjects performed the same perceptual judgment task as in the encoding task for 7.5 s. Each retrieval run also lasted 7 min. On days 1, 3, 4 and 5, subjects performed two rounds of encoding and retrieval tasks with different sets of 30 words, which were also separated by a working memory task. On day 2, the performed one round. The whole temporal order task lasted 46 min on days 1, 3, 4, and 5, and 20 min on day 2.

MRI acquisition

Imaging data were acquired on a 3.0 T Siemens Prisma MRI scanner with a 64-channel head-neck coil at the MRI Center at Peking University. High-resolution functional images were acquired using a simultaneous multi-slice EPI sequence (TR/TE/ θ = 2000 ms/30 ms/90°; FOV = 198 mm × 198 mm; matrix = 124 × 124; in-plane resolution = 1.6 × 1.6 mm; slice thickness = 1.6 mm; GRAPPA factor = 2; multi-band acceleration factor = 3). Ninety contiguous axial slices parallel to the AC-PC line were obtained to cover the whole cerebrum and partial cerebellum. A high-resolution structural image was acquired for the whole brain using a 3D, T1-weighted MPRAGE sequence (TR/TE/ θ = 2530 ms/2.98 ms/7°; FOV = 256 mm × 256 mm; matrix = 256 × 256; slice thickness = 1 mm; GRAPPA factor = 2). A high-resolution T2-weighted image was also acquired using a T2-SPACE sequence for hippocampus segmentation. The image plane was perpendicular to the long axis of the hippocampus and covered the whole MTL region (TR/TE/ θ = 13150 ms/82 ms/150°; FOV = 220 mm × 220 mm; matrix = 512 × 512; slice thickness = 1.5 mm; 60 slices). A field map was acquired for correction of magnetic field distortions using a Gradient Echo sequence (TR = 767 ms; θ = 60°; TE1/TE2 = 4.92 ms/7.38 ms; FOV = 198 mm × 198 mm; matrix = 124 × 124; slice thickness = 2 mm; 77 slices).

Image preprocessing

MRI data were first converted to Brain Imaging Data Structure (BIDS) format⁶³. The first 10 volumes before the task were automatically discarded by the scanner to allow for T1 stabilization. Image preprocessing was performed using FMRIPrep v1.4.0⁶⁴. Each T1 volume was corrected for intensity using N4BiasFieldCorrection⁶⁵ and skull-stripped using antsBrainExtraction.sh (OASIS template). Cortical surfaces were reconstructed using FreeSurfer v6.0.1⁶⁶. The T1 volume was then normalized to the ICBM 152 Nonlinear Asymmetrical template (version 2009c) through nonlinear registration with the ANTs v2.1.0⁶⁷. Functional data were slice time corrected using AFNI v16.2.07⁶⁸, motion-corrected using FSL’s MCFLIRT⁶⁹, and registered to the T1 image using a boundary-based registration with nine degrees of

freedom⁷⁰. For univariate analysis, data were spatially smoothed with a 6 mm full-width-at-half-maximum Gaussian kernel using FSL's SUSAN, filtered in the temporal domain using a nonlinear high-pass filter with a 100 s cutoff, and normalized to MNI Template space. For ROI analysis and representational similarity analysis, data were aligned to subjects' T1 image and kept at its native resolution. Slight spatial smoothing was applied to the data using a 1.6 mm full-width-at-half-maximum Gaussian kernel and filtered in the temporal domain using a nonlinear high-pass filter with a 100 s cutoff in order to obtain both high signal to noise ratio and anatomical specificity.

Hippocampal subfields segmentation

The hippocampus and surrounding medial temporal lobe areas were segmented into CA1, CA2, CA3, DG, EC and PHC using the automatic segmentation of hippocampal subfields (ASHS) toolbox with the UPenn atlas²⁵. Anatomical masks segmented by ASHS were coregistered to the functional image for further analyses. CA2, CA3 and DG were combined (i.e., CA23DG) because they could not be unambiguously distinguished. To further examine functional specificity of EC subregions, we segmented the EC into anterior-lateral EC (alEC) and posterior-medial EC (pmEC), using the masks from a previous publication²⁶. The masks were resampled and coregistered to each subject's functional space. They were then intersected with the EC masks generated by ASHS to improve segmentation precision. Finally, our ROIs included CA1, CA23DG, alEC, pmEC and PHC (1.6 mm³ resolution, numbers of voxels: CA1 = 683.93 ± 74.31; CA23DG = 440.76 ± 55.52; alEC = 93.45 ± 17.99; pmEC = 63.90 ± 13.00; PHC = 514.59 ± 83.70). Two experimenters visually verified the accuracy of the segmentations.

