

Reactivation strength during cued recall is modulated by graph distance within cognitive maps

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Abstract

Declarative memory retrieval is thought to involve reinstatement of neuronal activity patterns elicited and encoded during a prior learning episode. Furthermore, it is suggested that two mechanisms operate during reinstatement, dependent on task demands: individual memory items can be reactivated simultaneously as a clustered occurrence or, alternatively, replayed sequentially as temporally separate instances. In the current study, participants learned associations between images that were embedded in a directed graph network and retained this information over a brief 8-minute consolidation period. During a subsequent cued recall session, participants retrieved the learned information while undergoing magnetoencephalographic (MEG) recording. Using a trained stimulus decoder, we found evidence for clustered reactivation of learned material. Reactivation strength of individual items during clustered reactivation decreased as a function of increasing graph distance, an ordering present solely for successful retrieval but not for retrieval failure. In line with previous research, we found evidence that sequential replay was dependent on retrieval performance and was most evident in low performers. The results provide evidence for distinct performance-dependent retrieval mechanisms with graded clustered reactivation emerging as a plausible mechanism to search within abstract cognitive maps.

eLife assessment

This magnetoencephalography study reports **important** new findings regarding the nature of memory reactivation during cued recall. It replicates previous work showing that such reactivation can be sequential or clustered, with sequential reactivation being more prevalent in low performers. It adds **convincing** evidence, even though based on limited amounts of data, that high memory performers tend to show simultaneous (i.e., clustered) reactivation, varying in strength with item distance in the learned graph structure. The study will be of interest to scientists studying memory replay.

Introduction

Memory classically relies on three distinct stages: encoding (learning), consolidation (strengthening and transforming) and retrieval (reinstating) of information. New episodic memories are learned by encoding a representation, thought to be realized in a specific spatio-temporal neuronal firing pattern in hippocampal and neocortical networks (Frank et al., 2000 [↗](#); Preston & Eichenbaum, 2013 [↗](#)). These firing patterns are reactivated during subsequent rest or sleep, sometimes in fast sequential sequences, a process linked to memory consolidation (Born & Wilhelm, 2012 [↗](#); Feld & Born, 2017 [↗](#)). Similarly, during retrieval, the same firing patterns seen during encoding are replayed in a manner that predicts retrieval success (Carr et al., 2011 [↗](#); Foster, 2017 [↗](#)). Even though replay has been studied most intensely with respect to the hippocampus, the replay of memory traces in temporal succession is suggested as a general mechanism for planning, consolidation, and retrieval (Buhry et al., 2011 [↗](#)). While a rich body of evidence exists in rodents (Ambrose et al., 2016 [↗](#); Chen & Wilson, 2023 [↗](#); Foster & Knierim, 2012 [↗](#); Ólafsdóttir et al., 2018 [↗](#)), the contributions of replay to memory storage and retrieval in humans are only beginning to be examined (Brunec & Momennejad, 2022 [↗](#); Eichenlaub et al., 2020 [↗](#); Fuentemilla et al., 2010 [↗](#); Wimmer et al., 2020 [↗](#)).

One obstacle has been the difficulty in measuring sequential replay or general network reactivation in humans (NB here we follow the definition of Genzel et al., 2020, where *reactivation* is used as an umbrella term for any form of reoccurrence of a previously encoded neural pattern related to information-encoding, and *replay* refers to reactivation events with a temporally sequential nature). The most straightforward method is to use intracranial electroencephalography (iEEG), though this is generally only feasible within individuals undergoing evaluation for management of epilepsy (Axmacher et al., 2008 [↗](#); Engel et al., 2005 [↗](#); Staresina et al., 2015 [↗](#); Zhang et al., 2015 [↗](#)). Another approach is to use functional magnetic resonance imaging (Schuck & Niv, 2019 [↗](#); Wittkuhn & Schuck, 2021 [↗](#)) though the latter is burdened by the challenge posed by the sluggishness of the hemodynamic response. Researchers have recently started to leverage the spatio-temporal precision of magnetoencephalography (MEG), in combination with machine learning based brain decoding techniques, to reveal sequential human replay in humans across a range of settings that includes memory, planning and inference (Eldar et al., 2018 [↗](#); Kurth-Nelson et al., 2016 [↗](#); Liu et al., 2019 [↗](#); Liu, Mattar, et al., 2021; McFadyen et al., 2023 [↗](#); Nour et al., 2021 [↗](#); Wimmer et al., 2020 [↗](#), 2023 [↗](#); Wise et al., 2021 [↗](#)). Many of the latter studies deploy a novel statistical analysis technique, temporally delayed linear modeling (TDLM) (Liu, Dolan, et al., 2021). TDLM, and its variants, enable identification of sequential replay for previously experienced material during resting state (Liu et al., 2019 [↗](#); Liu, Mattar, et al., 2021), during planning of upcoming behavioral output (Eldar et al., 2020 [↗](#); Kurth-Nelson et al., 2016 [↗](#); McFadyen et al., 2023 [↗](#); Wise et al., 2021 [↗](#)) and during memory retrieval (Wimmer et al., 2020 [↗](#)).

Wimmer et al. (2020) [reported](#) sequential reactivation of episodic content following a single initial exposure during cued recall one day post-encoding. Specifically, they showed participants eight short, narrated stories, each consisting of four different visual story anchor elements taken from six different categories (faces, buildings, body parts, objects, animals, and cars) and a unique ending element. In a next day recall session, participants were shown two story elements and asked whether both elements were part of the same story, and whether the second element appeared before or after the first. At retrieval they showed stories were replayed in reverse order to the prompt (i.e., when prompting element 3 and element 5, successful retrieval would traverse element 5 through 4 and arrive at element 3). However, this effect was only found in those with regular performance, while in high performers there was no evidence for temporal succession. Instead, the latter group simultaneously reactivated all related story elements at in a clustered manner.

In memory research, declarative tasks often avail of item lists or paired associates (Barnett et al., 2016 [Cho et al., 2020](#) [Feld et al., 2013](#) [Kolibius et al., 2021](#) [Roux et al., 2022](#) [Schönauer et al., 2014](#) [Stadler et al., 1999](#) [1999](#)). When studying sequential replay, the task structure must have a linear element (Liu et al., 2019 [Liu, Mattar, et al., 2021](#); Wimmer et al., 2020 [Wise et al., 2021](#)) and such linearity is a defining feature of episodic memory (Tulving, 1993). By contrast, semantic memory is rarely organized linearly and instead involves complex and interconnected knowledge networks or cognitive maps (Behrens et al., 2018) motivating researchers to ask how memory works when organized into a complex graph structure (Eldar et al., 2020 [G. Feld et al., 2021](#) [Garvert et al., 2017](#) [Schapiro et al., 2013](#); for an overview see Momennejad, 2020). However, little is currently known regarding the contribution of replay to consolidation and retrieval processes for information that is embedded in a graph structures. In particular, the question remains how the brain keeps track of graph distances for successful recall and whether the previously found difference between high and low performers also holds true within a more complex graph learning context.

Here we examined the relationship between retrieval from a learned graph structure and reactivation and replay in a task where participants learned a directed, cyclic graph, represented by ten connected images. Eight nodes had exactly one direct predecessor and successor node, two *hub nodes*, each had two direct predecessors and successors (See **Figure 2B**). The task was arranged such that participants could not rely on simple pair mappings but needed to learn the context of each edge. Additionally, the graph-structure was never shown to participants as a ‘birds-eye-view’, encouraging implicit learning of the underlying structure. Following a retention period, consisting of eight minutes eyes closed resting state, participants then completed a cued recall task, which is the focus of the current paper.

Methods

Participants

We recruited thirty participants (15 men and 15 women), between 19 and 32 years old (mean age 24.7 years). Inclusion criteria were right-handedness, no claustrophobic tendencies, no current or previous diagnosed mental disorder, non-smoker, fluency in German or English, age between 18 and 35 and normal or corrected-to-normal vision. Caffeine intake was requested to be restricted for four hours before the experiment. Participants were recruited through the institute’s website and mailing list and various local Facebook groups. A single participant was excluded due to a corrupted data file and replaced with another participant. We acquired written informed consent from all participants, including consent to share anonymized raw and processed data in an open access online repository. The study was approved by the ethics committee of the Medical Faculty Mannheim of Heidelberg University (ID: 2020-609). While we had preregistered the study design and an analysis approach for the resting state data (<https://aspredicted.org/kx9xh.pdf>, #68915)

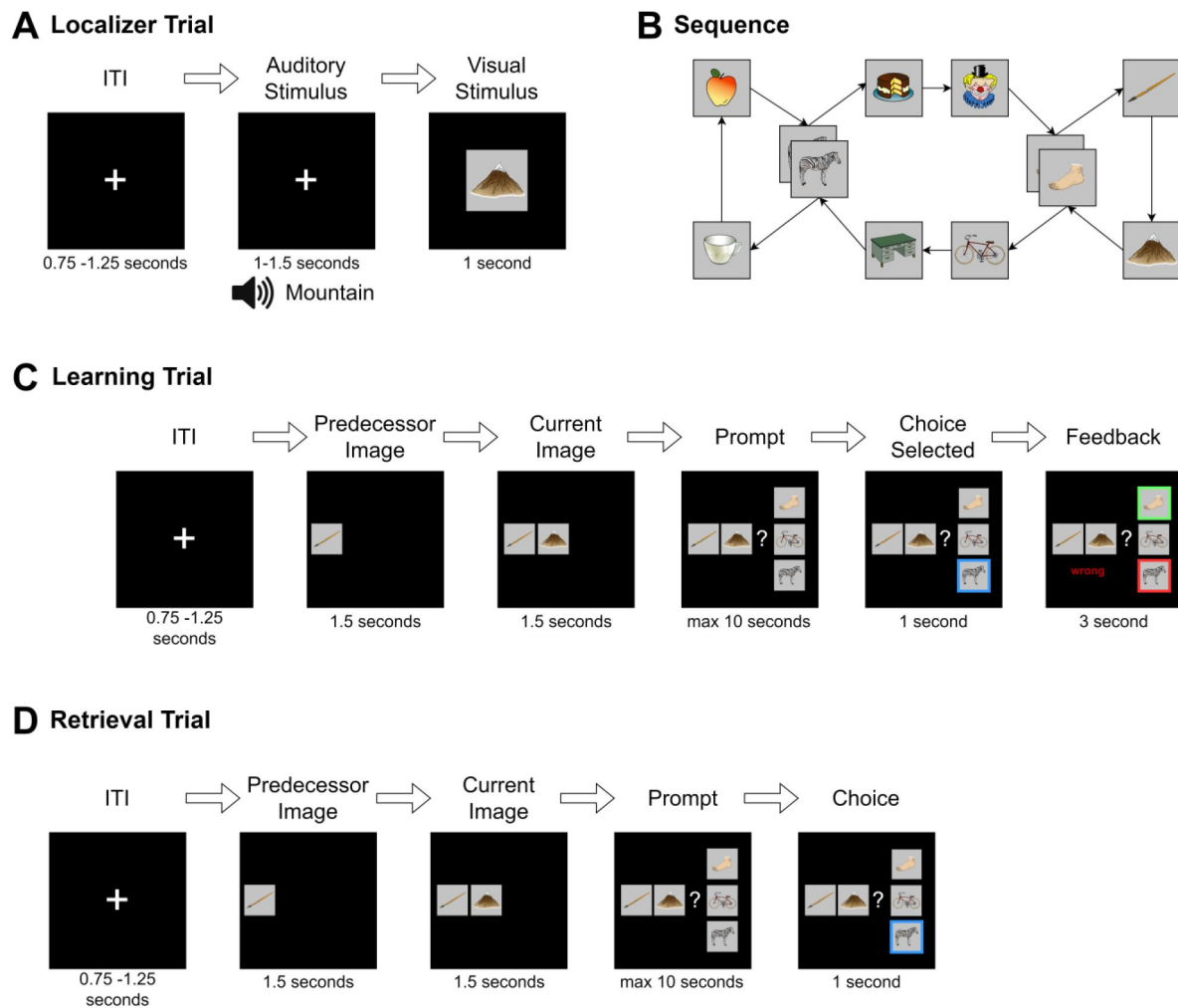


Figure 2

Task structure: A) During the localizer task, a word describing the stimulus was played via headphones and the corresponding visual item was then shown to the participant. In 4% of trials, the audio and visual cue did not match and in this case, participants were instructed to press a button on detection (attention check). B) Graph layout of the task. Two elements could appear in two different triplets. The graph was directed such that each tuple had exactly one successor (e.g., apple → zebra could only be followed by cake and not mug), but individual items could have different successors (zebra alone could be followed by mug or cake). Participants never saw the illustrated birds-eye-view. C) During learning, in each trial one node was randomly chosen as the current node. First, its predecessor node was shown, followed by the current node with the participant then given a choice of three items. They were then required to choose the node that followed the displayed cue tuple. Feedback was then provided to the participant. This process was repeated until the participant reached 80% accuracy for any block or reached a maximum of six blocks of learning. D) The retrieval followed the same structure as the learning task, except that no feedback was given.

here we report analyses of the retrieval period. The current analysis conceptually replicates the analyses and hypotheses of Wimmer et al. (2020) [\[link\]](#) focusing on the retrieval period albeit in a much more complex and therefore naturalistic paradigm and are therefore, despite not being preregistered, mainly of confirmatory nature. We wish to maintain transparency by acknowledging that the findings from the preregistered analysis concerning the resting state data, are being prepared for publication as part of a distinct submission.

