Supplementary Materials for

Cueing motor memory reactivation during NREM sleep engenders learningrelated changes in precuneus and sensorimotor structures

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SUPPLEMENTARY NOTES: BASELINE SRTT PERFORMANCE

Before sleep, no difference was found between the average reaction time of the cued and uncued sequence for either both hands (BH, t_{29} = -0.25, p = 0.801), left hand (LH, t_{29} = 0.27, p = 0.786) or right hand (RH, t_{29} = -0.50, p = 0.621) (paired-samples t-tests) dataset. Similar results were obtained where comparing random sequences before sleep for all datasets (BH: z = -0.57, p = 0.572; LH: z = -0.63, p = 0.530; Wilcoxon signed-rank tests; RH: t_{29} = -0.16, p = 0.872; paired-samples t-test). Thus, any post-sleep difference between the sequences can be regarded as the effect of TMR. Furthermore, average reaction times before sleep were significantly shorter for the last 4 sequence blocks than for the random blocks, confirming that the participants learned both sequences during S1 (BH cued: z = -4.74, BH uncued: z = -4.49; LH cued: z = -4.78, LH uncued: z = -4.33; p < 0.001 for all comparisons, Wilcoxon signed-rank test; RH cued: t_{29} = 7.21, RH uncued: 6.49; p < 0.001 for all comparisons, paired-samples t-test). Summary statistics for each sequence and dataset during S1 are presented in Table S1.

SUPPLEMENTARY NOTES: EXPLICIT MEMORY TASK

Given that TMR was shown to promote the emergence of explicit knowledge the next morning¹, we also set out to test whether this is true after a longer period. However, we found no difference between the free recall of the cued and uncued sequence (z = -0.568, p = 0.570, Wilcoxon signed-rank test), suggesting no TMR effect on the explicit knowledge of the sequence 20 days post-encoding (Fig.S1). Nevertheless, performance on both sequences differed from chance (cued: z = -4.14, p < 0.001; uncued: z = -4.29, p < 0.001; Wilcoxon signed-rank test), indicating that the participants learned both sequences explicitly over the course of the experiment.

SUPPLEMENTARY NOTES: QUESTIONNAIRES

The EHI confirmed that all participants were right-handed, as the laterality quotient score (ranging between -100 and +100, where the negative values indicate left-handers and positive right-handers) was +100% for all but one subject who scored +75%. PSQI global scores (on a 21-points scale) ranged between 1 and 7 points, with a mean of 3.67 (\pm 0.28), indicating, on average, a 'good quality' of sleep². The median answer to the SQ (with 1 and 9 indicating the highest and lowest level of alertness, respectively) was 2 (IQR: 1) for all sessions, suggesting similar levels of alertness throughout the study.

Participants did report hearing experimental sounds during the night: On a 3-points scale, the median answer was 2 (IQR: 2), with 33% of the participants not hearing any sounds (answer 1), 20% of the participants being unsure (answer 2), and 47% of the participants hearing them clearly (answer 3). However, when asked about the number of sounds they had heard, the maximum number selected was 4 (reported by 13% of the participants), with the median answer of 2 (IQR: 2) sounds.

SUPPLEMENTARY TABLES:

Table S1. SRTT summary statistics.

Mean reaction times (\pm SEM) (in ms) for the BH, LH and RH trials of the cued and uncued sequence blocks (24 blocks per sequence) as well as random blocks (2 blocks with tones matching the cued sequence and 2 blocks with tones matching the uncued sequence) during Session 1. Average reaction times (\pm SEM) for the last 4 blocks of each sequence are shown as well. BH: both hands; LH: left hand; RH: right hand. n = 30.