Univariate analysis

Models were constructed using the general linear model within the FILM (version 6.00) module of FSL. For both the encoding and retrieval tasks, two types of trials were modeled: remembered and forgotten trials. We excluded trials with a temporal error of 1 position, as it was unclear whether these errors were due to inaccurate memory or response errors. The "unsure" trials and orientation trials from both encoding and retrieval were modeled as regressors of no interest. The above regressors were convolved with a double gamma hemodynamic response function. Six movement parameters and the frame-wise displacement (FD) were modeled as confound regressors. Additional censor regressors were included for each volume with a FD greater than 0.3 mm. Each run was modeled separately in the first level analysis. Cross-run averages for each contrast image were created for each subject using a fixed-effects model. These contrast images were then used for group analyses with a random-effects model. Group images were thresholded using cluster detection statistics, with a height threshold of $z > 2.3$ and a cluster probability of $P < 0.05$, corrected for whole-brain multiple comparisons using Gaussian Random Field Theory.

Region-of-Interest (ROI) analysis

We conducted percent signal changes analysis within the predefined ROIs in subject's native space. Parameter estimates (β values) of remembered and forgotten trials from the GLM were each averaged across all voxels in a given ROI for each subject. Percent signal changes were calculated using the

following formula: $(\beta / \text{mean}) \times \text{ppheight} \times 100\%$, where ppheight is the peak height of the hemodynamic response versus the baseline level of activity ⁷¹.

Single-trial response estimations

Single-trial response estimations were done using the least-square separate method for each functional run ⁷². Each trial was estimated in a separate GLM, in which the given trial was modeled as a separate regressor whereas all the remaining trials were modeled as another regressor. The six movement parameters and FD were included in each GLM as confound regressors. Additional censor regressors were included for each volume with a FD greater than 0.3 mm. This resulted in a single β image for each trial, which was used for the representational similarity analysis.

Representational similarity analysis (RSA)

RSA ⁷³ was used to determine the neural pattern similarity between trial pairs from different conditions. The neural pattern similarities could be calculated between encoding-encoding, retrieval-retrieval, or encoding-retrieval tasks, using images from the same or different runs. The main analysis focused on examining similarities within predefined ROIs (CA1, CA23DG, aLEC, pmEC and PHC). Each trial's β values from the single-trial response estimations within the ROI were extracted. The Fisher Z-transformed Pearson's correlations across trials were used as the index of neural pattern similarity. These similarities were then grouped and averaged based on the spatial location, sequence distance, and temporal distance for further statistical analysis. We used all trials for the encoding task but only included remembered trials for the retrieval task to make sure the correct context representations were reinstated. All trials during retrieval were used when correlating the representations with behavioral performance. To test group-level significance, paired t-tests or repeated measures ANOVAs were used (see below).

In order to examine the effect beyond our predefined ROIs, a whole brain searchlight analysis was conducted ⁷⁴. Each searchlight was defined as a spherical cluster in subject's native space with a radius of 3 voxels (4.8 mm, 123 voxels in total) surrounding a target voxel. The neural pattern similarity difference of interest was calculated within the sphere and assigned to the center voxel. All contrast maps of all subjects were transformed into MNI space and entered into group analysis using non-parametric permutation for inference on the statistical map. Non-parametric permutations were conducted by Randomise in FSL with 5,000 permutations. The significance of the derived statistical map was determined by the threshold-free cluster enhancement (TFCE) algorithm with $P < 0.05$ (whole brain FWE corrected) ⁷⁵.

