Hippocampus activations reflect temporal contexts while medial prefrontal cortex activations reflect ordinal positions during sequence memory in humans

Running Head: SEQUENCE MEMORY REPRESENTATIONS IN THE HUMAN BRAIN

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ABSTRACT

Sequence memory is a core function of the hippocampus (HC) and medial prefrontal cortex (mPFC), but their distinct contributions remain unclear. One possibility is that HC and mPFC represent sequences with temporal contexts and ordinal representations, respectively. To test the unique contributions of the HC and mPFC to sequence memory, we used fMRI while participants performed a sequence memory task suited to investigate underlying representations. After studying sequences, participants indicated whether items were in sequence (InSeq) or out of sequence (OutSeq). In our task, temporal context memory (TCM) predicts graded behavioral performance as a function of temporal contiguity. Thus, we predicted graded impairments across lag distance for items that skipped ahead in the sequence (Skips, e.g., AB<u>D</u>), and that short lags would take the biggest hit. Consistent with TCM, subjects found it difficult to identify OutSeq items that skipped ahead short distances compared to longer distances. We found that the right anterior HC tracked forward lag distance, providing evidence that HC supports sequence memory with temporal contexts. To test ordinal representations, we used items that were transferred from one sequence to another, but retained their position (Ordinal Transfers; e.g., AB<u>3</u>). Ordinal Transfers proved to be more difficult than the other probe types but were detected above chance levels. We found mPFC activity was strongest when Ordinal Transfers were incorrectly identified as InSeq, suggesting mPFC concomitantly codes positions during sequence memory.

INTRODUCTION

The hippocampus (HC) and medial prefrontal cortex (mPFC) are integral to sequence memory, however, their precise contributions remain an open question. It is thought that temporal organization is a defining feature of memory which helps to arrange events into distinct episodes (Long & Kahana, 2018; Eichenbaum, 2017; Howard & Eichenbaum, 2013; Allen & Fortin, 2013; Tulving, 2002). While many of our experiences occur in the same places (e.g., our home), with the same objects (e.g., our dining table and TV), the temporal aspects of an event are unique. Memory for sequences of events is impaired in typical aging (Allen et al., 2015) and in disease, including Alzheimer's disease (Belassen et al., 2012) and schizophrenia (Polyn et al., 2015). Elucidating the functional contributions of the HC and mPFC to sequence memory will aid in our overall understanding of memory mechanisms, and our understanding of disorders that impact the temporal organization of memory.

The HC plays an important role in the neurobiology of sequence memory. HC lesions in rodents impair sequence memory, but not simple recognition memory (DeVito et al., 2011; Feinberg et al., 2011; Fortin et al., 2002; Kesner et al., 2002). CA1 neurons show item-specific sequential temporal firing patterns dubbed "time cells" (MacDonald et al., 2013; 2011; Pastolkova er al., 2009), place cells retain experiential order during replay (e.g., Wikenheiser and Redish, 2015; Dragoi & Buzsáki, 2006; Foster & Wilson, 2006), and CA1 neurons are sensitive to sequence memory violations (Allen et al., 2016). Likewise, human fMRI studies show that HC activations are related to temporal order memory (Hsieh et al., 2015; 2014; Kalm et al, 2013; Tubridy & Davachi, 2011; Jenkins & Ranganath, 2010; Lehn et al., 2009; Ross et al., 2009; Ekstrom & Bookheimer, 2007; Kumaran & Maguire, 2006), electrical stimulation in the human HC is sufficient to impair temporal memory (Goyal et al., 2018), and neuronal activity in the human medial temporal lobe reflects temporal contexts (Howard et al., 2012). But to understand the neurobiology of sequence memory further, we have to look to the larger network of brain structures.

mPFC also has an important role in sequence memory. Like HC lesions, mPFC lesions impair sequence memory (DeVito et al., 2011), mPFC neurons playback event sequences (Euston et al.,

2007), and time cells have been found in mPFC (Tiganj et al., 2017). Additionally, activations in human mPFC are sensitive to temporal order memory, and an HC-mPFC system shows robust functional connectivity during sequence memory (Hsieh & Ranganath, 2015; Preston & Eichenbaum, 2013). Based on this, and other work, it's thought that an HC-mPFC system underlies memory for sequences of events in the mammalian brain (Eichenbaum, 2017; Allen & Fortin, 2013). However, the distinct contributions of these two brain regions to coding sequences in memory remains unclear.

One possibility is that HC codes for temporal contexts while mPFC codes sequences with ordinal representations. To test these distinct roles in HC and mPFC, we measured BOLD fMRI while participants performed a sequence memory task tailored to investigate the underlying representations (Allen et al., 2016; 2015; 2014). Specifically, the sequence memory task presents participants with an unfolding sequence of events one item at a time. Participants must decide whether individual items are presented "in sequence" (InSeq; expected) or "out of sequence" (OutSeq; unexpected), based on memory for sequences they experienced during the study phase. By testing sequence memory in this way, we can present different types of out of sequence probe trials that place distinctive demands on temporal contexts and ordinal representations (Allen et al., 2014; Orlov et al., 2000).

We hypothesized that BOLD activations in HC would reflect item-specific associations via temporal contexts. By contrast, we hypothesized that BOLD activations in mPFC would reflect ordinal representations (1st, 2nd, 3rd, etc.). To test for temporal contexts, we looked at the probe trials with items that skipped ahead in the sequence (e.g., item "D" in AB<u>D</u>DEF) across different lag distances (+1 to +4). We predicted that subjects would find it more difficult to identify items that skipped ahead only a short distance (+1), compared to longer distances (+2, +3, +4), and that the BOLD activation patterns in HC would correlate with these forward lags. Theoretically, this reflects a decrease in the probability of retrieving items that occurred at longer temporal distances from each other (Kragel et al., 2015; Roberts et al., 2014; Howard & Kahana, 2002). For example, the probability of retrieval, after experiencing item "A", would be highest for the next item "B" (correct retrieval), and decrease across the remaining items "C-D-E-F" (incorrect retrievals). We found that activity in the right anterior HC tracked forward lag

distance, providing evidence that the HC supports sequence memory with temporal contexts. Next, we looked at ordinal representations by using probes in which an item was transferred from one sequence to another, but retained its ordinal position (e.g., item “2” in A2CDEF). If ordinal representations are used, (e.g., A-1, B-2, C-3, etc.) then this type of probe trial would be difficult to detect, but if temporal contexts are used, then this type of probe trial would be easy to identify as out of sequence (since “2” never occurred in sequence ABCDEF). First, we found that these probe trials were the most difficult for participants, but that their performance was better than chance levels. This behavioral pattern indicates that both types of representations are concomitant during the task. Importantly, we found mPFC activity was strongest when these trials were not detected (incorrectly treated as InSeq), suggesting mPFC codes ordinal representations during sequence memory.

The results of this study advance long standing questions about how the HC-mPFC system supports the temporal organization of memory by demonstrating that both temporal contexts and ordinal representations are concurrently engaged during sequence memory, and these representations are subserved by activity in HC and mPFC, respectively. We briefly discuss how these two representations might interact in support of episodic memory.

METHODS

Overview. We tested participants using a previously established sequence memory task (Allen et al., 2014). The task was designed to investigate the ability of humans to learn and remember arbitrary sequences of items. During task performance, BOLD fMRI data were collected.

Participants. Thirty-nine students were recruited from Florida International University (FIU). Participants were scanned during two different cognitive tasks (presented in counterbalanced order). Only the sequence memory task was analyzed and reported here. Participants were paid $20/hr, with sessions typically lasting ~2hrs. Of the 39 participants, five were excluded for failing to complete the sequence task (n = 1), or not demonstrating basic task competency (*G*-tests with $p < .05$, see below; n = 4). The remaining participants (n = 34) were composed of 56% female ($n = 19$) and 44% male ($n =$

15), with a mean age of 21 years (*SD* = 2). All procedures were completed in accordance with the FIU IRB.

Task Apparatus. The task was run on a Dell computer using Matlab (2015b) with custom scripts that included functions from Psychtoolbox (Psychtoolbox-3 distribution; http://www.psychtoolbox.org). Graphical images were back projected and viewed by participants with an angled mirror mounted on the head coil. Responses were recorded using a Current Designs MR-compatible 4-button inline response device (https://www.curdes.com).

Task Design. Participants were given scripted instructions. All participants began with a practice to become acquainted with the structure of the task. During the practice, participants viewed four easily predictable sequence sets each composed of six unique images (e.g., Fig1B, top, a black arrow that rotated clockwise in 60º steps). Each image in a sequence was presented one at a time in the center of the screen. The practice consisted of a study phase when the sequences were passively viewed, and participants were instructed to remember the sequences. The test phase was self-paced and required participant responses to advance. The task paused between sequences and the words “Press the button to begin” would appear. Participants were instructed to determine whether images were presented InSeq or OutSeq.

Following the practice participants began the experiment. During the experiment six total sequences were presented: two sequence sets of the easily predictable sequences mentioned before and four novel arbitrary sequences made of fractal images (Fig1B, bottom). The exact composition of the fractal sequence sets was different for each participant. Sets were selected randomly, without replacement, from a bank of 240 unique fractal images. For simplicity, we call the two easily predictable sequences the “low memory demand” sets, and the fractal image sequences the “high memory demand” sets. The experiment began like the practice, with a study phase followed by a test phase. During the test phase, each sequence was presented 40 times (for a total of 240 sequence presentations). Sequence sets were psuedorandomly interleaved and had the following two rules: (1) the first six sequences during the test phase was always Set 1 through Set 6 with all items presented

InSeq (i.e., in the same order as during the study phase); (2) in the first half of the test phase (the first 120 sequence sets), only Repeats and Skips (description below) could serve as the OutSeq probe trials. In the second half of the test phase (the last 120 sequence sets) all three OutSeq probe trials occurred including Repeats, Skips, and Ordinal Transfers. To initiate an image in the sequence, participants were required to press and hold the button and release it relative to a 1sec decision threshold. If the image was InSeq, participants were instructed to hold down the button until the image disappeared on its own at 1sec (Fig1C). If the item was OutSeq, participants were instructed to release the button prior to the decision threshold (<1sec), at which point the image would disappear upon button release (Fig1D). Both accuracy and response times (RT) were recorded for each image. Exactly half of the sequences had one image OutSeq. InSeq and OutSeq sets were randomly presented throughout the test phase. To provide participants a rest (<1min), the test phase was performed in blocks that timed out after 15mins of continuous performance. Thus, the number of testing blocks varied for each participant dependent on their pacing, but for 76.471% of participants there were four blocks, the remaining had either three or five.