Dataset	Cued sequence	Uncued sequence	Cued random	Uncued random	Cued sequence (last 4 blocks)	Uncued sequence (last 4 blocks)
вн	$\textbf{321.82} \pm \textbf{6.46}$	$\textbf{322.89} \pm \textbf{7.83}$	357.81 ± 6.63	$\textbf{360.10} \pm \textbf{6.69}$	286.05 ± 8.09	$\textbf{292.64} \pm \textbf{9.99}$
LH	$\textbf{335.98} \pm \textbf{7.18}$	$\textbf{334.90} \pm \textbf{7.82}$	$\textbf{376.86} \pm \textbf{7.45}$	$\textbf{371.60} \pm \textbf{6.60}$	$\textbf{297.44} \pm \textbf{9.19}$	$\textbf{303.92} \pm \textbf{10.55}$
RH	$\textbf{307.67} \pm \textbf{6.28}$	$\textbf{310.92} \pm \textbf{8.44}$	347.77 ± 6.95	348.60 ± 7.76	$\textbf{274.62} \pm \textbf{7.81}$	$\textbf{281.40} \pm \textbf{10.20}$

Table S2. Effect of and interaction between TMR, hand and session.

Results of the likelihood ratio tests between the full, linear mixed effects model and reduced models, i.e., models without the fixed effect of interest, or with an interaction. The full model was used to test the effect of TMR, hand and session on the early and late SSS. df: degrees of freedom; χ 2: chi-squared; AIC: Akaike Information Criterion; SSS: Sequence Specific Skill. *p < 0.05.

	df	χ2	p-value	AIC of a reduced model	AIC of a full model
A. Both hands					
i) Early SSS					
TMR	1	1.5450	0.2138	1632.4	1632.9
Session	2	175.77	<0.0001*	1804.6	1632.9
TMR x Session	2	0.0740	0.9637	1636.3	1632.9
ii) Late SSS					
TMR	1	11.009	0.0009*	1621.3	1612.3
Session	2	93.041	<0.0001*	1701.3	1612.3
TMR x Session	2	3.0133	0.2216	1613.3	1612.3
B. Left hand					
i) Early SSS					
TMR	1	0.1878	0.6648	1666.5	1668.3
Session	2	132.02	<0.0001*	1796.3	1668.3
TMR x Session	2	0.1044	0.9492	1672.2	1668.3
ii) Late SSS					
TMR	1	4.015	0.0451*	1646.3	1644.2
Session	2	64.529	<0.0001*	1704.8	1644.2
TMR x Session	2	1.9750	0.3725	1646.3	1644.2
C. Right hand					
i) Early SSS					
TMR	1	3.2309	0.0723	1651.0	1659.8
Session	2	181.60	<0.0001*	1827.4	1649.8
TMR x Session	2	0.3741	0.8294	1653.4	1649.8
ii) Late SSS					
TMR	1	15.458	<0.0001*	1636.8	1650.3
Session	2	96.024	<0.0001*	1728.9	1636.2
	2	3.9605	0.1380	1636.9	1636.8

i) Early SSS

Hand	1	22.423	<0.0001*	3286.3	3265.9
Hand x Session	2	9.1678	0.0102*	3260.7	3265.9
TMR x Hand	1	0.8955	0.3440	3267.0	3265.9
ii) Late SSS					
Hand	1	25.927	<0.0001*	3261.5	3237.5
Hand x Session	2	6.4262	0.0402*	3237.5	3235.9
TMR x Hand	1	2.1656	0.1411	3237.4	3237.5

Table S3. Effect of session on SSS.

Post-hoc pairwise comparisons between sessions for the early and late SSS, conducted on each dataset separately. P-values reported are Holm adjusted. SSS: Sequence Specific Skill; S2-4: Session 2-4; df: degrees of freedom. *p < 0.05.