Procedure

Participants came to the laboratory for a single study session of approximately 2.5 hours. After filling out a questionnaire about their general health, their vigilance state (Stanford Sleepiness Scale, Hoddes et al., 1973 [\[link\]](#)) and mood (PANAS, Watson et al., 1988 [\[link\]](#)), participants performed five separate tasks while in the MEG scanner. First, an eight-minute eyes-closed resting state was recorded. This was followed by a localizer task (~30 minutes), in which all 10 items were presented 50 times in pseudo-randomized order, using auditory and visual stimuli. Next, participants learned a sequence of the 10 visual items embedded into a graph structure until they achieved 80% accuracy or reached a maximum of six blocks (7 to 20 minutes). Following this, we recorded another eight-minutes eyes-closed resting state to allow for initial consolidation and, finally, a cued retrieval session (four minutes). For an overview see **Figure 1** [\[link\]](#).

Stimulus material

Visual stimuli were taken from the colored version (Rossion & Pourtois, 2001 [\[link\]](#)) of the Snodgrass & Vanderwart (1980) [\[link\]](#) stimulus dataset. To increase brain pattern discriminability, images were chosen with a focus on diversity of color, shape and category (see **Figure 2B** [\[link\]](#)) and for having short descriptive words (one or two syllables) both in German and English. Auditory stimuli were created using the Google text-to-speech API, availing of the default male voice (*SsmlVoiceGender.NEUTRAL*) with the image description labels, either in German or English, based on participants language preference. Auditory stimulus length ranged from 0.66 to 0.95 seconds.

Task description

Localizer task

In the localizer task, the ten graph stimulus items were shown to participants repeatedly in a pseudo-random order, where a DeBruijn-sequence (DeBruijn, 1946 [\[link\]](#)) ensured the number of transitions between any two stimuli was equal. Two runs of the localizer were performed per participant, comprising 250 trials with 25 item repetitions. Each trial started with a fixation cross followed by an inter-trial interval of 0.75 to 1.25 seconds. Next, to encourage a multi-sensory neural representation, the name of the to-be-shown image was played through in-ear head-phones (maximum 0.95 seconds) followed 1.25 to 1.75 seconds later by the corresponding stimulus image, shown for 1.0 second. As an attention check, in ~4% of the trials the auditory stimulus did not match the image and participants were instructed to press a button as fast as possible to indicate detection of an incongruent auditory-visual pair. A short break of maximum 30 seconds was scheduled every 80 trials. Between the two parts of the localizer task, another short break was allowed. Stimulus order was randomized and balanced between subjects. To familiarize the participant with the task, a short exemplar of the localizer task with dummy images was shown beforehand. All subsequent analyses were performed using the visual stimulus onset as a point of reference.

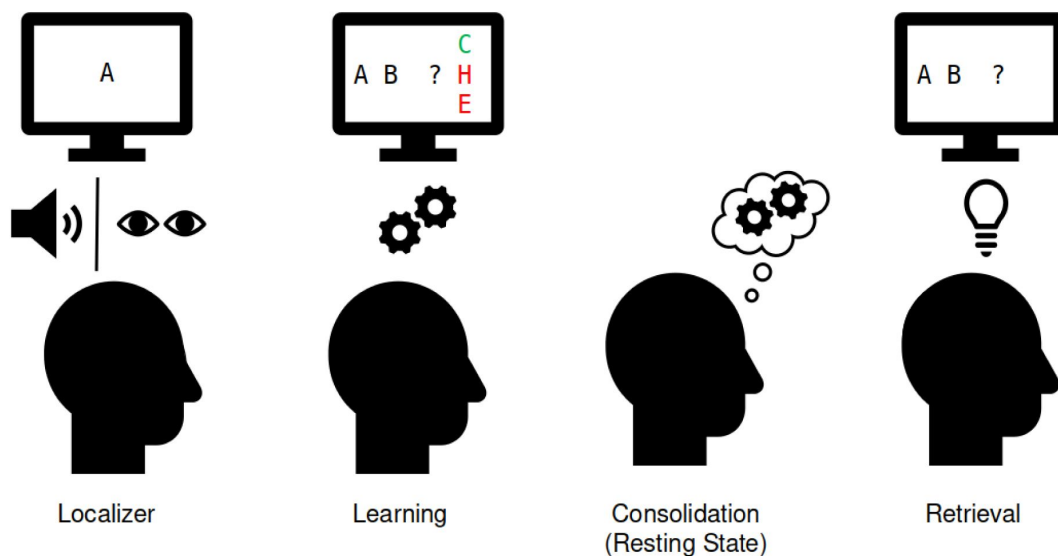


Figure 1

Experimental procedure in the MEG. Localizer task: The ten individual items were repeatedly presented to the participant auditorily and visually to extract multisensory activity patterns. Learning: Participants learned pseudo-randomly generated triplets of the ten items by trial and error. These triplets were determined by an underlying graph structure. Participants were unaware of the exact structure and graph layout. Consolidation: Eight minutes of resting state activity were recorded. Retrieval: Participants' recall was tested by cueing triplets from a sequence. The letters in the pictograms are placeholders for individual images.

Graph-Learning

The exact same images deployed in the localizer task were randomly assigned to nodes of the graph, as shown in **Figure 2** [Error! Reference source not found.](#) B. Participants were instructed to learn a randomized sequence of elements with the goal of reaching 80% performance within six blocks of learning. During each block, participants were presented with each of the twelve edges of the graph exactly once, in a balanced, pseudo-randomized order. After a fixation cross of 3.0 seconds a first image (predecessor) was shown on the left of the screen. After 1.5 seconds, the second image (current image) appeared in the middle of the screen. After another 1.5 seconds, three possible choices were displayed in vertical order to the right of the two other images. One of the three choice options was the correct successor of the cued edge. Of the two distractor stimuli, one was chosen from a distal location on the graph (five to eight positions away from the current item), and one was chosen from a close location (two to four positions away from the current item). Neither of the latter were directly connected to any of the other elements onscreen. Participants used a three-button controller to indicate their answer. The chosen item was then highlighted for 3.0 seconds, and the participant's performance was indicated ("correct" or "wrong") (see **Figure 2C** [Error! Reference source not found.](#)). No audio was played during learning. The participant was instructed to learn the sequence transitions by trial-and-error, and also instructed that there was no semantic connection between items (i.e., that the sequence did not follow any specific logic related to image content). Participants completed a minimum of two, and a maximum of six blocks of learning. To prevent ceiling effects, learning was discontinued if a participant reached 80% accuracy during any block. To familiarize participants with the task, a short example with dummy images was shown before the learning task. Triplets were shown in a random order and choices were displayed in a pseudo-random position that ensured the on-screen position of the correct item could never be at the same position for more than three consecutive trials. Distractor choices were balanced such that exposure to each individual item was approximately equal.

Resting State

After graph learning, participants completed a resting state session of eight minutes. Here, they were instructed to close their eyes and "to not think of anything particular". These resting state data are not reported here.

Retrieval

After the resting state, we presented subjects with a single retrieval session block, which followed the exact layout of the learning task with the exception that no feedback was provided as to whether entered choices were correct or incorrect (**Figure 2D** [Error! Reference source not found.](#)).

MEG Acquisition and Pre-Processing

MEG was recorded in a passively shielded room with a MEGIN TRIUX (MEGIN Oy, Helsinki, Finland) with 306 sensors (204 planar gradiometers and 102 magnetometers) at 1000 Hz with a 0.1–330 Hz band-pass acquisition filter at the ZIPP facility of the Central Institute for Mental Health in Mannheim, Germany. Before each recording, empty room measurements made sure that no ill-functioning sensors were present. Head movement was recorded using five head positioning coils. Bipolar vertical and horizontal electrooculography (EOG) as well as electrocardiography (ECG) was recorded. After recording, the MEGIN proprietary MaxFilter algorithm (version 2.2.14) was run using temporally extended signal space separation (tSSS) and movement correction with the MaxFilter default parameters (Taulu & Simola, 2006 [Error! Reference source not found.](#), raw data buffer length of 10 s, and a subspace correlation limit of .98. Bad channels were automatically detected at a detection limit of 7; none had to be excluded. The head movement correction algorithm used 200 ms windows and steps of 10 ms. The HPI coil fit accept limits were set at an error of 5 mm and a g-value of .98). Using the head movement correction algorithm, the signals were virtually re-positioned to the mean head position during the initial localizer task to ensure compatibility of sensor-level analysis

across the recording blocks. The systematic trigger delay of our presentation system was measured and visual stimuli appeared consistently 19 milliseconds after their trigger value was written to the stimulus channel, however, to keep consistency with previous studies that do not report trigger delay, timings in this publication are reported uncorrected (i.e., ‘as is’, not corrected for this delay).

Data were pre-processed using Python-MNE (version 1.1, [Gramfort, 2013](#)). Data were down-sampled to 100 Hz using the MNE function ‘*resample*’ (with default settings, which applies an anti-aliasing filter before resampling with a brick-wall filter at the Nyquist frequency in the frequency domain) and ICA applied using the ‘*picard*’ algorithm ([Ablin et al., 2018](#)) on a 1 Hz high-pass filtered copy of the signal using 50 components. As recommended, ICA was set to ignore segments that were marked as bad by *Autoreject* ([Jas et al., 2017](#)) on two-second segments. Components belonging to EOG or ECG and muscle artifacts were identified and removed automatically using MNE functions ‘*find_bads_eog*’, ‘*find_bads_ecg*’ and ‘*find_bads_emg*’, using the EOG and ECG as reference signals. Finally, to reduce noise and drift, data were filtered with a high-pass filter of 0.5 Hz using the MNE filter default settings (hamming window FIR filter, -6 dB cutoff at 0.25 Hz, 53 dB stop-band attenuation, filter length 6.6 seconds).

Trials from the localizer and retrieval task were created from -0.1 to 0.5 seconds relative to visual stimulus onset to train decoders. For the sequenceness analysis related to the retrieval, trials were created from 0 to 1.5 seconds after onset of the second visual cue image. No baseline correction was applied. To detect artifacts, *Autoreject* was applied using default settings, which repaired segments by interpolation in case artifacts were present in only a limited number of channels and rejected trials otherwise (see Supplement 1). Finally, to improve numerical stability, signals were re-scaled to similar ranges by multiplying values from gradiometers by $1e^{10}$ and from magnetometers by $2e^{11}$. These values were chosen empirically by matching histograms for both channel types. As outlier values can have significant influence on the computations, after re-scaling, values that were still above 1 or below -1 were “cutoff” and transformed to smaller values by multiplying with $1e^{-2}$. Anonymized and maxfiltered raw data are openly available at Zenodo (<https://doi.org/10.5281/zenodo.8001755>), code is made public on GitHub (<https://github.com/CIMH-Clinical-Psychology/DeSMRRest-clustered-reactivation>).

Decoding framework and training

In line with previous investigations ([Kurth-Nelson et al., 2016](#); [Liu et al., 2019](#); [Wimmer et al., 2020](#)) we applied Lasso regularized logistic regression on sensor-level data of localizer trials using the Python package Scikit-Learn ([Pedregosa et al., 2011](#)). Decoders were trained separately for each participant, and each stimulus, using *liblinear* as a solver with 1000 maximum iterations and a L1 regularization of $C=6$. This value was determined based on it giving the best average cross-validated peak accuracy across all participants when searching within the parameter space of $C = 1$ to 20 in steps of 0.5, using the same approach as outlined below (note that Scikit-Learn shows stronger regularization with lower C values, opposite to e.g., MATLAB). To circumvent class imbalance due to trials removed by *Autoreject*, localizer trials were stratified such that they contained an equal number of trials from each stimulus presentation by randomly removing trials from over-represented classes. Using a cross validation schema (leaving one trial out for each stimulus per fold, i.e., 10 trials left out per fold), for each participant the decoding accuracy was determined across time (**Figure 3A**). During cross validation, for each fold, decoders were trained on data of each 10 milliseconds time step and tested on left out data from the same time step. Therefore, decoding accuracy reflects the separability of the stimulus classes by the sensor values for each time step independently. Decoders were trained using a one-vs-all approach, which means that for each class, a separate classifier was trained using positive examples (target class) and negative examples (all other classes) plus null examples (data from before stimulus presentation, see below). This approach allows the decoder to provide independent estimates of detected events for each class.