Representational connectivity analysis

To examine representational connectivity, we generated the cross-run representational similarity matrix (RSM) ⁷³ (30×30) for each ROI and each processing stage (encoding and retrieval) (Fig. S7). We then correlated the RSMs from different regions within each processing stage (e.g., CA1 and PHC) or from different processing stages within the same region (CA1 encoding and CA1 retrieval) ⁷⁶. To test for group-level significance, non-parametric permutations were conducted by shuffling one of the representational

similarity patterns in a given RSM pair, e.g., PHC and CA1. Permutations were conducted 5,000 times and averaged to obtain the baseline correlation coefficient under the null hypothesis for each ROI pair and each subject. The p-value was obtained by paired t-tests between the original connectivity value and the baseline across subjects.

The representation of semantic information in the brain

To control the reorientations of word semantics, we conducted latent semantic analysis to generate a semantic similarity matrix of the words in the scanning session. For each word, its semantic features (i.e., word vectors) were extracted from a well-trained Chinese word embedding model, Directional Skip-Gram²⁷. In this model, each word vector consists of 200 values, and each value indicates the meaning of the word in one semantic dimension. The semantic similarities of the 60 words were estimated by calculating the Pearson correlation of these word vectors, resulting in a 60 by 60 semantic similarity matrix. The semantic similarity matrix was then correlated with the neural representational similarity patterns in hippocampal subfields and cortical regions using Pearson correlation. Based on previous studies, we focused on the following cortical regions that are related to episodic memory of words: inferior parietal lobule (IPL), superior parietal lobule (SPL), retrosplenial cortex (RSC), parahippocampal gyrus (PHG), medial temporal gyrus (MTG), inferior temporal gyrus (ITG), temporal pole (TP), lateral prefrontal cortex (IPFC), ventromedial prefrontal cortex (vmPFC), occipito-temporal cortex (OTC) (Fig. S9D). The IPL consisted of supramarginal gyrus and angular gyrus. For each subject, their individual cortical surface masks were extracted based on the Destrieux Atlas using FreeSurfer software⁷⁷.

We used a permutation test to assess group-level significance. During each permutation, we shuffled the orders of the words and generated a new 60 by 60 semantic similarity matrix, which was then correlated with the neural similarity matrix. The permutation was done 5,000 time for each region and each subject, and the value across the 5,000 permutations were averaged to form a baseline. A paired t-test was then conducted for each region to determine a group-level p-value. Results were shown in supplemental materials (Fig. S9).

Statistical analysis

All paired t-tests and repeated measures ANOVAs in our analysis were two-tailed and conducted by the afex package using type III sums of squares and using Greenhouse–Geisser correction to correct the degrees of freedom if necessary in R 3.6.1. Error bars in figures denote within-subject errors that account for heterogeneity of variance. FDR correction was performed to correct for multiple comparisons across the multiple ROIs.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Code availability

The code used to collect and analyze data from this study is available from the corresponding author upon reasonable request.

Declarations

Author contributions:

Conceptualization, G.X.; Methodology, G.X. and C. L., and Z.Y.; Software, C. L. and Z.Y.; Formal Analysis, G.X. and C. L., and Z.Y.; Investigation, Z.Y.; Resources G.X. and Z.Y.; Writing – Original Draft, G.X. and C. L.; Writing – Review & Editing, G.X., C.C and N. A.; Visualization, C. L; Supervision, G.X.; Funding Acquisition, G.X.

Competing interests:

The authors declare no competing interests.

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Figures

Figure 1

The MOL and experimental design. (A) Subjects completed five sessions on five consecutive days. On Day 1, they conducted a baseline test (two rounds of the temporal order memory task) and studied the MOL video for two hours. On Day 2, they reviewed the MOL method and practiced one round of the temporal order memory task. On Days 3 and 4 and during the fMRI scanning, they finished two rounds of the temporal order memory task. (B) Subjects were trained to remember a sequence of locations and then asked to make associations between these locations and the to-be-learned words. The same locations and route were used for all subjects. (C) During encoding, subjects studied a list of 30 words by mentally associating them with 10 well-trained locations, which was not presented to the subjects. Each word was

presented for 2 s, followed by a 4 s fixation cross. Subjects had 6 s to encode the word. During retrieval, a studied word was presented for 2 s and subjects were asked to indicate its exact temporal position in the list, by moving the red slider on the timeline with the “left” or “right” button in 4 s. Subjects could press the “don’t know” button if they could not remember the word or its list position. A slow event-related design (14 s/trial) was used for the encoding and retrieval phases. To prevent subjects from further processing the word, subjects performed a 7.5-s perceptual orientation judgment task after encoding or retrieval each word. The encoding-retrieval cycle was conducted twice with 30 different words in each run.