	Mean S2 (±SE) [ms]	Mean S3 (±SE) [ms]	Mean S4 (±SE) [ms]	Estimate (±SE)	df	t ratio	p-value (Holm adj)	Effect size
A. Both hands	S							
i) Early SSS								
S2-S3	39.2 (9.88)	81.7 (10.20)	-	-42.5 (6.49)	135	-6.553	<0.0001*	-1.320
S3-S4	-	81.7 (10.20)	163.1 (10.27)	-81.3 (6.63)	131	-12.270	<0.0001*	-2.520
ii) Late SSS								
S2-S3	129.0 (8.92)	154.0 (9.24)	-	-24.7 (6.14)	135	-4.018	0.0001*	-0.807
S3-S4	-	154.0 (9.24)	200 (9.30)	-46.2 (6.27)	131	-7.367	<0.0001*	-1.513
B. Left hand								
i) Early SSS								
S2 – S3	35.3 (11.10)	73.3 (11.40)	-	-37.9 (7.34)	135	-5.227	<0.0001*	-1.050
S3 – S4	-	73.3 (11.40)	144.1 (11.5)	-70.8 (7.50)	131	-9.553	<0.0001*	-1.960
ii) Late SSS								
S2 – S3	121.0 (10.20)	149.0 (10.50)	-	-27.4 (6.74)	135	-4.064	0.0001*	-0.817
S3 – S4	-	149.0 (10.50)	183 (10.6)	-34.4 (6.89)	131	-4.996	<0.0001*	-1.026
C. Right hand								
i) Early SSS								
S2 – S3	43.0 (9.13)	90.0 (9.53)	-	-46.9 (7.13)	136	-6.627	<0.0001*	-1.330
S3 – S4	-	90.0 (9.53)	182 (9.61)	-91.9 (7.30)	132	-12.670	<0.0001*	-2.600
ii) Late SSS								
S2 – S3	137.0 (8.27)	159.0 (8.69)	-	-21.9 (6.90)	137	-3.167	0.0019*	-0.634
S3 – S4	-	159.0 (8.69)	217 (8.77)	-58.1 (7.08)	132	-8.207	<0.0001*	-1.685

Table S4. Effect of TMR on late SSS during each session.

Post-hoc pairwise comparisons of late SSS between the cued and uncued sequence on each session (S2-S4), conducted on each dataset separately. Both the uncorrected and Holm adjusted p-values are reported. S2-4: Session 2-4; SSS: Sequence Specific Skill; df: degrees of freedom. *p < 0.05. $^{p} < 0.07$.

Cued vs Uncued	Mean cued (± SE) [ms]	Mean uncued (± SE) [ms]	Estimate (± SE)	df	t ratio	p value	p value (Holm adj)	Effect size
A. Both hands								
S2	135 (9.94)	123 (9.94)	-11.78 (7.95)	133	-1.482	0.1408	0.2815	-0.390
S3	157 (8.70)	147 (8.70)	-9.98 (8.71	133	-1.146	0.2537	0.2815	-0.331
S4	215 (11.60)	185 (11.60)	-29.13 (8.89)	133	-3.277	0.0013*	0.0040*	-0.965
B. Left hand								
S2	127 (11.20)	116 (11.20)	-11.02 (8.76)	133	-1.258	0.2106	0.4212	-0.3212
S3	146 (10.30)	145 (10.30)	-1.33 (9.60)	133	-0.139	0.8897	0.8897	-0.0401
S4	193 (13.10)	172 (13.10)	-20.29 (9.79)	133	-2.072	0.0401*	0.1206	-0.6101
C. Right hand								
S2	143 (9.73)	130 (9.73)	-12.50 (8.94)	133	-1.401	0.1634	0.1634	-0.369
S3	167 (8.37)	149 (8.37)	-18.60 (9.79)	133	-1.899	0.0597^	0.1193	-0.548
S4	237 (11.00)	198 (11.00)	-38.20 (9.99)	133	-3.822	0.0002*	0.0006*	-1.125

Table S5. Effect of time and hand on the cueing benefit.