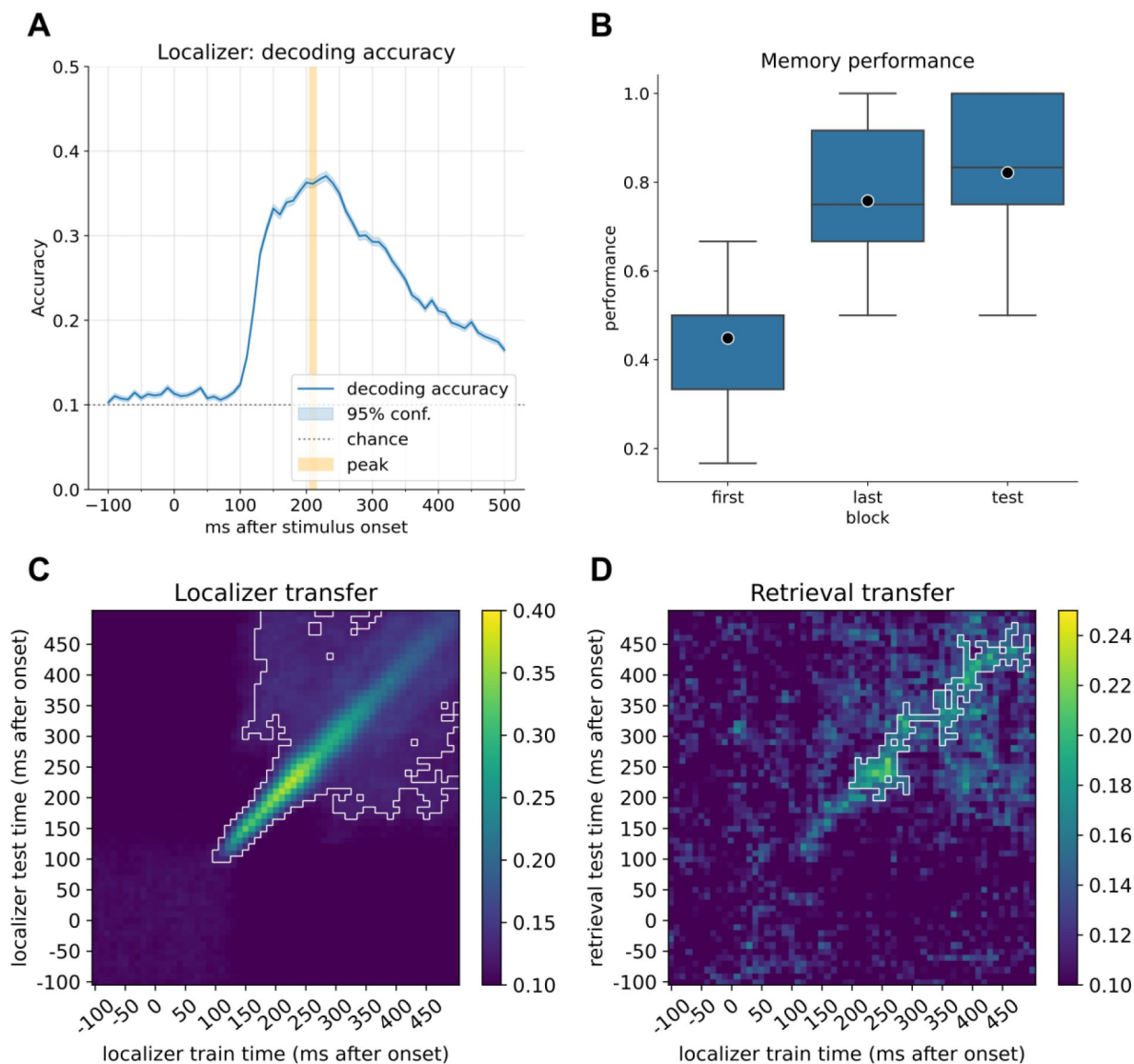


Figure 3

A) Decoding accuracy of the currently displayed item during the localizer task for participants with a decoding accuracy higher than 30% ($n=21$). The mean peak time point across all participants corresponded to 210 ms, with an average decoding peak decoding accuracy of 42% ($n=21$). Note that the displayed graph combines accuracies across participants, where peak values were computed on an individual level and then averaged. Therefore, the indicated individual mean peak does not match the average at a group level. B) Memory performance of participants after completing the first block of learning, the last block (block 2 to 6, depending on speed of learning), and the retrieval performance. C) Classifier transfer within the localizer when trained and tested at different time points determined by cross validation. D) Classifier transfer from the localizer session to the retrieval session when trained at different time points during training and tested at different time points during cue presentation of the first (predecessor) image cue during retrieval. For B and C: Within the white outline, classification was significantly above chance level (cluster permutation testing, $\alpha < 0.05$).

For each participant, a final set of decoders (i.e., 10 decoders per participant, for each stimulus one decoder) were trained at 210 milliseconds after stimulus onset, a time point reflecting the average peak decoding time point computed for all participants (for individual decoding accuracy plots see Supplement 3). For the final decoders, data from before the auditory stimulus onset was added as a negative class with a ratio of 1:2, based upon results from previous publications reaching better sensitivity with higher negative class ratio (Liu, Dolan, et al., 2021). Adding null data allows decoders to report low probabilities for all classes simultaneously in absence of a matching signal, and reduces false positives while retaining relative probabilities between true classes. Together with the use of a sparsity constraint on the logistic regression coefficients, this increases the sensitivity of sequence detection by reducing spatial correlations of decoder weights (see also Liu, Dolan, et al., 2021). For a visualization of relevant sensor positions see Supplement 5.

Decoders were then applied to trials of the retrieval session, starting from the time-point of onset of the second sequence cue (“current image”) and extending to just prior to onset of the selection prompt (1.5 seconds). For each trial, this resulted in ten probability vectors across the trial, one for each item, in steps of 10 milliseconds. These probabilities indicate the similarity of the current sensor-level activity to the activity pattern elicited by exposure to the stimulus and can therefore be used as a proxy for detecting active representations, akin to a representational pattern analysis approach (RSA, Grootswagers et al., 2017). As a sanity check, we confirmed that we could decode the currently on-screen image by applying the final trained decoders to the first image shown during retrieval (predecessor stimulus, see **Figure 3D**). Note that we only included data from the current image cue, and not from the predecessor image cue, as we assume the retrieval processes differ and should not be concatenated.

Sequential replay analysis

To test whether individual items were reactivated in sequence at a particular time lag, we applied temporally delayed linear modeling (TDLM, Liu, Dolan, et al., 2021) on the time span after the stimulus onset of the sequence cue (“current image”). In brief, this method approximates a time lagged cross-correlation of the reactivation strength in the context of a particular transition pattern, quantifying the strength of a certain activity transition pattern distributed in time. As input for the sequential analysis, we used the raw probabilities of the ten classifiers corresponding to the stimuli.

Using a linear model, we first estimate evidence for sequential activation of the decoded item representations at different time lags. For each item i at each time lag Δt up to 250 milliseconds we estimated a linear model of form:

$$Y_i = Y(\Delta t) \times \beta_i(\Delta t)$$

where Y_i contains the decoded probability output of the classifier of item i and $Y(\Delta t)$ is simply Y time lagged by Δt . When solving this equation for $\beta_i(\Delta t)$ we can estimate the predictive strength of $Y(\Delta t)$ for the occurrence of Y_i at each time lag Δt . Calculated for each stimulus i , we then create an empirical transition matrix $T_e(\Delta t)$ that indexes evidence for a transition of any item j to item i at time lag Δt (i.e., a 10x10 transition matrix per time lag, each column j contains the predictive strength of j for each item i at time lag Δt). These matrices are then combined with a ground truth transition matrix T (encoding the valid sequence transitions of interest) by taking the Frobenius inner product. This returns a single value $Z_{\Delta t}$ for each time lag, indicating how strongly the detected transitions in the empirical data follow the expected task transitions, which we term “sequenceness”. Using different transition matrices to depict forward (T_f) and backward (T_b) replay, we quantified evidence for replay at different time lags for each trial separately. This process is applied to each trial individually, and resulting sequenceness values are averaged to provide a final sequenceness value per participant for each time lag Δt . To test for statistical significance, we create a baseline distribution by permuting the rows of the transition matrix 1000 times (creating transition matrices with random transitions; identity-based permutation, Liu,

Dolan, et al., 2021) and calculate sequenceness across all time lags for each permutation. The null distribution is then constructed by taking the peak sequenceness across all time lags for each permutation.

Differential reactivation analysis

To test for clustered, non-sequential reactivation, we adopted the approach used in Wimmer et al. (2020) [\[1\]](#). Decoders were trained independently for each stimulus, and all decoders reacted to presentation of any visual stimulus to some extent. By using differences in reactivation between stimuli, this aggregated approach allowed us to examine whether near items are more strongly activated than distant items more closely, thereby quantifying non-sequential reactivation with greater sensitivity. For each trial, the mean probability of the two items following the current on-screen item was contrasted with the mean probability of all items further away by subtraction. We chose to combine the following pairs of items for two reasons: First, this doubled the number of included trials; secondly, using this approach the number of trials for each category (“near” and “distant”) was more balanced. The two items currently displayed on-screen (i.e., predecessor and current image) were excluded. As only few trials per participant were available for this analysis, the raw probabilities were noisy. Therefore, to address this we applied a Gaussian smoothing kernel (using `scipy.ndimage.gaussian_filter` with the default parameter of $\sigma = 1$ which corresponds approximately to taking the surrounding timesteps in both direction with the following weighting: current time step: 40%, ± 1 step: 25%, ± 2 step: 5%, ± 3 step: 0.5%) to the probability vectors across the time dimension. By shuffling the stimulus labels 1000 times, we constructed an empirical permutation distribution to determine at which time points the differential reactivation of close items was significantly above chance ($\alpha = 0.05$).

Graph reactivation analysis

To detect whether reactivation strength was modulated by underlying graph structure, we compared the raw reactivation strength of all items by distance on the directed graph. First, we calculated a time point of interest by computing the peak probability estimate of decoders across all trials, i.e., the average probability for each timepoint of all trials, of all distances except the previous onscreen item. Then, for each participant, for each trial we sorted all nodes based on their distance to the current on-screen item on the directed graph. Again, we smoothed probability values with a Gaussian kernel ($\sigma = 1$) and ignored the predecessor on-screen item. Following this, we evaluated the sorted decoder probabilities at the previously determined peak time point. Using a repeated measures ANOVA on the mean probability values per distance per participant, we then estimated whether reactivation strength was modulated by graph distance.

Exclusions

Replay analysis relies on a successive detection of stimuli where the chance of detection exponentially decreases with each step (e.g., detecting two successive stimuli with a chance of 30% leaves a 9% chance of detecting a replay event). However, one needs to bear in mind that accuracy is a “winner-takes-all” metric indicating whether the top choice also has the highest probability, disregarding subtle, relative changes in assigned probability. As the methods used in this analysis are performed on probability estimates and not class labels, one can expect that the 30% are a rough lower bound and that the actual sensitivity within the analysis will be higher. Additionally, based on pilot data, we found that attentive participants were able to reach 30% decodability, allowing its use as a data quality check. Therefore, we decided a priori that participants with a peak decoding accuracy of below 30% would be excluded from the analysis (nine participants in all) as obtained from the cross-validation of localizer trials. Additionally, as successful learning was necessary for the paradigm, we ensured all remaining participants had a retrieval performance of at least 50% (see Supplement 2).

Results

Behavioral

All but one participant learned the sequence of ten images embedded into the directed graph with partial overlap (Supplement 3). On average, participants needed 5 blocks of learning (range 2 to 6, see Supplement 4) and attained a memory performance of 76% during their last block of learning (range: 50% to 100%). After eight minutes of rest, retrieval performance improved marginally to a mean of 82% ($t=-2.053$, $p=0.053$, effect size $r=0.22$ **Figure 3B** [↗](#)). Note that since the last learning block included feedback, this marginal increase cannot necessarily be attributed to consolidation processes. Additionally, we have included an analysis showing how wrong answers participants provided were random in the first block and biased towards closer graph nodes in later blocks. This is consistent with participants actually learning the underlying graph structure as opposed to independent triplets (see figure and legend of Supplement 6 for details).

Decoder training

We first confirmed we could decode brain activity elicited by the ten items using a cross-validation approach. Indeed, decoders were able to separate the items presented during the localizer task (see **Figure 3A** [↗](#)) well, with an average peak decoding accuracy of ~42% across all participants (range: 32% to 57%, chance level: 10%, excluding participants with peak accuracy < 30%, for all participants see Supplement 3). We calculated the time point of the mean peak accuracy for each participant separately and subsequently used the average best time point, across all included participants, at 206 milliseconds (rounded to 210 milliseconds) for training of our final decoders. This value is very close in range to time points found in previous studies (Kurth-Nelson et al., 2016 [↗](#); Liu et al., 2019 [↗](#); Liu, Mattar, et al., 2021; Wimmer et al., 2020 [↗](#)). The decoders also transferred well to stimulus presentation during the retrieval trials and could effectively decode the current prompted image cue with above chance significance (cluster permutation test, see **Figure 3D** [↗](#)).

Sequential forward replay in subjects with lower memory performance

Next, we assessed whether there was evidence for sequential replay of the learned sequences during cued recall. Using TDLM we asked whether decoded reactivation probabilities followed a sequential temporal pattern, in line with transitions on the directed graph. Here we focused on all allowable graph transitions and analyzed the entire time window, of 1500 milliseconds, after onset of the retrieval cue ("current image"). We found positive sequenceness across all time lags for forward sequenceness, with a significant increase at around 40-50 milliseconds state to state lag for forward sequenceness (**Figure 4A** [↗](#)). As discussed in Liu, Dolan et al. (2021), correction for multiple comparisons for this sequenceness measure across time is non-trivial and the maximum of all permutations represents a highly conservative statistic. Due to this complexity, we also report the 95% percentile of sequenceness maxima across time per permutation. Nevertheless, as we did not have a pre-defined time lag of interest, and to mitigate multiple-comparisons, we additionally computed the mean sequenceness across all computed time lags for each participant (similar as previously proposed in the context of a sliding window approach in Wise et al., 2021 [↗](#)). This measure can help reveal an overall tendency for replay of task states that is invariant to a specific time lag. Our results show that across all participants, there is a significant increase in task-related forward sequential reactivation of states ($p=0.027$, two-sided permutation test with 1000 permutations; 95% of permutation maxima reached at 40-50 ms, **Figure 4B** [↗](#)). Following up on this, in a second analysis, we asked whether mean sequential replay was associated with memory performance and found a significant negative correlation between retrieval performance and forward replay (forward: $r=-0.46$, $p=0.031$; backward: $r=-0.13$, $p=0.56$,

see [Figure 4C](#)). In line with previous results ([Wimmer et al., 2020](#)) low-performing participants had higher forward sequenceness when compared to high-performing participants, whose mean sequenceness tended towards zero.