OutSeq Probe trials. Three distinct types of OutSeq probe trials were used during the test phase: Repeats, Skips, and Ordinal Transfers. The OutSeq probe types were counterbalanced across sequence sets. OutSeq probes were never presented in the first position (Pos1). The proportions of OutSeq probe trials across ordinal positions (Pos2 through Pos6) were distributed as follows: Pos1, 0%; Pos2, 15%; Pos3, 35%; Pos4, 50%; Pos5, 35%; Pos6, 15%.

Repeats occurred when an earlier item was repeated late in the sequence (e.g., ABCD<u>B</u>F). Repeats could be identified using multiple cognitive processes including working memory (WM) (e.g., in sequence ABC<u>A</u>EF, “A has already been presented”), temporal context memory (TCM) (e.g., “C does not lead to A”), or using ordinal representations (e.g., “A does not occur in the 5th position”). Repeats can be presented at different lag distances, ranging from a lag of -2 (e.g., ABC<u>B</u>EF is a repeat of an item 2 positions back in the sequence) to a lag of -5 (e.g., ABCDE<u>A</u> is a repeat of an item 5 positions

back in the sequence). A reverse lag distance of -1 was not used here because of the lack of an intervening item.

Skips occurred when an item was presented too early in the sequence (e.g., ABEDEF). These probe trials place a high demand on TCM because participants must anticipate the exact upcoming item and identify any items as OutSeq that are expected in a later position. Skips are presented with different forward lag distances ranging from a forward lag of +1 (e.g., ABCDFF is skipping one ahead) to a forward lag of +4 (e.g., AFCDEF is skipping 4 ahead).

Ordinal Transfers occurred when an item from one sequence (e.g., UVWXYZ) was transferred to another sequence (e.g., ABCDEF) while retaining its ordinal position (e.g., ABCDYF in which Y is presented in the 5th position it normally occupies, however, Y doesn't normally follow D; Fig4B). Response patterns to Ordinal Transfers parse the type of sequential representation supporting task performance including item-based temporal context associations and position-based associations (ordinal representations). Identifying Ordinal Transfers as OutSeq is indicative of item-based temporal context associations (i.e., E follows D, not Y), while chance accuracy levels are indicative of position-based associations (i.e., Y correctly occurred in the 5th position). Ordinal Transfers were evenly presented across positions (2 thru 6).

Sequence Memory Analysis. To examine whether participants demonstrated sequence memory, we compared observed and expected frequencies of InSeq and OutSeq responses using *G*-tests (Sokal and Rohlf, 1995). Responses to each item were sorted into a 2 x 2 matrix based on accuracy (correct/incorrect) and sequence condition (InSeq/OutSeq). For this analysis we only included the "high memory" sequence sets, because the "low memory" sequences were very easy and can be rule-based. Low memory sequences were included in the task, in part, simply to keep participants on task throughout the test phase. Responses to the first item of each sequence were excluded from analysis because those were always InSeq (there's no decision to make). Ordinal Transfers were also excluded from these tests since they were used to parse strategy.

To examine the overall sequence memory performance, we used a summary statistic called the Sequence Memory Index (SMI), calculated according to Equation 1 (Allen et al., 2015; 2014). The parameters of Equation 1 are as follows: Incor = InSeq correct, Ininc = InSeq incorrect, Outcor = OutSeq correct, and Outinc = OutSeq incorrect. SMI values can range between -1 and 1, where 1 represents perfect performance in the sequence memory task (a participant would have RT > 1s for all InSeq items and <1s for all OutSeq items), and zero represents chance performance. Note that an SMI score of -1 would reflect responding to all items (InSeq and OutSeq) perfectly incorrectly. Negative SMIs rarely occur, and when they do are very nearly zero. SMIs were also calculated for the "low memory" sequences to examine basic task competency.

Equation 1. $$SMI = \frac{(0.9\text{Incor})(0.1\text{Outcor}) - (0.9\text{Ininc})(0.1\text{Outinc})}{\sqrt{(0.9\text{Incor}+0.9\text{Ininc})(0.1\text{Outcor}+0.1\text{Outinc})(0.9\text{Incor}+0.1\text{Outinc})(0.9\text{Ininc}+0.1\text{Outcor})}}$$

Lag Analysis. To evaluate theoretical processes that could support performance on Skips and Repeats we used a lag analysis. For Skips, we evaluated performance across the *n*-forward lag distances (*n*-forward lags: +1, +2, +3, and +4). Smaller *n*-forward lags occurred more often because there are more combinations available, inherent to using item transpositions in sequences. We compared each n-forward lag position performance (accuracy and SMI) to chance using a one-sample t-test. We also tested for different performance levels across lags using repeated-measures ANOVAs. If Mauchly's Test of Sphericity indicated that sphericity had been violated ($p < .05$), a Greenhouse-Geisser correction was used. SMI was recalculated using only Skips as the OutSeq trials. For Repeats, we evaluated performance (accuracy and SMI) across the *n*-back lags (*n*-back lags: -2, -3, -4, and -5). Smaller *n*-back lags occurred more often since there are more combinations available, inherent to using item transpositions in sequences. To compare performance on each *n*-back lag to chance, we used a one-sample t-test. To test for different performance levels across lags, we performed repeated-

measures ANOVAs using accuracy and SMI scores. SMI was recalculated using only Repeats as the OutSeq trials.

For Ordinal Transfers, we evaluated performance across ordinal positions (Pos2 thru Pos6). To test for different performance levels relative to chance and across positions, we performed one-sample t-tests and repeated-measures ANOVAs using accuracy and SMI scores. SMI was recalculated using only Ordinal Transfers as the OutSeq trials.

Neuroimaging Acquisition. Neuroimaging data were collected on a General Electric Discovery MR750 3.0T scanner using a 32-channel head coil at the University of Miami Neuroimaging Facility. Structural T1-weighted images were collected (186 slices, flip angle 12°; FOV = 256 mm, slice thickness = 1.0mm). Whole-brain blood oxygen level dependent (BOLD) echo-planar imaging (EPI) functional data (42 slices, interleaved, bottom-up; flip angle = 75°; TE = 25ms; TR = 2000ms; matrix = 96x96 mm; FOV = 240 mm, slice thickness = 3.0mm, voxel size = 2.5 mm^2) were collected during the task.

Neuroimaging preprocessing. Preprocessing was performed using a pipeline developed in Nipype v0.1 (Gorgolewski et al., 2011), capitalizing on tools from Analysis of Functional Neuroimages (AFNI v16.3.18; Cox, 1996), FSL (v5.0.10), FreeSurfer (v5.1.0; Dale et al., 1999), Advanced Normalization Tools (v2.2.0; Avants et al., 2008), and artifact detection (ART). Following dicom conversion, cortical surface reconstruction and cortical/subcortical segmentation was performed on all participants' T1-weighted structural scans. Each participants' resulting surface reconstruction was visually inspected and errors were manually edited and resubmitted to the *recon_all* queue. Functional data were first 'despiked' removing and replacing intensity outliers in the functional time series. Then the data were simultaneously slice-time and motion corrected (Roche, 2011). An affine transformation matrix was calculated during coregistration of the mean of each participants' functional scans to their structural scan using Freesurfer's boundary-based registration algorithm (bbregister). Brain masks were created by binarizing the aparc+aseg file and dilating it by one voxel. The resulting binary masks were subsequently coregistered to the functional data by applying the inverse of the affine coregistration

matrix. Motion and intensity outliers were then identified using the rapid art artifact detection tool as implemented in nipype. Time points that either exceed 3 standard deviations of the mean intensity or greater than 1mm of composite frame-wise displacement were flagged as outliers to serve as subsequent regressors of no interest in the first-level general linear models. Finally, functional data were spatially filtered with a 5mm FWHM gaussian kernel using the *SUSAN* algorithm (FSL), which preserves the underlying structure by only averaging local voxels with similar intensities.

Neuroimaging normalization. A study specific template was generated from the full sample. Each participants' structural scan was skull-stripped by multiplying the T1-weighted structural scan by the binarized and dialated aparc+aseg file in structural space. Each skull-stripped brain was then rigid-body transformed (no scaling or shearing) to Montreal Neurological Institute (MNI) space using FSL's *FLIRT* algorithm. This first pass was used to minimize large spatial shifts between participants and generate a template close to a commonly used reference. Following visual inspection, the study template was created using ANTS *buildtemplateparallel.sh* script. After template generation, each participants' skull-stripped brain was normalized using the non-linear symmetric diffeomorphic mapping implemented by ANTS. The resulting warps were applied to contrast parameter estimates following fixed-effects modeling for subsequent group-level tests.