Results of the likelihood ratio tests between the full, linear mixed effects model and reduced models, i.e., models without the fixed effect of interest, or with an interaction. The full model was used to test the effect of hand and number of days post-TMR ('Time') on the cueing benefit (SSS for the uncued sequence subtracted from the cued sequence). χ 2: chi-squared; AIC: Akaike Information Criterion. df: degrees of freedom. *p < 0.05. ^p < 0.07.

	df	χ2	p-value	AIC of a reduced model	AIC of a full model
A. Both hands					
Time	2	3.965	0.046*	809.14	807.18
B. Left hand					
Time	2	0.736	0.391	825.46	826.73
C. Right hand					
Time	2	6.581	0.010*	834.01	829.43
D. Left and right hand combine	ed				
Hand	1	3.760	0.052^	1641.70	1639.90
Hand x Time	2	1.424	0.233	1639.9	1640.8

Table S6. Sleep parameters.

Total recording duration, total sleep time, time spent in each sleep stage and time scored as movement presented as average (minutes \pm SEM) and as percentage of the total recording duration. Total sleep time was calculated by subtracting the time spent awake from the total recording duration. N1-N3: stage 1 – stage 3 of NREM sleep. REM: Rapid Eye Movement sleep. n = 29.

	Percentage of total recording duration [%]	Mean duration ± SEM [min]
Total recording duration	100%	524.19 ± 10.29
Total sleep time	88.38%	463.29 ± 12.89
Wake	11.50%	60.90 ± 10.37
N1	4.52%	23.33 ± 1.73
N2	46.35%	242.45 ± 8.41
N3	19.91%	104.22 ± 4.05
REM	15.86%	83.43 ± 4.30
Movement	1.61%	8.43 ± 1.32

Table S7. Cueing benefit and the duration of N2 and N3.

Results of Pearson's correlations between cueing benefit and the percentage of time spent in N2 and N3. Both the uncorrected and FDR corrected p-values are reported. df: degrees of freedom; S2-4: Session 2-4; SSS: Sequence Specific Skill; N2-3: Stage 2-3 of NREM sleep. *p < 0.05.

		Time		Time spent in N3 [%]				
	df	Pearson's correlation	p-value	p-value (FDR corr)	df	Pearson's correlation	p-value	p-value (FDR corr)
A. Both hand	s							
S2	25	0.197	0.324	0.324	27	-0.015	0.939	0.939
S3	20	0.378	0.082	0.144	21	-0.031	0.887	0.939
S4	19	0.372	0.096	0.144	21	0.089	0.697	0.939
B. Left hand								

S2	23	0.101	0.632	0.632	25	0.036	0.857	0.974	
S3	19	0.432	0.045*	0.135	21	0.070	0.746	0.974	
S4	19	0.324	0.152	0.228	21	0.007	0.974	0.974	
C. Right hand									
S2	24	-0.030	0.884	0.884	26	-0.053	0.788	0.788	
S3	19	0.177	0.431	0.657	21	-0.125	0.562	0.788	
S4	19	0.377	0.092	0.276	21	0.144	0.511	0.788	

Table S8. Summary statistics for sleep spindles.

Average number and density (number/min) of spindles (± SEM). Results are presented for N2 and N3 of the cue (A) and no-cue (B) period. N2-3: stage 2-3 of NREM sleep.

		Density			Number			
	Average	Left	Right	Average	Left	Right		
A. Cue								
N2	5.28 ±0.27	5.43 ±0.29	5.13 ±0.19	124.02 ±11.31	127.68 ±11.70	120.35 ±11.23		
N3	4.24 ±0.16	4.31 ±0.16	4.17 ±0.16	191.58 ±13.14	195.39 ±13.88	187.77 ±12.74		
N2 & N3	4.20 ±0.22	4.43 ±0.25	3.96 ±0.24	286.91 ±18.50	301.06 ±19.81	272.76 ±18.70		
B. No-Cue								
N2	4.85 ±0.27	5.02 ±0.29	4.68 ±0.28	51.71 ±5.07	53.17 ±5.22	50.24 ±5.08		
N3	3.86 ±0.16	3.94 ±0.17	3.78 ±0.17	79.48 ±5.68	81.30 ±5.79	77.66 ±5.69		
N2 & N3	3.80 ±0.20	4.01 ±0.23	3.59 ±0.21	117.74 ±7.69	123.59 ±8.12	111.89 ±7.79		

Table S9. Cueing benefit and spindle density.