Closer nodes show stronger reactivation than distant nodes

Next, in a complementary analysis, we asked whether a non-sequential clustered reactivation of items occurs after onset of a cue image (as shown previously for high performers in [Wimmer et al., 2020](#)). We compared reactivation strength of the two items following the cue image with all items associated a distance of more than two steps, subtracting the mean decoded reactivation probabilities from each other. Using this differential reactivation, we found evidence consistent with near items being significantly reactivated compared to items further away within a time window of 220 ms to 260 ms after cue onset ([Figure 5A](#), $p < 0.05$, permutation test with 10000 shuffles).

To further explore the relation of reactivation strength and graph distances, we analyzed the mean reactivation strength by item distance at peak classifier probabilities and found reactivation strength significantly related to graph distance (repeated measures ANOVA, $F(4, 80) = 2.98$, $p = 0.023$ [Figure 5B](#)). When subdividing trials into correct and incorrect responses, we found that this relationship was only significant for trials where a participant successfully retrieved the currently prompted sequence excerpt (repeated measures ANOVA, $F(4, 80) = 5.0$, $p = 0.001$ for correctly answered trials, [Figure 5C](#)). For incorrect trials we found no evidence for this relationship ($F(4, 48) = 1.45$, $p = 0.230$ for incorrectly answered trials), albeit we found no interaction between distance and response type ($F(4, 48) = 1.8$, $p = 0.13$). Note, that the last two analysis are based on $n = 14$ since 7 participants had no incorrect trials.

To examine how the 8-minute consolidation period affected reactivation we, post-hoc, looked at relevant measures across learning trials in contrast to retrieval trials. For all learning trials, for each participant, we calculated differential reactivation for the same time point we found significant in the previous analysis (220-260 milliseconds). On average, differential reactivation probability increased from pre to post resting state ([Figure 5D](#)).

Questionnaire results

Participants were concentrated and alert as indicated by the Stanford Sleepiness Scale ($M = 2.3$, $SD = 0.6$, range 1-3). Participants' summed positive affect score was on average 33.2 ($SD = 4.5$), their summed negative affect score was on average 12.2 ($SD = 1.9$) (PANAS). Individual questionnaire answers for each included participants are available in the supplementary download in the code repository at GitHub.

Discussion

We combined a graph-based learning task with machine learning to study neuronal events linked to memory retrieval. Participants learned triplets of associated images by trial and error, where these were components of a simple directed graph with ten nodes and twelve edges. Using machine learning decoding of simultaneously recorded MEG data we asked what brain processes are linked to retrieval of this learned information, and how this relates to the underlying graph structure. We show that learned graph items are retrieved by a simultaneous, clustered, reactivation of items and that the associated reactivation strength relates to graph distances.

Memory retrieval is thought to involve reinstatement of previously evoked item-related neural activity patterns ([Danker & Anderson, 2010](#); [Johnson & Rugg, 2007](#); [Staresina et al., 2012](#)). Both spatial and abstract information is purported to be encoded into cognitive maps within the

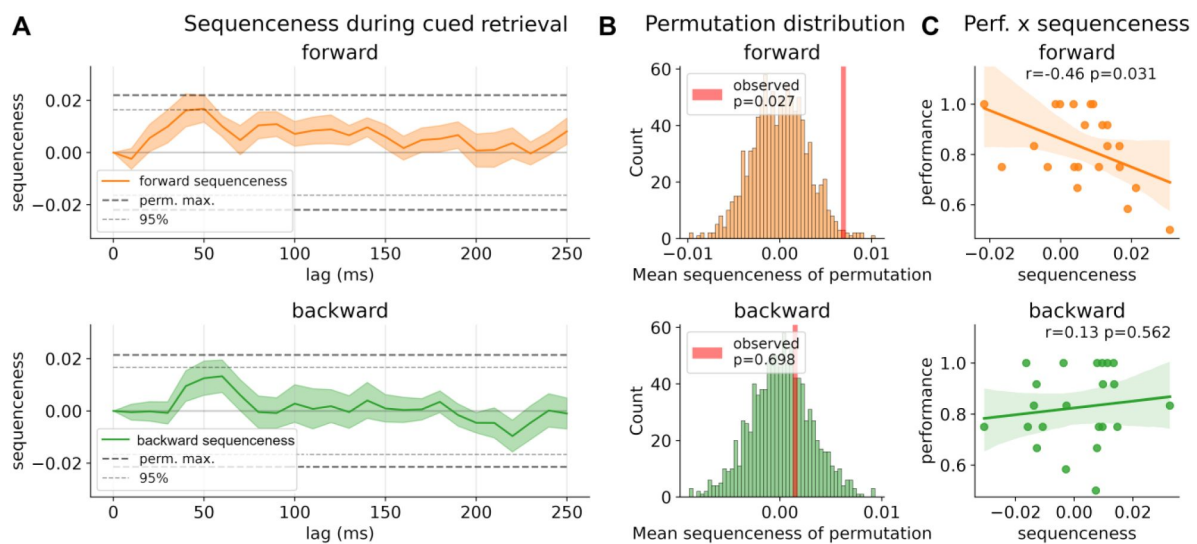


Figure 4

A) Strength of forward and backward sequenceness across different time lags up to 250 ms during the 1500 ms window after cue onset. Two significance thresholds are shown: Conservative threshold of the maximum of 1000 permutations of classification labels across all time lags and the 95% percentiles (see Methods section for details). B) Permutation distribution of mean sequenceness values across 1000 state permutations. Observed mean sequenceness is indicated with a red line. C) Association between memory performance and mean sequenceness value computed across all trials, and time lags, for each participant.

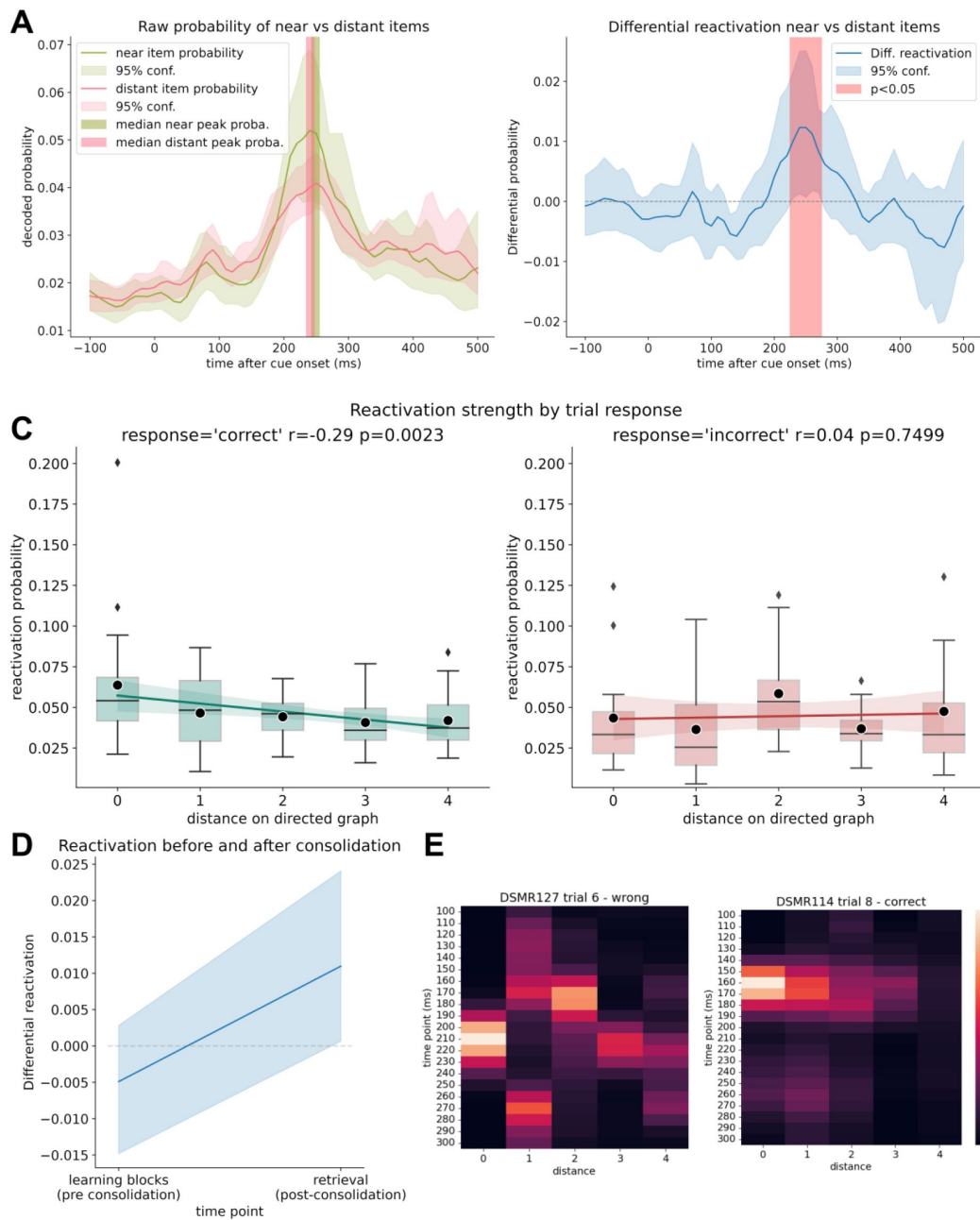


Figure 5

A) Decoded raw probabilities for off-screen items, that were up to two steps ahead of the current stimulus cue ('near',) vs. distant items that were more than two steps away on the graph, on trials with correct answers. The median peak decoded probability for near and distant items was at the same time point for both probability categories. Note that displayed lines reflect the average probability while, to eliminate influence of outliers, the peak displays the median. B) Differential reactivation probability between off-screen items that were up to two steps ahead of the current stimulus cue vs. distant items that were more than two steps away on the graph for trials with correct answers. Between 220 and 260 ms the next items are simultaneously reactivated significantly more than items that are further away ($p < 0.05$; permutation test with 10000 shuffles). C) Reactivation strength of items after retrieval cue onset by distance of items to the currently on-screen stimulus subdivided into trials in which participants answered correctly (left) and in which participants did not know the correct answer (right). A correlation between reactivation strength and distance can only be seen in case of successful retrieval (but see also limitations for a discussion of the low trial and participant number in this sub-analysis). Mean probability values are marked by black dots. D) Mean differential reactivation at peak time point (220-260 milliseconds) during all learning trials (before consolidation) compared to retrieval trials. E) Example activations of a successful retrieval (left) and a failed retrieval (right), sorted by distance to current cue. Colors indicate probability estimates of the decoders.

hippocampus and related structures (Behrens et al., 2018 [↗](#); Bellmund et al., 2018 [↗](#); Epstein et al., 2017 [↗](#); Garvert et al., 2017 [↗](#); O’Keefe & Nadel, 1979 [↗](#); Peer et al., 2021 [↗](#)). While, for example, spatial distance within cognitive maps is encoded within hippocampal firing patterns (Theves et al., 2019 [↗](#)), it is unclear how competing, abstract, candidate representations are accessed during retrieval (Kerrén et al., 2018 [↗](#), 2022 [↗](#); Spiers, 2020 [↗](#)). Two separate mechanisms seem plausible. First, depth-first search might enable inference in not yet fully consolidated cognitive maps by sequential replay of potential candidates (Mattar & Daw, 2018 [↗](#); Nyberg et al., 2022 [↗](#)). Second, breadth-first search could be deployed involving simultaneous activation of candidates when these are sufficiently consolidated within maps that support non-interfering co-reactivation of competing representations (Mattar & Lengyel, 2022 [↗](#)), or when exhaustive replay would be too expensive computationally. Indeed, consistent with this, Wimmer et al., (2020 [↗](#)) showed that for regular memory performance, sequential and temporally spaced reactivation of items seems to ‘piece together’ individual elements. This contrasted with high performers who showed a clustered, simultaneous, reactivation profile. We replicate this clustered reactivation and show that its strength reflects distance on a graph structure. This complements previous findings of graded pattern similarity during memory search representing distance within the search space (Manning et al., 2011 [↗](#); Tarder-Stoll et al., 2023 [↗](#)). As this effect was evident only for correct choices the finding points to its importance for task performance.