Neuroimaging analysis. Functional data were analyzed according to a general linear model approach using FMRIB's Software Library (www.fmrib.ox.ac.uk/fsl). Two separate models were used for first-level analyses: 1) a lag model and 2) a performance (correct/incorrect) model. All first-level models included event and nuisance regressors. The lag analysis consisted of eight event regressors of interest: Repeats at different *n*-back lags (*n*-back lags: -2, -3, and -4), Skips at different *n*-forward lags (*n*-forward lags: +1, +2, and +3), and InSeq and Ordinal Transfers irrespective of behavioral performance. We evaluated linear changes in activation across lags with the following contrast weights: -1, 0, 1 and 1, 0, -1. We did not include a lag of -5 or +4 as there were not sufficient trials per participant. The performance analysis also included eight event regressors of interest: correct and incorrect InSeq, Repeats, Skips, and Ordinal Transfers. Contrasts examined differences in activation

across the detected and undetected probe trials. Event regressors were convolved with FSL's double gamma hemodynamic response function with an onset beginning at the first stimulus item of a sequence and a duration equal to the length of time required to evaluate all items in that sequence (Mean = 9.917s per subject; self-paced). Regressors for baseline or low memory sequences were included in both models. Nuisance regressors in both models included motion parameters (x, y, z translations; pitch, roll, yaw rotations), the first and second derivatives of the motion parameters, the normalized motion, first, second, and third order Lagrange polynomials, as well as each outlier timepoint that exceeded the artifact detection thresholds identified during preprocessing. Following the first-level analysis a fixed effects analysis across experimental runs was performed for each participant for the respective contrasts of interest (e.g., lag analysis: linear contrasts; performance analysis: correct versus incorrect probe trials). Contrast parameter estimates from the fixed effects analysis were normalized to the study specific template and group-level analyses were performed using FSL's *randomise* threshold-free cluster enhancement (tfce) one sample t-test. To test *a priori* hypotheses with respect to the functional contributions of the HPC and mPFC during sequence learning, we constrained our voxel-wise analyses at the group level to the bilateral medial temporal lobe and mPFC using a small volume correction procedure. Whole-brain exploratory analyses were used to follow up our directed tests. In the performance analysis, some participants were missing key events of interest (e.g., correct Ordinal Transfers or incorrect repeat probes). These participants were not included in the relevant analyses, reducing the sample size for the correct versus incorrect Ordinal Transfer contrasts to n = 26 and n = 31 for the correct versus incorrect repeat probe trial contrasts.

RESULTS

Sequence memory performance

We tested whether participants could perform the sequence memory task better than chance. We calculated SMI separately for the low and high memory demand sequences (Fig1E). Participants

were significantly better than chance on both the low memory sequences (SMI_{low}: 0.751 ± 0.173; $SMI_{low\ vs.\ chance}$: $t_{(33)} = 37.065$, $p = 3.487 \times 10^{-23}$) and the high memory sequences (SMI_{high}: 0.582 ± 0.170; $SMI_{low\ vs.\ chance}$: $t_{(33)} = 19.690$, $p = 5.391 \times 10^{-20}$). Low memory sequences had significantly better performance than the high memory sequence performance ($SMI_{low\ vs.\ high}$: $t_{(33)} = -4.839$, $p = 2.958 \times 10^{-5}$). Sequence memory (examined using the high memory sequences) was not significantly affected by task order ($SMI_{1st\ block} = 0.571 \pm 0.190$, $SMI_{2nd\ block} = 0.594 \pm 0.152$; $SMI_{1st\ vs.\ 2nd}$: $t_{(32)} = -0.386$, $p = 0.702$), or sex ($SMI_{female} = 0.566 \pm 0.197$, $SMI_{male} = 0.602 \pm 0.133$; $SMI_{female\ vs.\ male} = t_{(32)} = -0.612$, $p = 0.545$), thus these groups were combined throughout the rest of the results.

We examined overall performance on the three different memory probes types using percent correct for ease of interpretation (Repeats: 78.584 ± 2.853%; Skips: 49.205 ± 2.686%; and Ordinal Transfers 34.927 ± 4.120%). Overall, participants performed each of the three trial types significantly better than chance (Repeats: $t_{(33)} = 24.201$, $p = 1.403 \times 10^{-22}$; Skips: $t_{(33)} = 14.771$, $p = 4.203 \times 10^{-16}$; Ordinal Transfers: $t_{(33)} = 6.164$, $p = 5.951 \times 10^{-7}$). For each of these one-sample t-tests chance was set at 9.534%, which was determined by calculating the probability of releasing the button before 1sec assuming subjects had no knowledge of the sequential status of an item. That is, chance was defined as the complement to an assumed response bias to hold for >1sec on positions 2 thru 6, irrespective of the trial type. The same pattern of results was found using SMI, which also controls for individual response patterns. For all three probe types, learning was fairly rapid (asymptotic in a few trials), and performance was steady throughout the duration of the imaging experiment (Fig1F). Thus, performance in the task and the accompanying brain imaging data primarily reflects memory and the associated behavioral decisions.

Evidence for multiple cognitive processes in sequence memory as a function of lag direction and distance.

Successful performance on the sequence memory task can be supported by multiple cognitive processes including WM, TCM, and ordinal representations. Theoretically, WM and TCM predict

different performance patterns across the different lags (Fig2A), which we examined here parsing both lag direction and lag distance. However, ordinal representations would theoretically contribute to performance across lags equally and therefore cannot be discriminated using a lag analysis.

Probe trials that skipped over items in the sequence (*n*-forward e.g., the "D" in ABDDEF) afford the opportunity to evaluate predictions of a TCM-based process in rapid sequence decisions. Specifically, successful performance on Skips requires participants to have precise expectations for the next item in the sequence. Performance on Skips is thought to be supported by TCM. TCM predicts that the likelihood of retrieval is highest for the next item in the forward direction (lag distance = 0) and drops off (in a graded fashion) as the distance of the forward skip increases (from +1 to +4; Fig2B, blue, above the diagonal). This prediction stands in contrast to temporary traces in WM that cannot predict upcoming items at all (Fig2B, red above the diagonal). In this task, TCM makes the simple prediction that performance on Skip OutSeq probe trials should be poorest on the shortest forward lag distance (+1) and get better at longer distances (+2, +3, or +4) (Fig2A, right side). This predicted inverse in performance in our task, compared to free recall tasks, stems from the idea that TCM is more likely to retrieve items that appeared in closer temporal proximity (e.g., Kragel et al. 2015; Howard & Kahana, 2002). Skips with a short forward lag are more likely to be retrieved, thus we predicted that short forward lags would be more difficult than longer lags.

We tested this prediction by examining performance across specific n-forward lag distances. First, all forward lags were performed at better than chance level (+1: 0.420 ± 0.178; $+1_{vs.\ chance}$: $t_{(33)} = 10.609$, $p = 3.608 \times 10^{-12}$; +2: 0.665 ± 0.156; $+2_{vs.\ chance}$: $t_{(33)} = 21.299$, $p = 7.417 \times 10^{-21}$; +3: 0.747 ± 0.248; $+3_{vs.\ chance}$: $t_{(33)} = 15.343$, $p = 1.402 \times 10^{-16}$; +4: 0.765± 0.431; $+4_{vs.\ chance}$: $t_{(33)} = 9.066$, $p = 1.780 \times 10^{-10}$), suggesting TCM retrieval does not completely impair OutSeq determinations at any forward lag distance. Second, subjects exhibited graded improvement as the skip distance increased (Fig2C, blue bars; $F_{(3, 99)} = 13.790$, $p = 13.790 \times 10^{-5}$). Further, we found that Skips with a +1 lag had the lowest accuracy compared to all other forward lags (*post hoc* LSD: +2: $p = 1.218 \times 10^{-9}$, +3: $p = 1.248 \times 10^{-7}$, +4: $p = 1.186 \times 10^{-4}$). These results are consistent with our predictions based on TCM (Fig2A&C).

Performance on Skips with a +2 lag were significantly lower than +3 trials (*post hoc* LSD: $p = 0.039$). However, no significant difference in performance was observed when comparing skips of +2 and +4 (*post hoc* LSD: $p = 0.187$) and +3 to +4 (*post hoc* LSD: $p = 0.814$) suggesting performance was approaching an asymptote. These behavioral results suggest that the participants' sequence memory was, at least in part, driven by TCM.

Repeats. OutSeq trials that repeated an item from earlier in the sequence (*n*-back; e.g., the "A" in AB<u>A</u>DEF) can be solved by either TCM, WM, or a mixture of the two (Fig2A, left side). Notably, the pattern of performance as a function of *n*-back lag distance might differentiate the two competing processes. TCM predictions on Repeats (somewhat mirroring predictions with Skips) are that short backward lag distances (e.g., -2) would have the poorest performance, while further *n*-back lag distances (e.g. -5) would be less consequential (Fig2B, blue, below the diagonal). In contrast, WM predicts the opposite pattern because the most recently experienced items would be most accessible (Fig2B, red, below the diagonal).

We found that all *n*-back lag distances were performed better than chance (-2: 0.859 ± 0.116; $-2_{vs.\ chance}$: $t_{(33)} = 38.333$, $p = 6.058 \times 10^{-29}$; -3: 0.805 ± 0.171; $-3_{vs.\ chance}$: $t_{(33)} = 24.138$, $p = 1.522 \times 10^{-22}$; -4: 0.782 ± 0.233; $-4_{vs.\ chance}$: $t_{(33)} = 17.204$, $p = 4.821 \times 10^{-18}$; -5: 0.529 ± 0.507; $-5_{vs.\ chance}$: $t_{(33)} = 4.996$, $p = 4.996 \times 10^{-5}$). We also observed a decrease in accuracy as the *n*-back lag distance increased ($F_{(3, 99)} = 9.760$, $p = 0.001$) (Fig2C, purple bars), suggestive of WM contributions. Items with an *n*-back lag closest to their original position (e.g., ABC<u>B</u>EF; lag = -2) had the highest performance compared to all other *n*-back lag positions (*post hoc* LSD lag -2 compared to: lag -3: $p = 0.020$; lag -4: $p = 0.025$; lag -5: $p = 4.262 \times 10^{-4}$). In contrast, items with an *n*-back lag farthest away from their original position (e.g. ABCDE<u>A</u>; lag = -5) had the lowest accuracy compared to all other *n*-back lag positions (*post hoc* LSD lag -5 compared to: lag -4: $p = 0.009$; lag -3: $p = 0.005$; lag -2: $p = 4.262 \times 10^{-4}$). No significant differences in performance between -3 and -4 *n*-back lags were observed (*post hoc* LSD: $p = 0.532$). Taken together, performance at the *n*-back lag extremes (-2 and -5) support the notion that WM plays an important role in Repeats performance. The absence of a graded performance across *n*-back lags of

-3 and -4 lends support to the idea that some mixture of cognitive processes is occurring, but overall these analyses do not support TCM as an isolated process driving Repeats.