Results of Pearson's (A) and Spearman's (B) correlations (both FDR corrected and not) between the cueing benefit for BH dataset during each of the post-stimulation sessions (S2, S3, S4) and spindle density averaged over all motor channels during the cue (A) and no-cue (B) period. N2 (green) and N3 (blue) were analysed separately and together (N23, purple). df: degrees of freedom; S2-4: Session 2-4; N2-N3: stage 2 - stage 3 of NREM sleep; BH: Both Hands.

Dataset	Session	Sleep stage	Correlation coefficient	p-value	df	p-value (FDR corr)					
A. Cue period											
BH	S2	N2	0.237	0.224	26	0.288					
вн	S3	N2	-0.254	0.231	22	0.288					
ВН	S4	N2	-0.231	0.288	21	0.288					
ВН	S2	N3	-0.04	0.840	25	0.840					
ВН	S3	N3	-0.337	0.126	20	0.192					
ВН	S4	N3	-0.343	0.128	19	0.192					
ВН	S2	N23	0.196	0.309	27	0.322					
вн	S3	N23	-0.277	0.191	22	0.322					
ВН	S4	N23	-0.216	0.322	21	0.322					
			B. No-cue period	ł							
BH	S2	N2	0.135	0.485	27	0.485					
ВН	S3	N2	-0.317	0.132	22	0.396					
ВН	S4	N2	-0.227	0.296	21	0.444					
ВН	S2	N3	0.227	0.235	27	0.546					
ВН	S3	N3	-0.171	0.422	22	0.546					
вн	S4	N3	-0.132	0.546	21	0.546					

BH	S2	N23	0.189	0.326	27	0.639	
BH	S3	N23	-0.172	0.419	22	0.629	
вн	S4	N23	-0.042	0.851	21	0.851	

Table S10. Functional TMR-related activity.

Clusters showing increased (inc) or decreased (dec) activity for the cued relative to uncued sequence alone (A - S2 and S3, B - S2) or when considering covariates of cueing benefit during S2 (C) and cueing benefit during S4 (E). (D) Decrease in activity from S2 to S3 for the [cued > uncued] contrast when considering behavioural cueing benefit at S4 as a covariate. No significant clusters were found when considering cueing benefit during S3 as a covariate.

Region	MNI x, y, z (mm)	Number of voxels	F/T peak	P _{FWE} peak
. Main effect of TMR for S2 and S3				
i. Right Precuneus (inc)	8, -72, 58	9	22.67	0.032
. [Cued > Uncued at S2]				
i. Left Precuneus (inc)	-9 -62, 66	9	4.79	0.020
. [Cued > Uncued at S2 * cueing bene	fit at S2]			
i. Left Precuneus (inc)	-4, -78, 46	40	5.18	0.009
ii. Left Precuneus (inc)	-18, -68, 36	1	4.44	0.046
iii. Right Cerebellum (inc)	28, -58, -30	1	4.94	0.044
iv. Left Putamen (inc)	-24, 4, 6	3	4.41	0.034
6. $[\Delta \text{ Cued} > Uncued from S2 to S3 * cu$	ueing benefit at S4]			
i. Left Precuneus (dec)	-2, -54, 10	18	6.66	0.002
. [Cued > Uncued at S3 * cueing bene	fit at S4]			
i. Right Postcentral gyrus (inc)	58, -18, 38	7	5.50	0.022

Regions listed were significant at peak voxel threshold of $p_{FWE} < 0.05$, after correction for multiple voxel-wise comparisons within pre-defined bilateral ROI for bilateral precuneus (A.i, B.i, C.i, C.ii, D.i), bilateral hippocampus and parahippocampus (E.ii), bilateral cerebellum (C.iii), bilateral dorsal striatum (C.iv), bilateral sensorimotor cortex (E.i). Peak voxel MNI coordinates and peak F (A) and T (B-E) values are given. n = 22 for (A), n = 28 for (B) and (C), n = 23 for (E), n = 21 for (D).