As per Wimmer et al. (2020 [↗](#)), we found that the strength of replay related to weaker memory performance. This suggests that the expression of sequential replay or simultaneous reactivation depends on the stability of an underlying memory trace. However, we acknowledge that it remains unclear which factors enable recruitment of either of these mechanisms. A crucial step in consolidation encompasses an integration of memory representations into existing networks (Dudai et al., 2015 [↗](#); Sekeres et al., 2017 [↗](#)). In Wimmer et al. (2020 [↗](#)), participants had little exposure to the learning material and replay was measured after a substantial retention period that included sleep, where the latter is considered to strengthen and transform memories via repeated replay (Diekelmann & Born, 2010 [↗](#); Feld & Born, 2017 [↗](#)). This contrasts with the current task design, which solely involved several blocks of learning and retrieval and only a relatively brief period of consolidation.

Intriguingly, it has been speculated that retrieval practice may elicit the same transformation of memory traces as offline replay (Antony et al., 2017 [↗](#)). In line with this reasoning, it is possible that both consolidation during sleep and repeated practice have similar effects on the transformation of memories, and consequently on mechanisms that support their subsequent retrieval. This possibility is especially interesting in the light of retrieval practice enhancing memory performance more than is the case for restudy (McDermott, 2021 [↗](#)), a finding also in line with evidence that replay during rest prioritizes weakly learned memories (Schapiro et al., 2018 [↗](#)). It is known that retrieval practice reduces the pattern similarity of competing memory traces in the hippocampus (Hulbert & Norman, 2015 [↗](#)) and, as in the case of our graph-based task, may enable clustered reactivation since differences in timing of reactivation are no longer required to distinguish correct from incorrect items. Therefore, we speculate that clustered reactivation may be a physiological correlate of retrieval facilitated either by repeated retrieval testing-based learning (as in our study) or by sleep dependent memory consolidation (as in Wimmer et al., 2020 [↗](#)). This implies that there may be a switch from sequential replay to clustered reactivation corresponding to when learned material can be accessed simultaneously without interference. This suggestion could be systematically investigated by, for example, manipulating retrieval practice, retention interval, and the difficulty of a graph-based task. Nevertheless, even though our results show a nominal increase in reactivation from learning to retrieval (see **Figure 5D** [↗](#)), due to experimental design features our data do not enable us to test for an hypothesized switch for sequential replay (see also “limitations” and Supplement 8). Finally, even though we primarily focused on the mean sequenceness scores across time lags, there appears to be a (non-

significant) peak at 40-60 milliseconds. While simultaneous forward and backward replay is theoretically possible, we acknowledge that it is somewhat surprising and, given our paradigm, could relate to other factors such as autocorrelations (Liu, Dolan, et al., 2021).

Limitations

There are limitations to our study, many of which originate from a suboptimal study design that resulted in a relatively limited number of trials for the retrieval session per participant. Additionally, as we performed criteria learning, a sub-group analysis as in Wimmer et al., (2020) [was not feasible](#), as median performance in our sample was 83% (mean 81%), with six participants exactly at that threshold, resulting in a very high cut-off. Our design also meant participants had different number of learning blocks (two to six blocks, see Supplement 4), making a comparison of learning progress across participants difficult. Finally, while we closely follow the analysis approach taken in Wimmer et al., (2020) [we did not explicitly preregister the confirmatory analysis of the retrieval data as such](#). We do acknowledge that only a somewhat limited number of trials were available for analysis, affecting especially the analysis of incorrect answers. In addition, the number of low-performing participants was low in our study, which would render a performance-dependent sub-analysis underpowered.

Conclusion

Our findings support a role for a clustered reactivation mechanism for well-learned items during memory retrieval. When interconnected semantic information is retrieved, the retrieval process seems to resemble a breadth-first search, with items sorted by neural activation strength. Additionally, we find that the presence of sequential replay related to low memory performance. The likely coexistence of two types of retrieval process, recruited dependent on the participants' learning experience, is an important direction for future research. The use of more complex memory tasks, such as explicitly learned associations of graph networks, should enable a more systematic study of this process. Finally, we suggest that accessing information embedded in a knowledge network may benefit from recruitment of either process, replay or reactivation, on the fly.

Data availability

MaxFiltered and anonymized MEG raw data as well as behavioural results are available at Zenodo (<https://doi.org/10.5281/zenodo.8001755>).

Code availability

The code of the analysis as well as the experiment paradigm and the stimulus material is available at <https://github.com/CIMH-Clinical-Psychology/DeSMRRest-clustered-reactivation>.

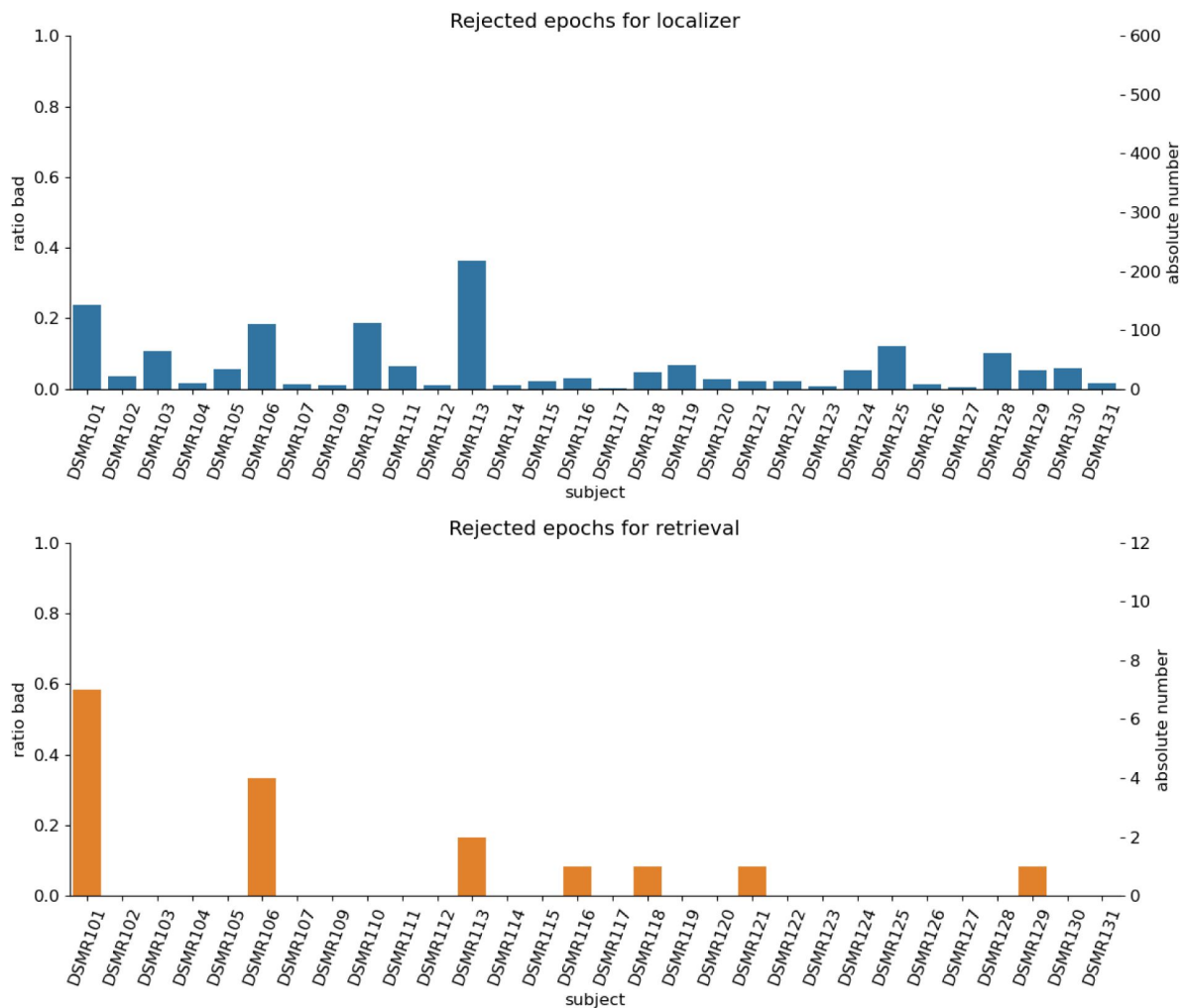
Supplement figures

For supplementary figures of this draft, see after references.

Acknowledgements

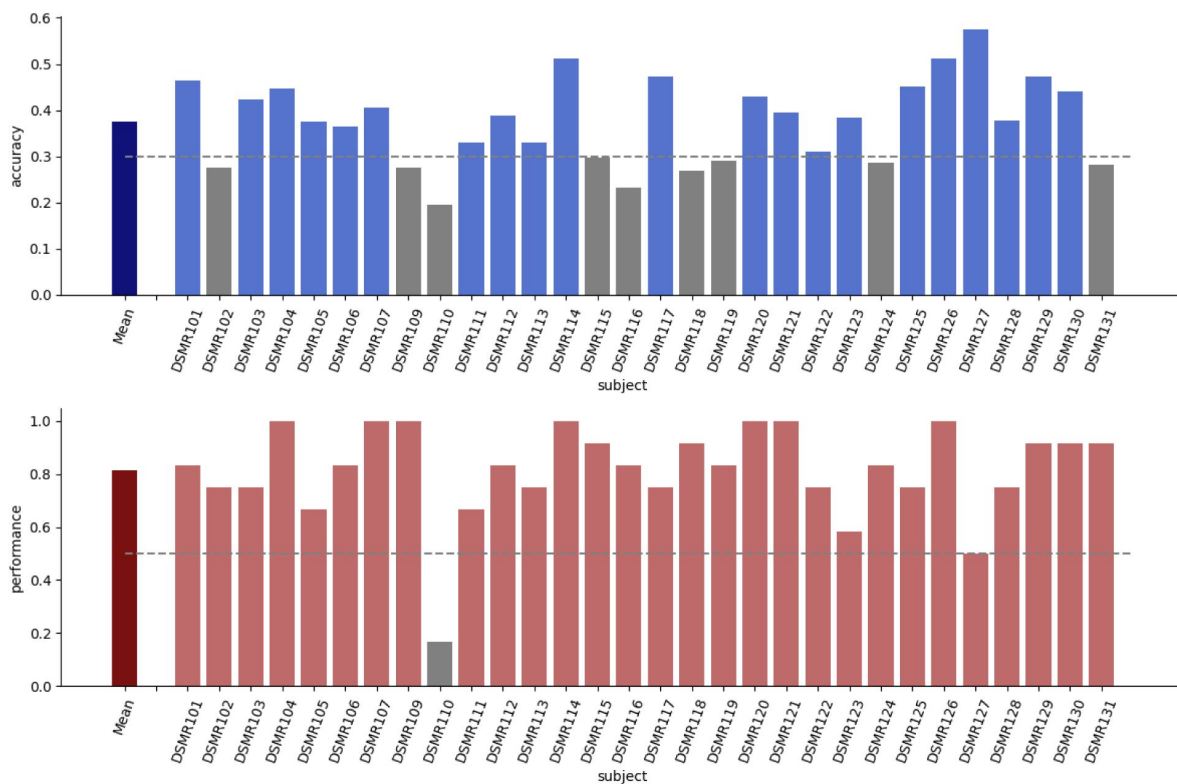
This research was supported by an Emmy-Noether research grant awarded to GBF by the DFG (FE1617/2-1) and a project grant by the DGSM as well as a doctoral scholarship of the German Academic Scholarship Foundation, both awarded to SK. Additionally, we want to thank the ZIPP core facility of the Central Institute of Mental Health for their generous support of the study.

Supplement



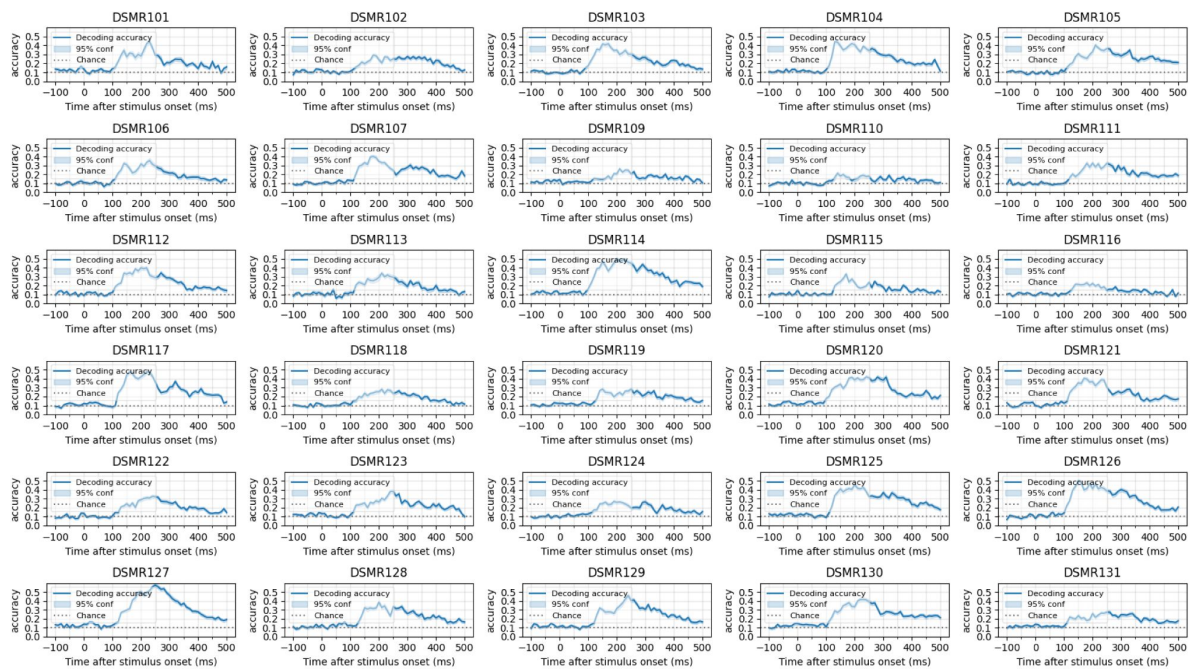
Supplement 1

Percentage of rejected trials for each participant. Artifacts were detected automatically by AutoReject. If possible, channels were interpolated for the affected time span, else the trial was rejected. The figure displays the ratios as well as the absolute number of rejected epochs for each participant in the study. The analysis is based on the remaining non-rejected epochs. For the retrieval, on average 11.5 epochs were available, in total 252 across the study.