Overall accuracy, linear regression slopes, and variability in residuals suggest TCM supports Skips, but a mixture of cognitive processes best captures Repeats performance

Differences in overall accuracy and the residuals from a lag-based linear regression model can help further elucidate the contributions of TCM, WM, or their mixture during OutSeq probe trials. First, we predicted that overall performance on Repeats would be higher than Skips since TCM and WM complement each other (Fig2A, left, purple dash). Second, we predicted that a linear regression in Skips would positively increase across forward lags, whereas a similar analysis across *n*-back lags for Repeats would be essentially flat. Third, we predicted that the residuals from the linear regressions would be, perhaps counterintuitively, highest on Repeats compared to Skips reflecting increased performance variability. This is because Repeats can possibly be detected based on two cognitive processes (e.g., TCM and WM), whereas Skips involves a single process (e.g., TCM). To test these predictions, we compared overall accuracy between the two probe trial types, calculated a linear regression based on the *z*-scores (accounting for individual baseline performance levels) across different lag positions, and averaged the squared residuals as a measure of performance variability across lags. Participants were significantly more accurate at identifying Repeats ($M_{accuracy} = 0.786 \pm 0.166$) compared to Skips ($M_{accuracy} = 0.492 \pm 0.157$) ($t_{(33)} = 14.435$, $p = 8.137 \times 10^{-16}$). However, consistent with our hypotheses, linear regression in Skips positively increases across forward lags ($R^2 = 0.572$, $\beta = 0.757$, $p = 3.711 \times 10^{-20}$), whereas a linear regression across backward lags for Repeats had no significant slope ($R^2 = 0.024$, $\beta = 0.154$, $p = 0.139$). When quantifying the variability, Repeats ($M_{residual} = 0.593 \pm 0.243$) were significantly more variable in their performance across different lag positions when compared to the Skips ($M_{residual} = 0.285 \pm 0.478$) ($t_{(30)} = 3.051$, $p = 0.004$). The pattern in accuracy and variability further support the conclusion that multiple cognitive processes likely contribute to performance on Repeats while TCM underlies performance on Skips.

Activation in the HC consistent with TCM during Skips

Converging evidence supports the medial temporal lobe, specifically the HC formation, as playing a disproportionate role in TCM in the brain (Hsieh et al., 2014). To test whether regions of the medial temporal lobe contribute to TCM during Skips and Repeats we looked for linear changes in activation across the different lag distances (Fig3A). We observed a significant cluster in the right anterior HC following small volume corrections (bilateral MTL, FWE-tfce $p < 0.05$) that increased its activation across n-forward lags (Fig3B). Similar patterns of activation across n-back lags did not survive corrections for multiple comparisons (Fig3C). Further, following an anatomical ROI analysis of the bilateral anterior HC we observed greater activation for linear changes in Skips ($M = 0.678 \pm 1.421$) relative to Repeats ($M = -0.129 \pm 1.539$) when testing for patterns of BOLD activation ($t_{(33)} = -2.079$, $p = 0.045$, Fig3D). These results suggest that the right anterior HC contributes to TCM during Skips.

Performance during Ordinal Transfers demonstrates use of ordinal representation

Ordinal Transfers (e.g., A**2**CDEF) provide a unique opportunity to evaluate strategies in sequence memory behavior. If an ordinal representation is being used (e.g., A goes in the 1st position, B goes in the 2nd position, etc.) then this type of probe trial would be difficult to identify as OutSeq (e.g., 2 goes in the 2nd position). By contrast, if a TCM process was being employed, the ordinal probe would deviate from all item-based expectations and would be easy to identify as OutSeq. Moreover, if an ordinal representation was used, we would not expect there to be differences in accuracy as a function of transfer position. First, Ordinal Transfers were performed better than chance (Pos2: 0.314 ± 0.357; $Pos2_{vs.\ chance}$: $t_{(33)} = 3.566$, $p = 0.001$; Pos3: 0.338 ± 0.313; $Pos3_{vs.\ chance}$: $t_{(33)} = 4.512$, $p = 7.712 \times 10^{-5}$; Pos4: 0.347 ± 0.258; $Pos4_{vs.\ chance}$: $t_{(33)} = 5.670$, $p = 2.549 \times 10^{-6}$; Pos5: 0.354 ± 0.283; $Pos5_{vs.\ chance}$: $t_{(33)} = 5.334$, $p = 6.892 \times 10^{-6}$; Pos6: 0.373 ± 0.336; $Pos6_{vs.\ chance}$: $t_{(33)} = 4.811$, $p = 3.212 \times 10^{-5}$) and Repeats ($Pos2_{vs.\ Repeats}$: $t_{(33)} = -7.711$, $p = 7.019 \times 10^{-9}$; $Pos3_{vs.\ Repeats}$: $t_{(33)} = -8.338$, $p = 1.244 \times 10^{-9}$; $Pos4_{vs.\ Repeats}$: $t_{(33)} = -9.917$, $p = 1.996 \times 10^{-11}$; $Pos5_{vs.\ Repeats}$: $t_{(33)} = -8.919$, $p = 2.621 \times 10^{-10}$; $Pos6_{vs.\ Repeats}$: $t_{(33)} = -7.175$, $p = 3.185 \times 10^{-8}$) regardless of position (Fig4C). Consistent with our prediction, accuracy during Ordinal Transfers did not significantly differ across positions ($F_{(4, 132)} = 0.331$, $p = 0.786$), and showed high

variability between participants across positions ($M_{residual}$ = 0.795 ± 0.074). Thus, the observed behavioral performance suggests ordinal representations.

mPFC activations reflect ordinal representations within a sequence

If an ordinal representation was being used during the task, we hypothesized that Ordinal Transfers would be difficult to detect because the position association remained accurate, but the item itself was inaccurate. Thus, we predicted the use of an ordinal representation would be most evident in mPFC when contrasting incorrect versus correct trials. Consistent with our hypothesis, we observed activations throughout mPFC bilaterally (prelimbic cortex, anterior cingulate cortex, medial superior frontal gyrus) following the comparison of undetected (InSeq determinations) versus detected (OutSeq determinations) Ordinal Transfers (Fig4F; FWE-tfce $p < 0.05$). Next, we posited that Skips and Repeats would be correctly identified using an ordinal representation. Thus, we compared mPFC activity on correct versus incorrect trials for Skips and Repeats. No clusters survived corrections for multiple comparisons in mPFC when comparing correct versus incorrect Skips (Fig4G) and Repeats (not shown) (FWE-tfce $p > 0.05$). These results suggest that successful performance on Skips and Repeats was not wholly reliant on an ordinal representation in mPFC. We further show that clusters within mPFC did survive corrections for multiple comparisons when contrasting incorrect to correct Skips (FWE-tfce $p < 0.05$). However, these clusters were more laterally observed when performing a whole-brain exploratory analysis, while no significant clusters were found contrasting incorrect to correct Repeats (Supplementary Fig1, Supplementary Table 1). Lastly, we performed an anatomical ROI analysis across mPFC to further evaluate mPFC activity across the three probe types (Fig.3B). mPFC was significantly more active during Ordinal Transfers relative to both Skips and Repeats for contrasts that assumed an ordinal strategy ($F_{(2, 56)} = 4.038$, $p = 0.034$). Post hoc tests (Tukey HSD) supported the conclusion that mPFC is most active during the contrast comparing incorrect versus correct Ordinal Transfers (M = 0.708 ± 1.998) relative to correct versus incorrect Skips (M = -0.649 ± 1.445, $p = 0.051$) and Repeats (M = -0.431 ± 1.728, $p = 0.005$). No significant difference was identified in mPFC

activation between correct versus incorrect Skips and Repeats (p = 0.890). These results indicate that activation in mPFC reflects ordinal representations within a sequence memory.

DISCUSSION

Summary of main findings

In this study we used behavioral measures and BOLD fMRI to perform a detailed theoretical analysis of the underlying cognitive strategies and neurobiological representations in memory for sequences of events. First, we focused on a lag analysis of behavior on Repeats (e.g., AB<u>A</u>) and Skips (e.g., AB<u>D</u>). Theoretically, WM and TCM is distinguishable using a lag analysis on Repeats, but only TCM patterns are predicted on Skips. Behavioral performance matched predictions of TCM on Skips showing significantly better performance at farther, compared to closer, forward lags. Behavioral performance on Repeats fit more with a mixture of WM and TCM, as performance was, highly accurate, flat across lags, and highly variable. Second, we looked at BOLD fMRI in the medial temporal lobe using a lag analysis and predicted that activity would track lag distance, similar to behavior. Our results show that the right anterior HC had a significant positive slope across forward lags (Skips), but there was no correlation in activation with backward lags (Repeats) anywhere in the medial temporal lobe. Thus, we conclude that HC activations reflect TCM. Third, we focused on Ordinal Transfers (e.g., AB<u>3</u>) in behavior. Theoretically, sequence memory can be supported by ordinal representations (e.g., A-1, B-2, C-3, etc.), which we tested using an ordinal distractor. We found that Ordinal Transfers were most difficult, clear evidence for a positional coding because an InSeq (incorrect) determination on these trials can only be supported by the item's ordinal association. Fourth, we looked at BOLD fMRI in mPFC focusing on trials in which subjects incorrectly identified Ordinal Transfers as InSeq (reflecting positional coding). Our results show that mPFC had extensive activity on incorrect compared to correct Ordinal Transfer probe trials, which could not be accounted for by inaccuracy generally across other OutSeq probe trial types. Thus, we conclude that mPFC activity reflects representations of ordinal positions in sequence memory.

HC activation during Skips is consistent with temporal context memory (TCM)

The HC contributes to sequence memory through temporal contexts. TCM was originally developed to describe recency and associativity, two fundamental components of free recall and episodic memory (Howard and Kahana, 2002). TCM has emerged as an important framework for elucidating the neurobiological mechanisms of sequence memory. According to TCM, the HC associates items into sequences through a slowly drifting contextual representation (Polyn and Kahana, 2008; Howard et al., 2005). Electrophysiological evidence supports this claim, as population activity in the CA1 grows progressively more dissimilar over both small- and large-time frames (Mau et al., 2018; Rubin et al., 2015; Ziv et al., 2013; Mankin et al., 2012; Manns et al., 2007). The evolving population activity in the CA1, combined with time cells in the HC (MacDonald et al., 2011), lends compelling evidence that a key contribution of the HC to sequence learning is the process of linking temporally contiguous items through temporal contexts.