Figure 2

Behavioral results during the fMRI scan. (A) Proportions of remembered, forgotten, and unsure trials during fMRI scanning. The dashed line shows the chance level (3.33%). (B) Mean accuracy for each location. (C) Mean accuracy for each set. There was a significant fan or load effect, i.e., memory performance decreased when more items were associated with the same location. (D) Mean accuracy for different serial positions of the locations (Initial: locations 1 to 4; Middle: locations 5 to 7; Final: locations 8 to 10). There were significant recency and primacy effects across the 10 locations. Each dot represents one subject and the bars represent group means. Error bars indicate averaged within-subject errors. ** $P < 0.01$. *** $P < 0.001$. (E) Pattern of response errors. Most errors occurred between adjacent locations, followed by within-loci swap errors, as indicated by the red arrows.

Figure 3

ROIs segmentation and univariate effects of temporal memory. (A) Segmentation of hippocampus and adjacent medial temporal lobe areas. The top two panels show an example of segmentation from one subject, which was overlaid onto the subject's T2 image (coronal plane). The hippocampus and surrounding medial temporal lobe areas were segmented into CA1, CA2, CA3, DG, EC and PHC using ASHS 25. CA2, CA3 and DG were combined because they could not be unambiguously distinguished. The bottom panel shows an example of an EC segment, which was overlaid onto the subject's T1 image (sagittal plane). The EC was segmented into anterior lateral EC (aIEC) and posterior medial EC (pmEC), based on published masks 26. The masks were resampled and coregistered to each subject's native space, and were further intersected with the EC mask generated by ASHS to improve precision. (B) Subsequent memory effects (remembered > forgotten). (C) Retrieval success effects (remembered > forgotten). Each dot represents one subject and the bars represent group means. Error bars indicate averaged within-subject errors. * $P < 0.05$ uncorrected. ** $P < 0.01$ uncorrected.

Figure 4

Hippocampal representations of structured location sequences during encoding and retrieval. (A) Schematic depiction of word pairs depending on the distance of the associated MOL locations. Words in a pair are always from different runs. Same: same-location pairs; Near: near-distance pairs (ordinal distance = 1); Middle: middle-distance pairs ($2 \leq \text{ordinal distance} \leq 3$); Far: far-distance pairs (ordinal distance > 3). (B) Spatial pattern separation in PHC: higher similarity for same-location than near-distance pairs during encoding, but reversed pattern during retrieval. (C) Spatial pattern separation in CA1, but not CA23DG. Each line represents one subject and the bars represent the group means. (D) Sequence pattern separation in CA23DG, but not CA1. The CA23DG region showed higher pattern similarity for far-distance pairs than near- and middle-distance pairs. Each dot represents one subject and the bars represent the group means. Error bars indicate averaged within-subject errors. * $P < 0.05$. ** $P < 0.01$.

Figure 5

Sequence boundary representation during encoding. (A) Schematic depiction of within-boundary and cross-boundary word pairs. The black lines show the boundaries of the location sequence. (B) Pattern similarity for within-boundary and cross-boundary pairs in CA1 and CA23DG, showing higher pattern similarity for within-boundary pairs than cross-boundary pairs. Each line represents one subject and the bars represent the group means. Error bars indicate averaged within-subject errors. ** $P < 0.01$. *** $P < 0.001$.

Figure 6

Temporal context reinstatement during retrieval. (A) Schematic depiction of temporal distance during encoding. For all pairs of trials with a given temporal distance during retrieval (TDr, ranging from 1 to 20),

they could be grouped, based on their temporal distance during encoding (TDe), into short-distance ($TDe \leq 3$) or long-distance ($4 \leq TDe \leq 6$) pairs. The pattern similarity for each temporal distance during retrieval was first averaged, and then averaged across all temporal distances. (B) Pattern similarity for short and long TDe pairs during retrieval. The CA1 area showed greater pattern similarity for short-distance pairs than long-distance pairs. Each line represents one subject and the bars represent the group means. Error bars indicate averaged within-subject errors. * $P < 0.05$.

Supplementary Files

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- [SupplementalMaterials.docx](#)