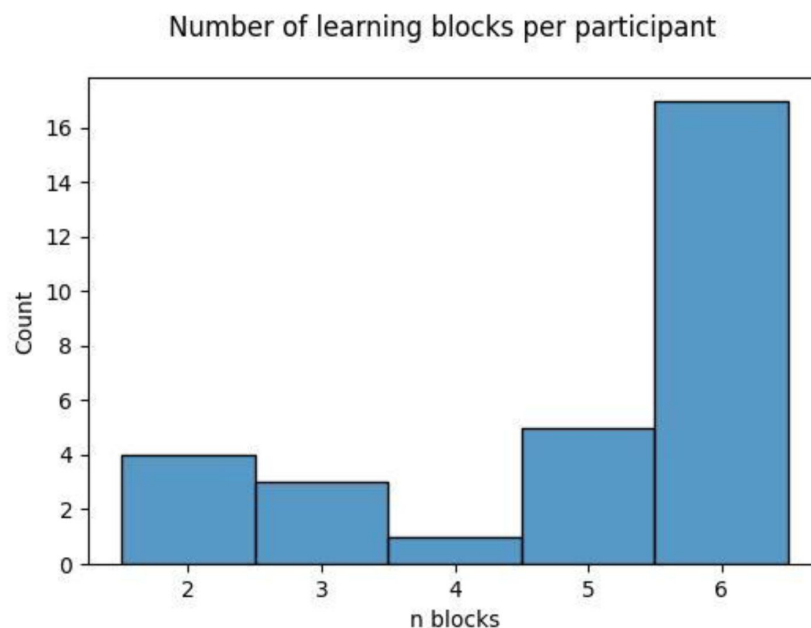
Supplement 2

Excluded participants based on decoding accuracy and memory performance during retrieval. Peak decoding accuracy was determined by a leave-one-per-class cross validation across time for each participant. Memory performance was the percentage of correct responses during the twelve retrieval trials.



Supplement 3

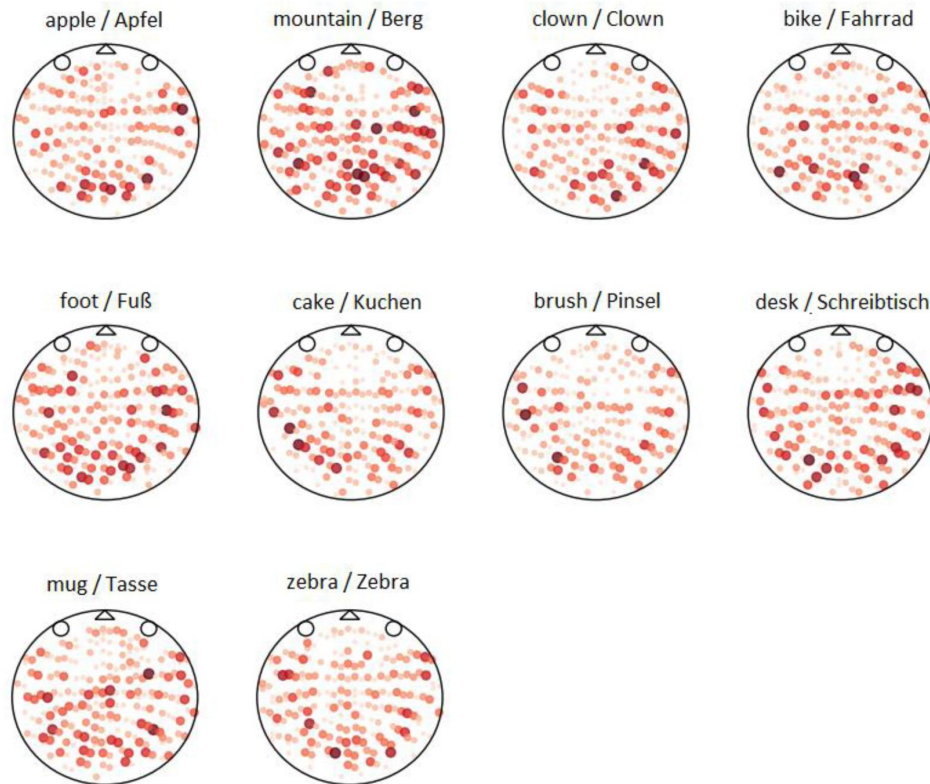
Decoding accuracy across time determined by a leave-one-per-class-out cross-validation per participant. For details on decoder training see the methods section.



Supplement 4

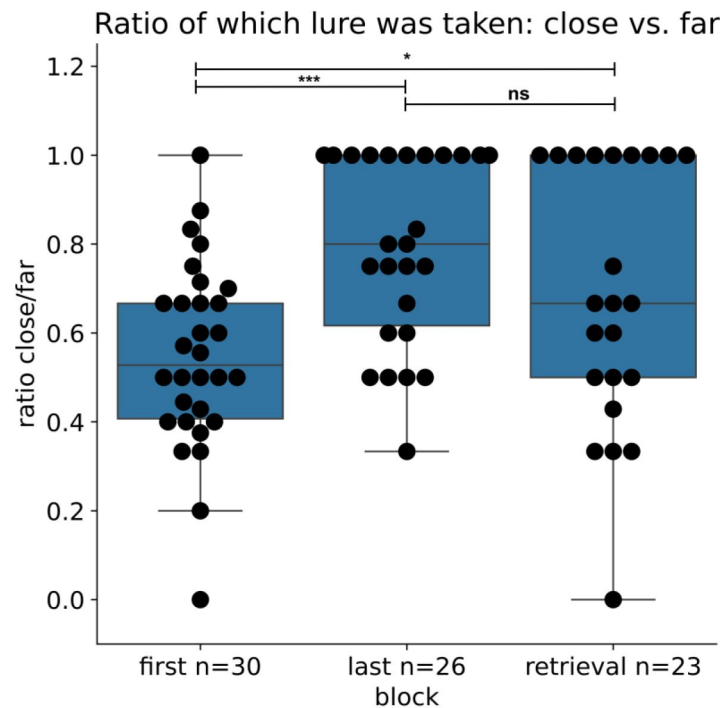
Number of learning blocks that each participant completed. The number of learning blocks was adapted to the speed of the participant such that each participant had a similar performance at their last block. Learning was stopped if participants reached at least 80% memory performance in a block or if they reached 6 blocks. A minimum of two blocks were shown, even if participants reached above 80% in their first block (by chance).

Sensor distribution for different images



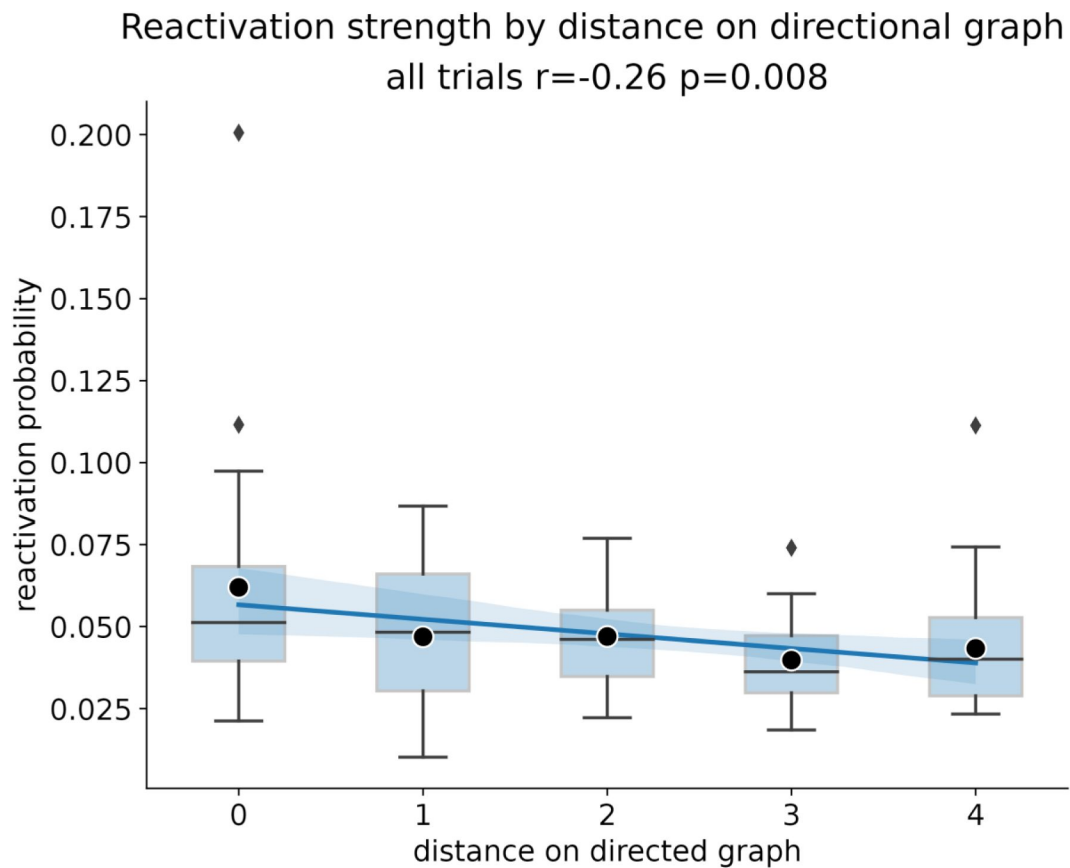
Supplement 5

Percentage of sensors relevant for each image across all participants (beta weight of sensor location unequal to zero). Larger/darker dots indicate more participants' decoders' used information from this sensor. LASSO/L1 regularization forces individual regressor values of the classifier belonging to a specific sensor to 0, such that only a sparse number of sensors contribute information to the decision process. The plot shows the average ratio that a sensor was included across participants, giving a rough estimate for location of stimulus processing. The largest dot indicates that this sensor was used for all participants for this image for this image. The smallest/lightest dot indicates that almost no participant's decoder used information from this sensor. Please note that the MEG head positioning was not aligned between participants such that the average dots do not indicate a specific location but only a broad region.



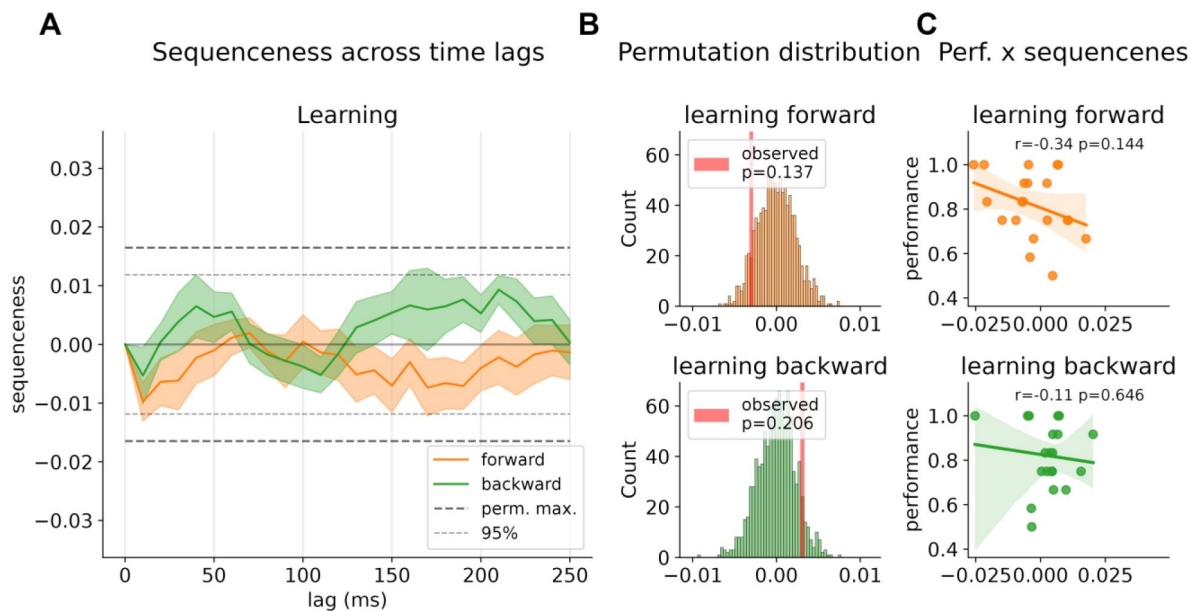
Supplement 6

During the learning and retrieval blocks, participants were presented two lures next to the correct answer to complete the triplet, one of which was closer to the target and one further away on the graph. To show that participants indeed learned the graph structure and not just triplets, the figure shows the ratio of close lures chosen vs lures that were further away on the graph. In the first learning block, the chosen lure is random as participants have not learned the graph structure yet. On the last learning block, many participants exclusively choose the closer lure, indicating that they are aware of approximate distances of the presented stimuli. Note however that the analysis relies solely on trials with incorrect responses. Therefore, the apparent (nonsignificant) drop of the ratio from the last block to the retrieval block can be attributed to participants reaching ceiling performance. Additionally, the number of blocks was determined by the learning speed of the participant (with a minimum of two learning blocks), making it hard to compare between participants with different numbers of learning blocks. Therefore, we have decided to plot the first, last and retrieval blocks, as they were defined for each participant. An ANOVA indicated that the three blocks were significantly different ($F=7.5$, $p=0.001$), a posthoc T-test indicated a significant difference between the first and last ($t=-4.3$, $p<0.0001$) and the first and retrieval session ($t=-2.0$, $p=0.046$) and no difference between the last and retrieval block ($t=1.4$, $p=0.16$).