A recent study in mice further supports this conclusion showing that events close in time were linked by overlapping populations of CA1 neurons (Cai et al., 2016). Work in humans has also shown that population activity representing adjacent items grows more similar over successive presentations (Paz et al., 2010). Multivariate approaches in human neuroimaging studies have also shown that similarity of BOLD activation patterns is greater for items rated as temporally closer (Ezzyat and Davachi, 2014) or that share temporal community structure (Schapiro et al., 2016), and has been shown to predict subsequent order memory (DuBrow and Davachi, 2014). Pattern similarity has also been shown to be graded, ranging from high similarity for same object position repetitions to reduced similarity with increasing lag (Hsieh et al., 2014). The neurobiological findings summarized above provide a mechanism for the characteristic response pattern, where temporally adjacent items are more likely to be recalled following the recall of an item, during free recall tasks (Howard & Kahana, 2002).

In the current task, OutSeq probe trials were used to selectively discern the competing neurobiological mechanisms underlying sequence memory. For example, we hypothesized that during the course of the task InSeq items would reinstate the original temporal context and neighboring items

that shared the same context. Thus, Skip probe trials would show a graded impairment in performance as the n-forward lag increased and the contextually linked neurobiological representation gradually weaker with increasing forward lag. Our behavioral results are consistent with this prediction: small lags were associated with worse performance in detecting Skip probe trials as OutSeq and improved as the forward lag increased. Similar patterns in performance have been observed during prior tasks probing temporal memory (Allen et al., 2015; 2014; DuBrow and Davachi, 2014). Our neuroimaging results also coincide with this prediction where univariate activation in the HC shows a graded response with n-forward lag distance, in line with a multivariate approach reported by Hsieh and colleagues (2014).

Positional coding in mPFC during sequence memory

Ample evidence shows that mPFC is important for temporal order memory in humans (Hsieh et al., 2014; Shimamura et al; 1990; Milner et al., 1985) and rodents (DeVito & Eichenbaum, 2011; Barker et al., 2007; Hanneson et al., 2004; Chiba et al., 1997), however it remains unclear what exactly mPFC is doing. We suggest that mPFC, in part, provides abstract positional representations of sequences (e.g., 1st, 2nd, 3rd, 4th, 5th, etc.). Positional representations can be used to form ordinal-item associations which aid in the retrieval of sequences.

Positional coding is a fundamental mnemonic representation (Allen et al., 2014; Chen et al., 1997) and reflected in behaviorally-relevant neural activity (Heusser et al., 2016). In a notable example of the behavioral importance of ordinal representations, Orlov and colleagues (2000) demonstrated that when monkeys reproduced learned sequences ordinal distractors (similar to Ordinal Transfers in our study) led to errors early and often, but subsided with further training. These results suggest that item-position associations play a critical role in episodic memory formation (Tulving, 2002).

In order to test the behavioral relevance of positional coding, we used Ordinal Transfer probe trials in which items from the same position were transferred between sequences (e.g. AB3). If a positional strategy dominates, then Ordinal Transfers would be considered InSeq (e.g., item 3 correctly occurs in the 3rd position), however if TCM strategies dominate, these would be considered OutSeq (e.g., **3** did not occur in temporal proximity to B). Importantly, these probe trials place TCM and

positional strategies in direct conflict, whereas TCM and positional strategies are in agreement on Repeats and Skips (i.e., with respect to InSeq/OutSeq decisions). Supporting a positional strategy, Ordinal Transfers are the most difficult OutSeq probes (Fig1F; see also Allen et al., 2015; 2014). However, it should be noted that performance is consistently better than chance, suggesting TCM is also engaged. Further, we found that performance was essentially flat across positions 2 thru 6 and lacking any obvious recency or primacy effects more associated with TCM. We also observed a high degree of variability in performance consistent with the notion that TCM and ordinal coding created conflict (Fig3E-G).

At the neurobiological level, we hypothesized that mPFC activity would be greatest when Ordinal Transfers were incorrectly identified as InSeq because, from the vantage point of positional coding, Ordinal Transfers are InSeq. To test this hypothesis, we contrasted Ordinal Transfer incorrect and correct trials. The contrast revealed significant BOLD activation throughout mPFC. mPFC did not show this increased activity on Skips and Repeats, suggesting that when the conflict presented by Ordinal Transfers drives mPFC, an ordinal strategy dominated behavior. mPFC did not show similar ordinal activation on Skips, nor if we looked at OutSeq inaccuracy generally. These results agree with other findings in the literature suggesting a role for mPFC in ordinal representations. For example, Hsieh et al., (2015) showed significant mPFC multi-voxel pattern similarity when comparing object pairs that shared the same positions within their respective sequences but were otherwise unique sensory objects.

HC-mPFC interplay during sequence memory

Functional interactions between HC and mPFC are likely an important neurobiological mechanism supporting sequence memory. Functional coupling between HC and mPFC has been consistently implicated in long-term memory formation, memory-guided decision making, and retrieval-mediated integration (Jadhav et al., 2016; Euston et al., 2012; Bonnici et al., 2012; Benchenane et al., 2010; Wang and Morris, 2010; van Kesteren et al., 2010; Zeithamova and Preston, 2010). During sequence memory, we believe that similar mechanisms are likely engaged (Boucquey, 2016). Thus,

HC sequence representations including TCM (Hsieh et al., 2015; 2014; Kalm et al, 2013; Tubridy & Davachi, 2011; Jenkins & Ranganath, 2010; Lehn et al., 2009; Ross et al., 2009; Ekstrom & Bookheimer, 2007; Kumaran & Maguire, 2006) and mPFC sequence representations including positional coding may be compared and or integrated to direct behavior (e.g., InSeq/OutSeq decisions). However, the mechanisms by which the two systems interact in support of sequence memory is unknown.

Several competing possibilities of HC-mPFC interactions in sequence memory are viable. First, mPFC might provide positional representations to HC useful for encoding conjunctive item-position associations in HC neurons. The advantage to episodic memory is the formation of rapid sequences by using a pre-existing temporal “flow” of events. Second, mPFC might drive position-based retrieval in HC, consistent with the general role of mPFC in retrieval (e.g., Euston et al., 2012). During the retrieval process, the current position would be compared with the remembered position. Third, HC might provide mPFC event sequences that become generalized to position sequences, and then mPFC itself compares the current and predicted positions. These processes are neither mutually dependent or exclusive, but are testable. For example, the first two scenarios predict position coding in HC, but not the last scenario. Interestingly, positional representations have been reported in both human HC using BOLD fMRI (Hsieh et al., 2014) and in rat CA1 using single-unit recordings (Allen et al., 2016). However, future studies are required to tease out whether the posited representations are truly positional, either by increasing the demand on positional strategies or eliminating the utility of positional information (e.g., by randomly starting anywhere in a circular sequence of events).

Conclusion

Our results provide evidence for multiple cognitive strategies and neurobiological representations serving memory for sequences of events. The unique utilization of different OutSeq probe trials (Skips, Repeats, and Ordinal Transfers) in this study facilitated our ability to elucidate multiple behavioral and neurobiological mechanisms supporting sequence memory. The HC exhibited patterns of activation consistent with TCM, while mPFC demonstrated activation patterns consistent

with ordinal representations. These findings demonstrate that temporal contexts in HC and ordinal representations in mPFC concurrently support sequence memory. Further experiments in both animals and humans are necessary to delineate the precise mechanisms by which TCM and ordinal representations are instantiated and interact in the brain to support the temporal organization of memories.

REFERENCES

Allen TA, Fortin NJ. 2013. The evolution of episodic memory. Proc Natl Acad Sci U S A. 110(suppl 2):10379-10386.

Allen TA, Morris AM, Mattfeld AT, Stark CEL, Fortin NJ. 2014. A sequence of events model of episodic memory shows parallels in rats and humans. Hippocampus. 24:1178-1188.

Allen, TA, Morris AM, Fortin NJ Stark CEL. 2015. Memory for sequences of events impaired in typical aging. Learn Mem, 22.138-148.

Allen TA, Salz DM, McKenzie SA, Fortin NJ. 2016. Nonspatial sequence coding in CA1 neurons. J Neurosci. 36(5):1547-1563.

Avants BB, Epstein CL, Grossman M, Gee JC. 2008. Symmetric diffeomorphic image registration with cross-correlation: Evaluating automated labeling of elderly and neurodegenerative brain. Med Image Anal. 12(1):26-41.

Barker GR, Bird F, Alexander V, Warburton EC. 2007. Recognition memory for objects, place, and temporal order: a disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex. J Neurosci. 27(11):2948-2957.

Belassen V, Iloi K, de Souza LC, Dubois B, Rondi-Reig L. 2012. Temporal order memory assessed during spatiotemporal navigation as a behavioral cognitive marker for differential Alzheimer's disease diagnosis. Neurobiol Dis. 32(6):1942-1952.

Benchenane K, Peyrache A, Khamassi M, Tierny PL, Gioanni Y, Battaglia FP, Wiener SI. 2010. Coherent theta oscillations and reorganization of spike timing in the hippocampal- prefrontal network upon learning. Neuron. 66(6):921-936.

Bonnici HM, Chadwick MJ, Lutti A, Hassabis D, Weiskopf N, Maguire EA. 2012. Detecting representations of recent and remote autobiographical memories in vmPFC and hippocampus. J Neurosci. 32(47):16982-16991.

Boucquey V. 2016. Investigation of the human neural correlates of memory for sequences of events and their changes in typical aging. UC Irvine. ProQuest ID: Boucquey_uci_0030D_14133. Merritt ID: ark:/13030/m57h66h6. Retrieved from https://escholarship.org/uc/item/72t2k0rs.

Cai DJ, Aharoni D, Shuman T, Shobe J, Biane J, Song W, Wei B, Veshkini, La-Vu M, Lou J, Flores SE, Kim I, Sano Y, Zhou M, Baumgaertel K, Lavi A, Kamata M, Tuszynski M, Mayford M, Golshani P, Silva AJ. 2016. A shared neural ensemble links distinct contextual memories encoded close in time. Nature. (534):115-118.

Chen S, Swartz KB, Terrace HC. 1997. Knowledge of the ordinal position of list items in rhesus monkeys. Psychol Sci. 8(2):80-86.