Supplement 7

Reactivation strength of items after retrieval cue onset by distance of items to the currently on-screen stimulus. A significant negative correlation between distance on a directional graph and reactivation strength can be seen ($p=0.008$). The correlation is shown for both, correct and incorrect answers. For a sub-analysis of correct and incorrect analysis, see



Supplement 8

sequential replay for all learning blocks. A) Strength of forward and backward sequenceness across different time lags. (see Methods section and [Figure 4](#) for details). B) Permutation distribution of mean sequenceness values across 1000 state permutations. C) Association between memory performance and mean sequenceness value computed across all trials, and time lags, for each participant. Note: As the paradigm applied criteria learning, participants had different amount of blocks and hence different exposure at different time points (see Supplement [Figure 4](#)), making a block-wise comparison between participants conceptually difficult. Therefore, to alleviate the bias of different learning speeds, we combined all trials of the learning blocks.

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Reviewer #1 (Public Review):

Summary:

Previous work in humans and non-human animals suggests that during offline periods following learning, the brain replays newly acquired information in a sequential manner. The present study uses an MEG-based decoding approach to investigate the nature of replay/reactivation during a cued recall task directly following a learning session, where human participants are trained on a new sequence of 10 visual images embedded in a graph structure. During retrieval, participants are then cued with two items from the learned sequence, and neural evidence is obtained for the simultaneous or sequential reactivation of future sequence items. The authors find evidence for both sequential and clustered (i.e., simultaneous) reactivation. Replicating previous work, low-performing participants tend to show sequential, temporally segregated reactivation of future items, whereas high-performing participants show more clustered reactivation. Adding to previous work, the authors show that an image's reactivation strength varies depending on its proximity to the retrieval cue within the graph structure.

Strengths:

As the authors point out, work on memory reactivation has largely been limited to the retrieval of single associations. Given the sequential nature of our real-life experiences, there is clearly value in extending this work to structured, sequential information. State-of-the-art decoding approaches for MEG are used to characterize the strength and timing of item reactivation. The manuscript is very well written with helpful and informative figures in the main sections. The task includes an extensive localizer with 50 repetitions per image, allowing for stable training of the decoders and the inclusion of several sanity checks demonstrating that on-screen items can be decoded with high accuracy.

Weaknesses:

Of major concern, the experiment is not optimally designed for analysis of the retrieval task phase, where only 4 min of recording time and a single presentation of each cue item are available for the analyses of sequential and non-sequential reactivation. In their revision, the authors include data from the learning blocks in their analysis. These blocks follow the same trial structure as the retrieval task, and apart from adding more data points could also reveal a possible shift from sequential to clustered reactivation as learning of the graph structure progresses. The new analyses are not entirely conclusive, maybe given the variability in the number of learning blocks that participants require to reach criterion. In principal, they suggest that reactivation strength increases from learning (pre-rest) to final retrieval (post-rest).

On a more conceptual note, the main narrative of the manuscript implies that sequential and clustered reactivation are mutually exclusive, such that a single participant would show either one or the other type. With the analytic methods used here, however, it seems possible

to observe both types of reactivation. For example, the observation that mean reactivation strength (across the entire trial, or in a given time window of interest) varies with graph distance does not exclude the possibility that this reactivation is also sequential. In fact, the approach of defining one peak time window of reactivation may bias towards simultaneous, graded reactivation. It would be helpful if the authors could clarify this conceptual point. A strong claim that the two types of reactivation are mutually exclusive would need to be substantiated by further evidence, for instance a suitable metric contrasting "sequenceness" vs "clusteredness".

On the same point, the non-sequential reactivation analyses use a time window of peak decodability that is determined based on the average reactivation of all future items, irrespective of graph distance. In a sequential forward cascade of reactivations, it could be assumed that the reactivation of near items would peak earlier than the reactivation of far items. In the revised manuscript, the authors now show the "raw" timecourses of item decodability at different graph distances, clearly demonstrating their peak reactivation times, which show convincingly that reactivation for near and far items occurs at very similar time points. The question that remains, therefore, is whether the method of pre-selecting a time window of interest described above could exert a bias towards finding clustered reactivation.

<https://doi.org/10.7554/eLife.93357.2.sa1>

Reviewer #2 (Public Review):

Summary:

The authors investigate replay (defined as sequential reactivation) and clustered reactivation during retrieval of an abstract cognitive map. Replay and clustered reactivation were analysed based on MEG recordings combined with a decoding approach. While the authors state to find evidence for both, replay and clustered reactivation during retrieval, replay was exclusively present in low performers. Further, the authors show that reactivation strength declined with an increasing graph distance.

Strengths:

The paper raises interesting research questions, i.e., replay vs. clustered reactivation and how that supports retrieval of cognitive maps. The paper is well written, well structured and easy to follow. The methodological approach is convincing and definitely suited to address the proposed research questions.

The paper is a great combination between replicating previous findings (Wimmer et al. 2020) with a new experimental approach but at the same time presenting novel evidence (reactivation strength declines as a function of graph distance).

What I also want to positively highlight is their general transparency. For example, they pre-registered this study but with a focus on a different part of the data and outlined this explicitly in the paper.

The paper has very interesting findings. However, there are some shortcomings especially in the experimental design. These are shortly outlined below but are also openly and in detail discussed by the authors.

Weaknesses:

The individual findings are interesting. However, due to some shortcomings in the experimental design they cannot be profoundly related to each other. For example, the authors show that replay is present in low but not in high performers with the assumption

that high performers tend to simultaneously reactivate items. But then, the authors do not investigate clustered reactivation (= simultaneous reactivation) as a function of performance due to a low number of retrieval trials and ceiling performance in most participants. As a consequence of the experimental design, some analyses are underpowered (very low number of trials, $n = \sim 10$, and for some analyses, very low number of participants, $n = 14$).

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Author response:

The following is the authors' response to the original reviews.

Reviewer 1

(1) Given the low trial numbers, and the point of sequential vs clustered reactivation mentioned in the public review, it would be reassuring to see an additional sanity check demonstrating that future items that are currently not on-screen can be decoded with confidence, and if so, when in time the peak reactivation occurs. For example, the authors could show separately the decoding accuracy for near and far items in Fig. 5A, instead of plotting only the difference between them.

We have now added the requested analysis showing the raw decoded probabilities for near and distant items separately in Figure 5A. We have also chosen to replace Figure 5B with the new figure as we think it provides more information than the previous Figure 5B. Instead, we have moved Figure 5B to the supplement. The median peak decoded accuracy for near and distant items is equivalent. We have added the following description to the figure:

“Decoded raw probabilities for off-screen items, that were up to two steps ahead of the current stimulus cue (‘near’,) vs. distant items that were more than two steps away on the graph, on trials with correct answers. The median peak decoded probability for near and distant items was at the same time point for both probability categories. Note that displayed lines reflect the average probability while, to eliminate influence of outliers, the peak displays the median.”

(2) The non-sequential reactivation analyses often use a time window of peak decodability, and it was not entirely clear to me what data this time window is determined on, e.g., was it determined based on all future reactivations irrespective of graph distance? This should be clarified in the methods.

Thank you for raising this. We now clarify this in the relevant section to read: “First, we calculated a time point of interest by computing the peak probability estimate of decoders across all trials, i.e., the average probability for each timepoint of all trials (except previous onscreen items) of all distances, which is equivalent to the peak of the differential reactivation analysis”

(3) Fig 4 shows evidence for forward and backward sequential reactivation, suggesting that both forward and backward replay peak at a lag of 40-50msec. It would be helpful if this counterintuitive finding could be picked up in the discussion, explaining how plausible it is, physiologically, to find forward and backward replay at the same lag, and whether this could be an artifact of the TDLM method.

This is an important point and we agree that it appears counterintuitive. However, we would highlight this exact time range has been reported in previous studies, though t never for both

forward and backward replay. We now include a discussion of this finding. The section now reads:

“[...] Even though we primarily focused on the mean sequenceness scores across time lags, there appears to be a (non-significant) peak at 40-60 milliseconds. While simultaneous forward and backward replay is theoretically possible, we acknowledge that it is somewhat surprising and, given our paradigm, could relate to other factors such as autocorrelations (Liu, Dolan, et al., 2021).”

(4) It is reported that participants with below 30% decoding accuracy are excluded from the main analyses. It would be helpful if the manuscript included very specific information about this exclusion, e.g., was the criterion established based on the localizer cross-validated data, the temporal generalisation to the cued item (Fig. 2), or only based on peak decodability of the future sequence items? If the latter, is it applied based on near or far reactivations, or both?

We now clarify this point to include more specific information, which reads:

“[...] Therefore, we decided a priori that participants with a peak decoding accuracy of below 30% would be excluded from the analysis (nine participants in all) as obtained from the cross-validation of localizer trials”

(5) Regarding the low amount of data for the reactivation analysis, the manuscript should be explicit about the number of trials available for each participant. For example, Supplemental Fig. 1 could provide this information directly, rather than the proportion of excluded trials.

We have adapted the plot in the supplement to show the absolute number of rejected epochs per participant, in addition to the ratio.

(6) More generally, the supplements could include more detailed information in the legends.

We agree and have added more extensive explanation of the plots in the supplement legends.

(7) The choice of comparing the 2 nearest with all other future items in the clustered reactivation analysis should be better motivated, e.g., was this based on the Wimmer et al. (2020) study?

We have added our motivation for taking the two nearest items and contrasting them with the items further away. The paragraph reads:

“[...] We chose to combine the following two items for two reasons: First, this doubled the number of included trials; secondly, using this approach the number of trials for each category (“near” and “distant”) was more balanced. [...]”

Reviewer 2

(1) Focus exclusively on retrieval data (and here just on the current image trials).

If I understand correctly, you focus all your analyses (behavioural as well as MEG analyses) on retrieval data only and here just on the current image trials. I am surprised by that since I see some shortcomings due to that. These shortcomings can likely be addressed by including the learning data (and predecessor image trials) in your analyses.

a) Number of trials: During each block, you presented each of the twelve edges once. During retrieval, participants then did one "single testing session block". Does that mean that all your results are based on max. 12 trials? Given that participants remembered, on average, 80% this means even fewer trials, i.e., 9-10 trials?

This is correct and a limitation of the paper. However, while we used only correct trials for the reactivation analysis, the sequential analysis was conducted using all trials disregarding the response behaviour. To retain comparability with previous studies we mainly focused on data from after a consolidation phase. Nevertheless, despite the trial limitation we consider the results are robust and worth reporting. Additionally, based on the suggestion of the referee, we now include results from learning blocks (see below).

b) Extend the behavioural and replay/reactivation analysis to predecessor images.

Why do you restrict your analyses to the current image trials? Especially given that you have such a low trial number for your analyses, I was wondering why you did not include the predecessor trials (except the non-deterministic trials, like the zebra and the foot according to Figure 2B) as well.

We agree it would be great to increase power by adding the predecessor images to the current image cue analysis, excluding the ambiguous trials, we did not do so as we considered the underlying retrieval processes of these trial types are not the same, i.e. cannot be simply combined. Nevertheless, we have performed the suggested analysis to check if it increases our power. We found, that the reactivation effect is robust and significant at the same time point of 220-230 ms. However, the effect size actually decreased: While before, peak differential reactivation was at 0.13, it is now at 0.07. This in fact makes conceptual sense. We suspect that the two processes that are elicited by showing a single cue and by showing a second, related, cue are distinct insofar as the predecessor image acts as a primer for the current image, potentially changing the time course/speed of retrieval. Given our concerns that the two processes are not actually the same we consider it important to avoid mixing these data.

We have added a statement to the manuscript discussing this point. The section reads:

"Note that we only included data from the current image cue, and not from the predecessor image cue, as we assume the retrieval processes differ and should not be concatenated."

c) Extend the behavioural and replay/reactivation analysis to learning trials.

Similar to point 1b, why did you not include learning trials in your analyses?

The advantage of including (correct and incorrect) learning trials has the advantage that you do not have to exclude 7 participants due to ceiling performance (100%).

Further, you could actually test the hypothesis that you outline in your discussion: "This implies that there may be a switch from sequential replay to clustered reactivation corresponding to when learned material can be accessed simultaneously without interference." Accordingly, you would expect to see more replay (and less "clustered" reactivation) in the first learning blocks compared to retrieval (after the rest period).