Chiba AA, Kesner RP, Gibson CJ. 1997. Memory for temporal order of new and familiar spatial location sequences: role of the medial prefrontal cortex. Learn Mem. 4(4):311-317.

Cox RW, 1996. AFNI: software for analysis and visualization of functional magnetic resonance Neuroimages. Comput Biomed Res. 29(3):162-173.

Dale AM, Fischl B, Sereno MI. 1999. Cortical surface-based analysis. I. Segmentation and surface reconstruction. Neuroimage 9:179-194.

D'Amato MR, Colombo M. 1988. Representation of serial order in monkeys (Cebus apella). J Exp Psychol Anim B. 14(2):131-139.

Davachi L, Dubrow S. 2015. How the hippocampus preserves order: the role of prediction and context. Trends Cogn Sci. 19(2):92-99.

Davachi L, Wagner AD. 2002. Hippocampal contributions to episodic encoding: insights from relational and item-based learning. J Neurophysiol. 88(2):982-990.

Dragoi G, Buzsáki G. 2006. Temporal encoding of place sequences by hippocampal cell assemblies. Neuron. 50(1):145-157.

DeVito LM, Eichenbaum H. 2011. Memory for the order of events in specific sequences: Contributions of the hippocampus and medial prefrontal cortex. J Neurosci. 31(9):3169-3175.

Dubrow S, Davachi L. 2013. The influence of context boundaries on memory for the sequential order of events. J Exp Psychol Gen. 142(4):1277-1286.

Dubrow S, Davachi L. 2016. Temporal binding within and across events. Neurobiol Learn Mem. 134:107-114.

Eichenbaum H. 2017. On the integration of space, time and memory. Neuron. 95(5):1007-1018.

Ekstrom AD, Bookheimer SY, 2007. Spatial and temporal episodic memory retrieval recruit dissociable functional networks in the human brain. Learn Mem. 14(10):645-654.

Euston DR, Gruber AJ, McNaughton BL. 2012. The role of medial prefrontal cortex in memory and decision making. Neuron. 76(6):1057-1070.

Euston DR, Tatsuno M, McNaughton BL. 2007. Fast-forward playback of recent memory sequences in prefrontal cortex during sleep. Science. 318(5853):1147-1150.

Ezzyat Y, Davachi L. 2011. What constitutes an episode in episodic memory? Psychol Sci. 22:243-252.

Ezzyat Y, Davachi L. 2014. Similarity breeds proximity: Pattern similarity within and across contexts is related to later mnemonic judgements of temporal proximity. Neuron. 81(5):1179-1189.

Feinberg LM, Allen TA, Ly D, Fortin NJ. 2011. Recognition memory for social and non-social odors: differential effects of neurotoxic lesions to the hippocampus and perirhinal cortex. Neurobiol Learn Mem. 97(1):7-16.

Fortin NJ, Agster KL, Eichenbaum HB. 2002. Critical role of the hippocampus in memory for sequences of events. Nat Neurosci. 5:458-462.

Foster DJ, Wilson MA. 2006. Reverse replay of behavioral sequences in hippocampal place cells during the awake state. Nature. 440:680-683.

Golomb J, Deleon MJ, Kluger A, George AE, Tarshish C, Ferris SH. 1993. Hippocampal atrophy in normal aging: an association with recent memory impairment. Arch Neurol. 50:967-973.

Gorgolewski K, Burns CD, Madison C, Clark D, Halchenko YO, Waskom ML, Ghosh SS. 2011. Nipype: a flexible, lightweight extensible neuroimaging data processing framework in python. Front Neuroinform. 5:13.

Goyal A, Miller J, Watrous AJ, Lee SA, Coffey T, Sperling MR, Sharan A, Worrell G, Berry B, Lega B, Jobst BC, Davis KA, Inmann C, Sheth SA, Wanda PA, Azzyat Y, Das SR, Stein J, Gorniak R, Jacobs J. 2018. Electrical stimulation in hippocampus and entorhinal cortex impairs spatial and temporal memory. J Neurosci. 38(19):4471-4481.

Hanneson DK, Howland JG, Phillips AG. 2004. Interaction between perirhinal and medial prefrontal cortex is required for temporal order but not recognition memory for objects in rats. J Neurosci. 24(19):4596-4604.

Heusser AC, Poeppel D, Ezzyat Y, Davachi L. 2016. Episodic sequence memory is supported by a theta-gamma phase code. Nat Neurosci. 19(10):1374-1380.

Hsieh LT, Gruber MJ, Jenkins LJ, Ranganath C. 2014. Hippocampal activity patterns carry information about objects in temporal context. Neuron. 81: 1165-1178.

Hsieh LT, Ranganath C. 2015. Cortical and subcortical contributions to sequence retrieval: Schematic coding of temporal context in the neocortical recollection network. Neuroimage. 121:78-90.

Howard MW, Eichenbaum H. 2013. The hippocampus, time and memory across scaled. J Exp Psychol. 142(4):1211-1230.

Howard MW, Kahana MJ. 2002. A distributed representation of temporal context. J Math Psychol. 46: 269-299.

Howard MW, Viskontas IV, Shankar KH, Fried I. 2012. Ensembles of human MTL neurons “jump back in time” in response to a repeated stimulus. Hippocampus. 22:1833-1847.

Jadhav SP, Rothschild G, Roumis DK, Frank LM. 2016. Coordinated excitation and inhibition of prefrontal ensembles during awake hippocampal sharp-wave ripple events. Neuron. 90(1):113-127.

Jenkins LJ, Ranganath C. 2010. Prefrontal and medial temporal lobe activity at encoding predicts temporal context memory. J Neurosci. 30(46):15558-15565.

Kahana MJ. 1996. Associate retrieval processes in free recall. Memory & cognition. 24(1):103-110.

Kalm K, Davis MH, Norris D. 2013. Individual sequence representations in the medial temporal lobe. J Cogn Neurosci. 25:1111-1121.

Kesner RP, Gilbert PE, Barua LA. 2002. The role of the hippocampus in memory for the temporal order of a sequence of odors. Behav Neurosci. 116(2):286-290.

Kragel JE, Morton NW, Polyn SM. 2015. Neural activity in the medial temporal lobe reveals the fidelity of mental time travel. J Neurosci. 35(7):2914-2926.

Kumaran D, Maguire EA. 2006. An unexpected sequence of events: Mismatch detection in the human hippocampus. Plos Biol. 4(12):2372-2382.

Lehn H, Steffenach HA, Strien NM, Veltman DJ, Witter MP, Haberg AK. 2009. A specific role of the human hippocampus in recall of temporal sequences. J Neurosci. 29(11):3475-3484.

Long HMK, Kahana NM. 2018. Hippocampal contributions to serial-order memory. Hippocampus. Epub.

MacDonald CJ, Carrow S, Place R, Eichenbaum H. 2013. Distinct hippocampal time cell sequences represent odor memories in immobilized rats. J Neurosci., 33:14607–14616.

MacDonald CJ, Lepage KQ, Eden UT, Eichenbaum H. 2011. Hippocampal “time cells” bridge the gap in memory for discontiguous events. Neuron. 71:737–749.

Mankin EA, Sparks FT, Slayyeh B, Sutherland RJ, Leutgeb S, Leutgeb JK. 2012. Neuronal code for extended time in the hippocampus. Proc Natl Acad Sci U S A. 109(47):19462-19467.

Manns JR, Howard MW, Eichenbaum H. 2007. Gradual changes in hippocampal activity support remembering the order of events. Neuron. 56(3):530-540.

Mau W, Sullivan DW, Kinsky NR, Hasselmo ME, Howard MW, Eichenbaum H. 2018. The same hippocampal CA1 population simultaneously codes temporal information over multiple timescales. Curr Biol. 28(10):1499-1508.

Milder B, Petrides M, Smith ML. 1985. Frontal lobes and temporal organization of memory. Hum Neurobiol. 4:137-142.

Orlov T, Yakovlev V, Hochstein S, Zohary E. 2000. Macaque monkeys categorize images by their ordinal number. Nature. 404:77-80.

Roberts JM, Ly M. Murray E, Yassa MA. 2014. Temporal discrimination as a function of lag interference in older adults. Hippocampus, 24(10):1189-1196.

Rubin A, Geva N, Sheintuch L, Ziv Yaniv. 2015. Hippocampal ensemble dynamics timestamp events in long-term memory. eLife. 4: e12247.

Pastalkova E, Itskov V, Amarasingham, Buzsáki G. 2008. Internally generated cell assembly sequences in the rat hippocampus. Science 321:1322-1327.

Paz R, Gelbard-Sagiv H, Mukamel R, Harel M, Malach R, Fried I. 2010. A neural substrate in the human hippocampus for linking successive events. Proc Natl Acad Sci U S A. 107(13):6046-6051.

Polyn SM, McCluey JD, Morton NW, Woolard AA, Luksik AS, Heckers S. 2015. Temporal context and the organizational impairment of memory search in schizophrenia. Cogn Neuropsychiatry. 20(4):296-310.

Polyn SM, Kahana MJ. 2008. Memory search and the neural representation of context. Trends Cogn Sci. 12(1):24-30.

Preston AR, Eichenbaum H. 2013. Interplay of hippocampus and prefrontal cortex in memory. Curr Biol. 23(17):764-773.

Roche A. 2011. A four-dimensional registration algorithm with application to joint correction of motion and slice timing in fMRI. IEE Trans Med Imaging.

Ross RS, Brown TI, Stern CE. 2009. The retrieval of learned sequences engages the hippocampus: Evidence from fMRI. Hippocampus. 19:790-799.

Schapiro AC, Turk-Browne NB, Normal KA, Botvinick MM. 2016. Statistical learning of temporal community structure in the hippocampus. Hippocampus. 26(1):3-8.

Shimamura AP, Janowsky JS, Squire LR. 1990. Memory for the temporal order of events in patients with frontal lobe lesions and amnesic patients. Neuropsychologia. 28:803–814.

Sokal RR, Rohlf FJ. 1995. Biometry: the principles and practices of statistics in biological research. New York: W.H. Freeman and Company.

Thomas JG, Milner HR, Haberlandt KF. 2003. Forward and backward recall: Different response time patterns, same retrieval order. Psychol Sci. 14(2):169-174.

Terrace HS. 2005. The simultaneous chain: A new approach to serial learning. Trends Cogn Sci. 9(4):202-210.

Tiganj Z, Kim J, Jung MW, Howard MW. 2017. Sequential firing codes for time in rodent medial prefrontal cortex. Cereb Cortex. 27(12):5663-5671.

Tubridy S, Davachi L. 2011. Medial temporal lobe contributions to episodic sequence encoding. Cerebral Cortex. 21:272-280.

Tulving E. 2002. Episodic memory: from mind to brain. Annu Rev Psychol. 53:1-25.

Van Kesteren MTR, Fernandez G, Norris DG, Hermans EJ. 2010. Persistent schema-dependent hippocampal-neocortical connectivity during memory encoding and postencoding rest in humans. Proc Natl Acad Sci U S A. 107(16):7550-7555.

Wang SH & Morris RG. 2010. Hippocampal-neocortical interactions in memory formation, consolidation, and reconsolidation. Annu Rev Psychol. 61:49-79.

Wikenheiser AM, Redish AD. 2015. Hippocampal theta sequences reflect current goals. Nat Neurosci. 18(2):289-94.

Zeithamova D & Preston AR. 2010. Flexible memories: differential roles for medial temporal lobe and prefrontal cortex in cross-episode binding. J Neurosci. 30(44):14676-14684.

Ziv Y, Burns LD, Cocker ED, Hamel EO, Ghosh KK, Kitch LJ, El Gamal A, Schnitzer MJ. 2013. Long-term dynamics of CA1 hippocampal place codes. Nat Neurosci. 16(3):264-266.

FIGURES

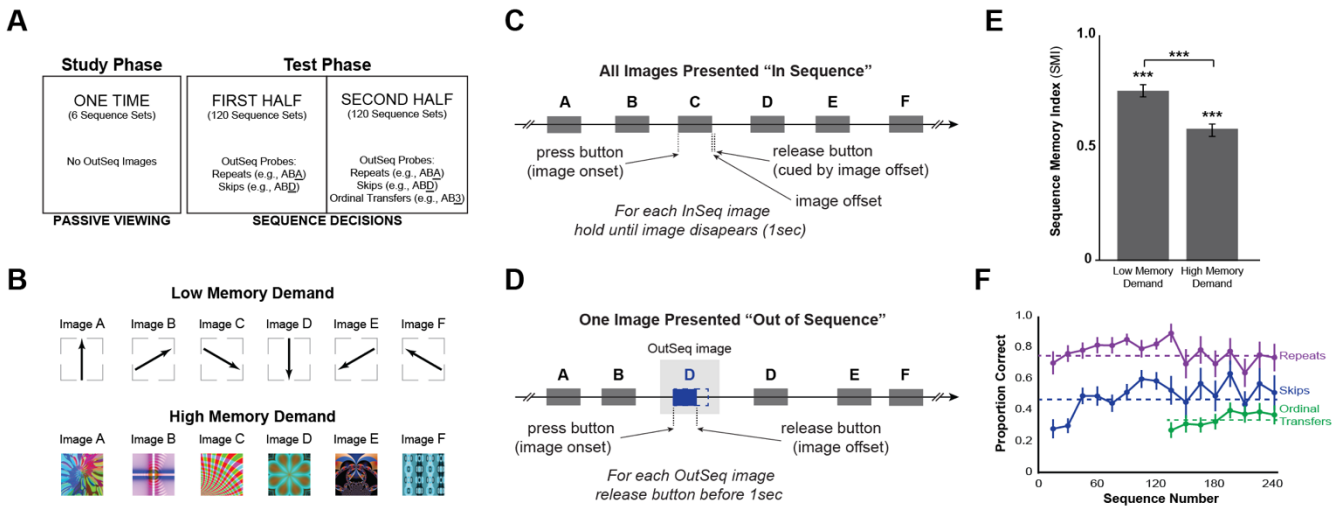

Figure 1. *Sequence memory task and overall performance levels*. Participants were tested on a sequence memory task which uses an array of out of sequence probe trial types to assess the cognitive strategies and representations underlying memory for sequences of events. ***A,*** During the study phase participants were instructed to remember the sequences for later testing (viewing sequences without responding). Next, the test phase was self-paced and participants were instructed to determine whether images were presented "in sequence" (InSeq) or "out of sequence" (OutSeq). Sequences were presented 40 times each (for a total of 240 presentations) with half containing one OutSeq item. OutSeq items consisted of repeated items (Repeats, e.g., AB<u>A</u>), items that occurred too early in the sequence (Skips; e.g., AB<u>D</u>), and items transferred from one sequence into another while remaining in their ordinal position (Ordinal Transfers; e.g., AB<u>3</u>). The first half of the test phase contained Repeats and Skips, and the second half contained all three OutSeq types. ***B,*** Sequences were either "low memory demand" (e.g., an arrow moving clockwise), or "high memory demand", composed of arbitrary sequences of fractal images. ***C,*** Half of the time, all items were presented InSeq during the test phase. For each InSeq item subjects were required to press and hold a button for 1sec. ***D,*** Half of the time, one item was presented OutSeq. For each OutSeq item, subjects were required to release a button before 1sec. ***E,*** Overall, subjects performed at levels that were significantly higher than chance (SMI = 0) on

both the low and high memory demand sequences. Subjects were significantly better with low memory demand sequences compared to high memory demand sequences. ***F,*** Subjects performed best on Repeats, followed by Skips, with the lowest performance on Ordinal Transfers. Performance was stable across the duration of the task. Symbol(s): *** $p < 0.001$.

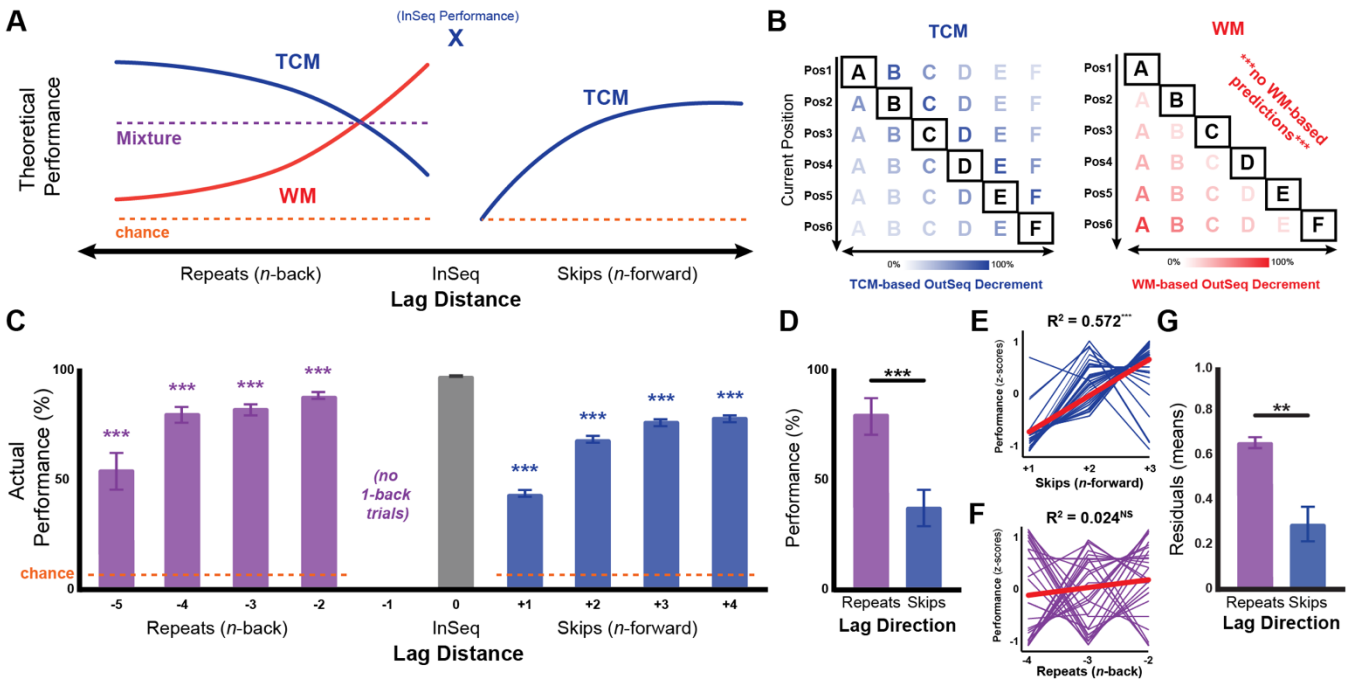

Figure 2. *Conceptual model and lag analysis.* ***A,*** Theoretical predictions based on temporal context memory (TCM) and working memory (WM) for different lag distances on Repeats (e.g., AB<u>A</u>) and Skips (e.g., AB<u>D</u>). TCM (blue lines) predicts that item retrieval in sequences is graded by lag distance (for both Repeats and Skips). According to TCM, OutSeq probe trials with small lags would be more difficult than those with larger lags reflecting the fact that they were more temporally contiguous in the original sequence. Conversely, WM (red line) predicts that items with small lags would be easier than those with larger lags, but WM can only contribute to performance on Repeats. A mixture of TCM and WM is shown in the dashed purple line. ***B,*** Theoretical sequence memory decrements with TCM (blue) and WM (red) are depicted. Correct items for a given trial (rows) are shown in black type within a black box, and the intensity of color for surrounding items (blue or red) shows the relative predicted performance decrements across items. TCM predicts performance decrements on small lags in both directions. WM predicts performance decrements on large lags but only contributes in the backward direction. ***C,*** Average actual performance accuracy for all participants on Repeats and Skips at different lags. ***D,*** We predicted and found that overall performance on Repeats would be higher than for Skips since the TCM

and WM complement each other. We also predicted and found that performance would show a positive slope on Skips reflecting TCM, **(*E)*** and would be flat on Repeats reflecting a mixture of TCM and WM **(*F)***. Specifically, a linear regression in Skips performance positively increases across forward lags but does not have a significant slope on Repeats. ***G,*** Lastly, we predicted and found significantly higher variability in Repeats compared to Skips which is thought to reflect the integration of variance from both TCM and WM. Symbol(s): ns, not significant; *, $p < 0.05$, **, $p < 0.01$, *** $p < 0.001$.