To track reactivation and replay over the course of learning is a great idea. We have given a lot of thought as to how to integrate these findings but have not found a satisfying solution. Thus, analysis of the learning data turned out to be quite tricky: We decided that each participant should perform as many blocks as necessary to reach at least 80% (with a limit of six and lower bound of two, see Supplement figure 4). Indeed, some participant learned 100%

of the sequence after one block (these were mostly medical students, learning things by hard is their daily task). With the benefit of hindsight, we realise our design means that different blocks are not directly comparable between participants. In theory, we would expect that replay emerges in parallel with learning and then gradually changes to clustered reactivation as memory traces become consolidated/stronger. However, it is unclear when replay should emerge and when precisely a switch to clustered reactivation would happen. For this reason, we initially decided not to include the learning trials into the paper.

Nevertheless, to provide some insight into the learning process, and to see how consolidation impacts differential reactivation and replay, we have split our data into pre and post resting state, aggregating all learning trials of each participant. While this does not allow us to track processes on a block basis, it does offer potential (albeit limited) insight into the hypothesis we outline in the discussion.

For reactivation, we see emergence of a clear increase, further strengthening the outlined hypothesis, however, for replay the evidence is less clear, as we do not know over how many learning blocks replay is expected.

We calculated individual trajectories of how reactivation and replay changes from learning to retrieval and related these to performance. Indeed, we see an increase of reactivation is nominally associated with higher learning performance, while an increase in replay strength is associated with lower performance (both non-significant). However, due to the above-mentioned reasons we think it would premature to add this weak evidence to the paper.

To mitigate problems of experiment design in relation to this question we are currently implementing a follow-study, where we aim to normalize the learning process across participants and index how replay/reactivation changes over the course of learning and after consolidation.

We have added plots showing clustered reactivation sequential replay measures during learning (Figure 5D and Supplement 8)

The added section(s) now read:

“To provide greater detail on how the 8-minute consolidation period affected reactivation we, post-hoc, looked at relevant measures across learning trials in contrast to retrieval trials. For all learning trials, for each participant, we calculated differential reactivation for the same time point we found significant in the previous analysis (220-260 milliseconds). On average, differential reactivation probability increased from pre to post resting state (Figure 5D). [...]

Nevertheless, even though our results show a nominal increase in reactivation from learning to retrieval (see Figure 5D), due to experimental design features our data do not enable us to test for an hypothesized switch for sequential replay (see also “limitations” and Supplement 8).”

d) Introduction (last paragraph): "We examined the relationship of graph learning to reactivation and replay in a task where participants learned a ..." If all your behavioural analyses are based on retrieval performance, I think that you do not investigate graph learning (since you exclusively focus the analyses on retrieving the graph structure). However, relating the graph learning performance and replay/reactivation activity during learning trials (i.e., during graph learning) to retrieval trials might be interesting but beyond the scope of this paper.

We agree. We have changed the wording to be more accurate. Indeed, we do not examine graph learning but instead examine retrieval from a graph, after graph learning. The mentioned sentence now read

“[...] relationship of retrieval from a learned graph structure to reactivation [...]”

e) It is sometimes difficult to follow what phase of the experiment you refer to since you use the terms retrieval and test synonymously. Not a huge problem at all but maybe you want to stick to one term throughout the whole paper.

Thank you for pointing this out. We have now adapted the manuscript to exclusively refer to “retrieval” and not to “test”.

(2) Is your reactivation clustered?

In Figure 5A, you compare the reactivation strength of the two items following the cue image (i.e., current image trials) with items further away on the graph. I do not completely understand why your results are evidence for clustered reactivation in contrast to replay.

First, it would be interesting to see the reactivation of near vs. distant items before taking the difference (time course of item probabilities).

(copied answer from response to Reviewer 1, as the same remark was raised)

We have added the requested analysis showing the raw decoded probabilities for near and distant items separately in Figure 5A. We have chosen to replace Figure 5B with the new figure as we think that it offers more information than the previous Figure 5B. Instead, we have moved Figure 5B to the supplement. The median peak decoded accuracy for near and distant items is equivalent. We have added the following description to the figure:

“Decoded raw probabilities for off-screen items, that were up to two steps ahead of the current stimulus cue (‘near’,) vs. distant items that were more than two steps away on the graph, on trials with correct answers. The median peak decoded probability for near and distant items was at the same time point for both probability categories. Note that displayed lines reflect the average probability while, to eliminate influence of outliers, the peak displays the median. .”

Second, could it still be that the first item is reactivated before the second item? By averaging across both items, it becomes not apparent what the temporal courses of probabilities of both items look like (and whether they follow a sequential pattern). Additionally, the Gaussian smoothing kernel across the time dimension might diminish sequential reactivation and favour clustered reactivation. (In the manuscript, what does a Gaussian smoothing kernel of $\sigma = 1$ refer to?). Could you please explain in more detail why you assume non-sequential clustered reactivation here and substantiate this with additional analyses?

We apologise for the unclear description. Note the Gaussian kernel is in fact only used for the reactivation analysis and not the replay analysis, so any small temporal successions would have been picked up by the sequential analysis. We now clarify this in the respective section of the sequential analysis and also explain the parameter of $\sigma = 1$ in the reactivation analysis section. The paragraph now reads

“[...] As input for the sequential analysis, we used the raw probabilities of the ten classifiers corresponding to the stimuli. [...]”

[...] Therefore, to address this we applied a Gaussian smoothing kernel (using `scipy.ndimage.gaussian_filter` with the default parameter of $\sigma=1$ which corresponds

approximately to taking the surrounding timesteps in both direction with the following weighting: current time step: 40%, ± 1 step: 25%, ± 2 step: 5%, ± 3 step: 0.5%) [...]"

(3) Replay and/or clustered reactivation?

The relationship between the sequential forward replay, differential reactivation, and graph reactivation analysis is not really apparent. Wimmer et al. demonstrated that high performers show clustered reactivation rather than sequential reactivation. However, you did not differentiate in your differential reactivation analysis between high vs. low performers. (You point out in the discussion that this is due to a low number of low performers.)

We agree that a split into high vs low performers would have been preferably for our analysis. However, there is one major obstacle that made us opt for a correlational analysis instead: We employed criteria learning, rendering a categorical grouping conceptually biased. Even though not all participants reached the criteria of 80%, our sample did not naturally split between high and low performers but was biased towards higher performance, leaving the groups uneven. The median performance was 83% (mean ~81%), with six of our subjects (~1/4th of included participant) having this exact performance. This makes a median or mean split difficult, as either binning assignment choice would strongly affect the results. We have added a limitations section in which we extensively discuss this shortcoming and reasoning for not performing a median split as in Wimmer et al (2020). The section now reads:

"There are some limitations to our study, most of which originate from a suboptimal study design. [...], as we performed criteria learning, a sub-group analysis as in Wimmer et al., (2020) was not feasible, as median performance in our sample would have been 83% (mean 81%), with six participants exactly at that threshold. [...]"

It might be worth trying to bring the analysis together, for example by comparing sequential forward replay and differential reactivation at the beginning of graph learning (when performance is low) vs. retrieval (when performance is high).

Thank you for the suggestion to include the learning segments, which we think improves the paper quite substantially. However, analysis of the learning data turned out to be quite tricky> We had decided that each participant should perform as many blocks as necessary to reach at least 80% accuracy (with a limit of six and lower bound of two, see Supplement figure 4). Some participants learned 100% of the sequence after one block (these were mostly medical students, learning things by hard is their daily task). This in hindsight is an unfortunate design feature in relation to learning as it means different blocks are not directly comparable between participants.

In theory, we would expect that replay emerges in parallel with learning and then gradually change to clustered reactivation, as memory traces get consolidated/stronger. However, it is unclear when replay would emerge and when the switch to reactivation would happen. For this reason, we initially decided not to include the learning trials into the paper at all.

Nevertheless, to give some insight into the learning process and to see how consolidation effects differential reactivation and replay, we have split our data into pre and post resting state, aggregating all learning trials of each participant. While this does not allow us to track measures of interest on a block basis, it gives some (albeit limited) insight into the hypothesis outlined in our discussion.

For reactivation, we see a clear increase, further strengthening the outlined hypothesis. However, for replay the evidence is less obvious, potentially due to that fact that we do not know across how many learning blocks replay is to be expected.

The added section(s) now read:

“To examine how the 8-minute consolidation period affected reactivation we, post-hoc, looked at relevant measures during learning trials in contrast to retrieval trials. For all learning trial, for each participant, we calculated differential reactivation for the time point we found significant during the previous analysis (220-260 milliseconds). On average, differential reactivation probability increased from pre to post resting state (Figure 5D).

[...]

Nevertheless, even though our results show a nominal increase in reactivation from learning to retrieval (see Figure 5D), our data does not enable us to show an hypothesized switch for sequential replay (see also “limitations” and Supplement 8).”

Additionally, the main research question is not that clear to me. Based on the introduction, I thought the focus was on replay vs. clustered reactivation and high vs. low performance (which I think is really interesting). However, the title is more about reactivation strength and graph distance within cognitive maps. Are these two research questions related? And if so, how?

We agree we need to be clearer on this point. We have added two sentences to the introduction, which should address this point. The section now reads:

“[...] In particular, the question remains how the brain keeps track of graph distances for successful recall and whether the previously found difference between high and low performers also holds true within a more complex graph learning context.”

(4) Learning the graph structure.

I was wondering whether you have any behavioural measures to show that participants actually learn the graph structure (instead of just pairs or triplets of objects). For example, do you see that participants chose the distractor image that was closer to the target more frequently than the distractor image that was further away (close vs. distal target comparison)? It should be random at the beginning of learning but might become more biased towards the close target.

Thanks, this is an excellent suggestion. Our analysis indeed shows that people take the near lure more often than the far lure in later blocks, while it is random in the first block.

Nevertheless, we have decided to put these data into the supplement and reference it in the text. This is because analysis of the learning blocks is challenging and biased in general. Each participant had a different number of learning blocks based on their learning rate, and this makes it difficult to compare learning across participants. We have tried our best to accommodate and explain these difficulties in the figure legend. Nevertheless, we thank the referee for guidance here and this analysis indeed provides further evidence that participants learned the actual graph structure.

The added section reads

“Additionally, we have included an analysis showing how wrong answers participants provided were random in the first block and biased towards closer graph nodes in later blocks. This is consistent with participants actually learning the underlying graph structure as opposed to independent triplets (see figure and legend of Supplement 6 for details).”

(5) Minor comments

a) "Replay analysis relies on a successive detection of stimuli where the chance of detection exponentially decreases with each step (e.g., detecting two successive stimuli with a chance of 30% leaves a 9% chance of detecting the replay event). " Could you explain in more detail why 30% is a good threshold then?

Thank you. We have further clarified the section. As we are working mainly with probabilities, it is useful to keep in mind that accuracy is a class metric that only provides a rough estimate of classifier ability. Alternatively, something like a Top-3-Accuracy would be preferable, but also slightly silly in the context of 10 classes.

Nevertheless, subtle changes in probability estimates are present and can be picked up by the methods we employ. Therefore, the 30% is a rough lower bound and decided based on pilot data that showed that clean MEG data from attentive participants can usually reach this threshold. The section now reads:

“(e.g., detecting two successive stimuli with a chance of 30% leaves a 9% chance of detecting a replay event). However, one needs to bear in mind that accuracy is a “winnertakes-all” metric indicating whether the top choice also has the highest probability, disregarding subtle, relative changes in assigned probability. As the methods used in this analysis are performed on probability estimates and not class labels, one can expect that the 30% are a rough lower bound and that the actual sensitivity within the analysis will be higher. Additionally, based on pilot data, we found that attentive participants were able to reach 30% decodability, allowing us to use decodability as a data quality check. “

b) Could you make explicit how your decoders were designed? Especially given that you added null data, did you train individual decoders for one class vs. all other classes (n = 9 + null data) or one class vs. null data?

We added detail to the decoder training. The section now reads

“Decoders were trained using a one-vs-all approach, which means that for each class, a separate classifier was trained using positive examples (target class) and negative examples (all other classes) plus null examples (data from before stimulus presentation, see below). In detail, null data was.”

c) Why did you choose a ratio of 1:2 for your null data?

Our choice for using a higher ratio was based upon previous publications reporting better sensitivity of TDLM using higher ratios, as spatial sensor correlations are decreasing. Nevertheless, this choice was not well investigated beforehand. We have added more information to this to the manuscript

d) You could think about putting the questionnaire results into the supplement if they are sanity checks.

We have added the questionnaire results. However, due to the size of the tables, we have decided to add them as excel files into the supplementary files of the code repository. We have mentioned the existence file in the publication.

e) Figure 2. There is a typo in D: It says "Precessor Image" instead of "Predecessor Image".

Fixed typo in figure.

f) You write "Trials for the localizer task were created from -0.1 to 0.5 seconds relative to visual stimulus onset to train the decoders and for the retrieval task, from 0 to 1.5 seconds after onset of the second visual cue image." But the Figure legend 3D starts at -0.1 seconds for the retrieval test.

We have now clarified this. For the classifier cross-validation and transfer sanity check and clustered analysis we used trials from -0.1 to 0.5s, whereas for the sequenceness analysis of the retrieval, we used trials from 0 to 1.5 seconds