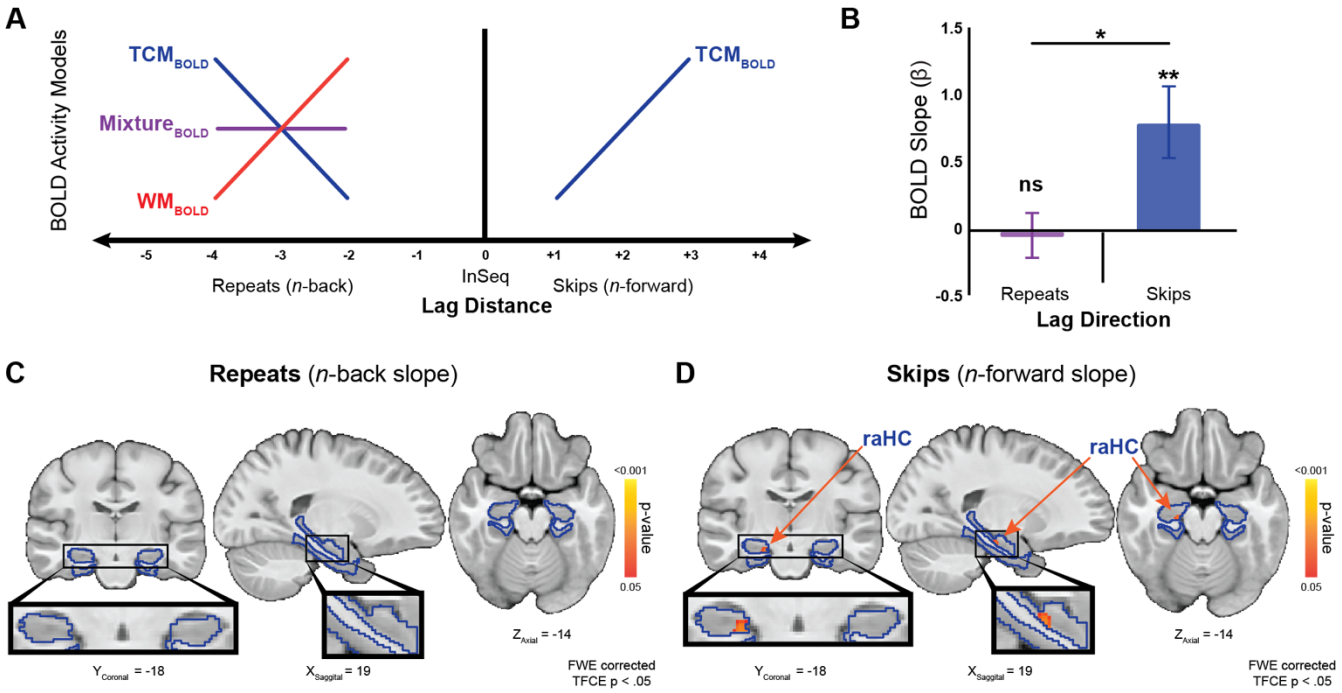

Figure 3. *BOLD fMRI analysis indicates that right anterior hippocampus (raHC) tracks temporal context across forward lags.* ***A,*** Theoretical linear contrast models in BOLD fMRI analyses representing TCM, WM or a mixture of the models at different lags. Note that the *-5*-back and *+4*-forward lags were not included in the fMRI analysis because there was only one trial per participant. ***B,*** BOLD fMRI analysis of Skips with a linear contrast of -1, 0, 1 for lags of +1, +2, +3 respectively (right blue line in A) using bilateral MTL as mask (outlined in blue in C and D) revealed significant activation in the raHC. ***C,*** BOLD fMRI analysis of Repeats with a linear contrast of 1, 0, -1 for lags of -4, -3, -2 respectively (red line in 3A) using bilateral MTL as mask showed no significant clusters. ***D,*** BOLD fMRI ROI analysis of the bilateral anterior HC was significantly higher in Skips compared to Repeats when using a linear contrast based on TCM. Thus, raHC activity tracks with temporal context predictions. Symbol(s): ns, not significant; **, $p < 0.01$.

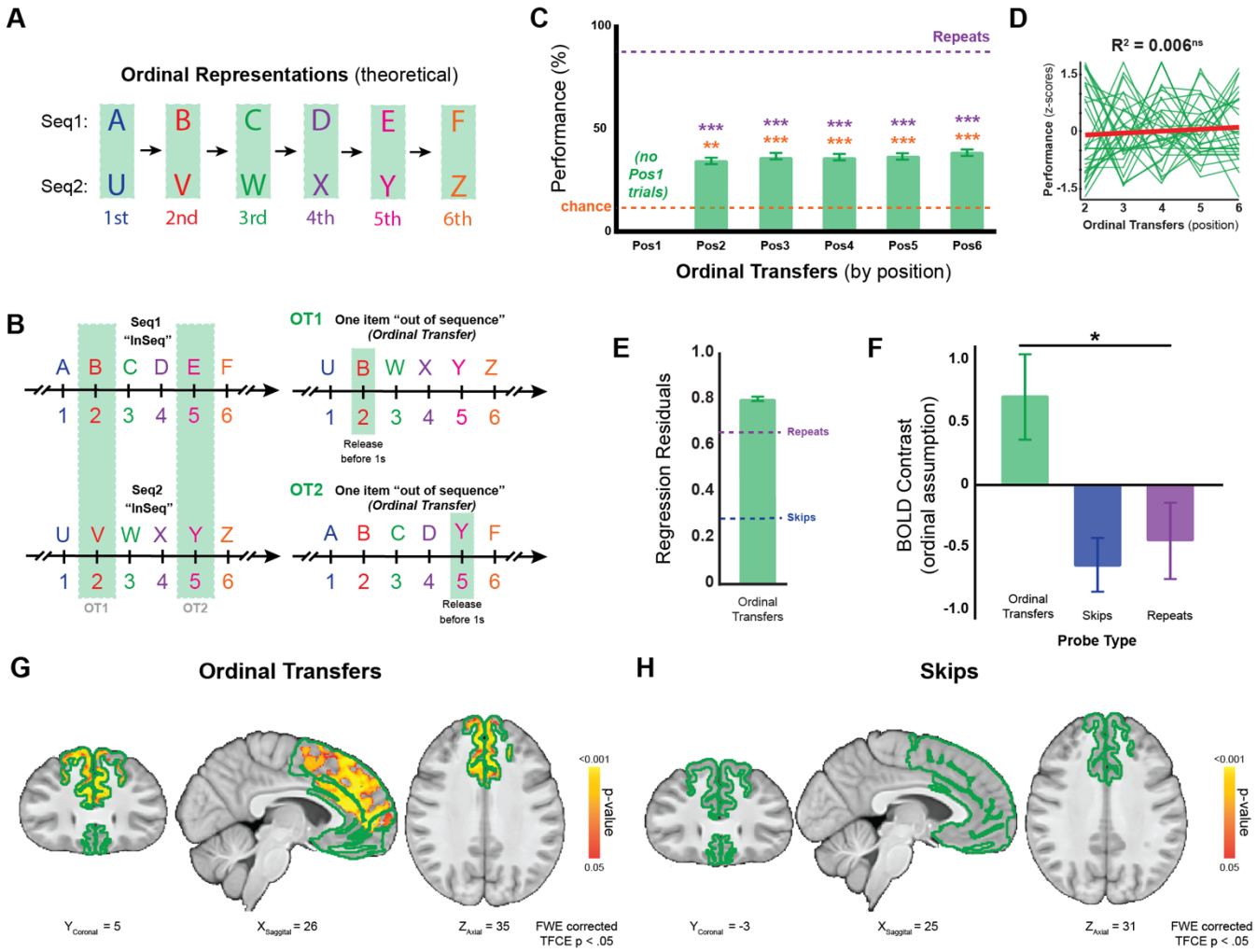

Figure 4. *mPFC activates with ordinal representations during sequences memory.* ***A,*** Theoretical model of ordinal representation for sequences in which items associate with ordinal positions (e.g., A = 1st, B = 2nd, etc.). ***B,*** Ordinal representations were probed using Ordinal Transfer trials. Ordinal Transfers occurred when an item from one sequence (e.g., UVWXYZ) was transferred to another sequence (e.g., ABCDEF) while retaining the item's original ordinal position (e.g. ABCDYF). Ordinal Transfers are identified as InSeq when an ordinal association is used and identified as OutSeq when an item-based association is used. ***C,*** Ordinal Transfers occurred in every position in a sequence except the first. For each position (2 thru 6) the average performance was significantly higher than chance (one minus the calculated response bias), and significantly lower than the average performance on

Repeats (best performance levels). There were no significant differences in performance across positions. ***D,*** There was no significant slope across positions, and there was high variability overall. High average performance variability suggests conflicting strategies (Repeats and Skips variability levels plotted for comparison in **E**). For the subsequent BOLD analysis, we focused on contrasts assuming participants were using an ordinal strategy. When an ordinal representation is used participants will identify Ordinal Transfers as InSeq but they would identify Repeats and Skips as OutSeq. ***F***, a BOLD fMRI mPFC ROI analysis showed that the Ordinal Transfers Incorrect – Correct contrast activation was significantly higher compared to Repeats and Skips (Correct – Incorrect Contrasts). Notably, mPFC activation did not simply reflect inaccuracy on OutSeq trials (see Supplemental Fig1 and Supplemental Table 1). ***G,*** a BOLD fMRI analysis using mPFC as a mask (outlined in green) resulted in significant activity in a large area of mPFC. To investigate whether there was a difference between these probe trials, we compared Ordinal Transfers Incorrect – Correct, Repeats Correct – Incorrect, and Skips Correct – Incorrect. ***H,*** No significant activation was found when comparing Repeats Correct – Incorrect (not shown) and Skips Correct – Incorrect. Symbol(s): ns, not significant; *, $p < 0.05$, **, $p < 0.01$, *** $p < 0.001$.