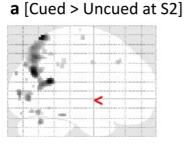
Table S11. Structural brain changes over time.

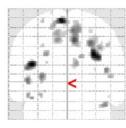
Clusters showing increased (inc) or decreased (dec) changes in grey matter volume over time and when considering covariates of cueing benefit during S3 and S4. No significant clusters were found when considering cueing benefit during S2 as a covariate. No significant clusters were found in white matter either.

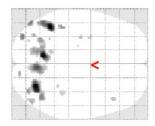
	Region	MNI x, y, z (mm)	Number of voxels	T peak	P _{FWE} peak			
A.	A. [Δ GM volume from S1 to S3 * cueing benefit at S4]							
	Right Precentral gyrus (inc)	42, -2, 45	5	6.21	0.020			
в.	3. [Δ GM volume from S1 to S3 * cueing benefit at S3]							
	Left Fusiform (dec)	-28, - 26, -24	5	5.51	0.025			

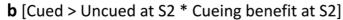
Regions listed were significant at peak voxel threshold of $p_{FWE} < 0.05$, after correction for multiple voxel-wise comparisons within pre-defined ROI for bilateral sensorimotor cortex (A) and bilateral hippocampus and parahippocampus (B). Since fusiform gyrus was not our ROI, the result in (B) has likely arisen due to the imperfection of the method and is thus not discussed any further. Peak voxel MNI coordinates, and peak T values are given. n = 24 for (A), n = 29 for (B).

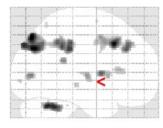
SUPPLEMENTARY FIGURES:

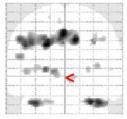


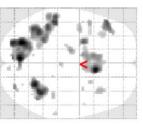












c [Δ Cued > Uncued from S2 to S3 * Cueing benefit at S4]



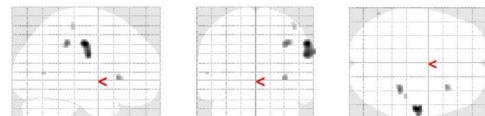


Fig. S1. Glass brain fMRI results. SPM fMRI results in glass brain projection displayed at p < 0.001, uncorrected, for the same contrasts as in Fig.5 and Fig.6A-B. **(A)** TMR-dependent increase in brain activity 24 h post-stimulation. **(B)** Brain activity for the cued > uncued contrast at S2 was positively associated with behavioural cueing benefit at the same time point. **(C)** A change in brain activity from S2 to S3 for the cued > uncued contrast was negatively associated with behavioural cueing benefit at S4. **(D)** Brain activity for the cued > uncued contrast at S3 was positively associated with behavioural cueing benefit at S4. S2-4: Session 2-4; n = 28 for (A-B), n = 21 for (C), n = 23 for (D).

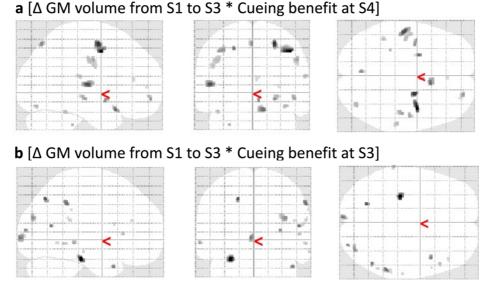


Fig. S2. Glass brain VBM results. SPM VBM results in glass brain projection displayed at p < 0.001, uncorrected, for the same contrasts as in Fig.6C-D (A) and Table S11 (A, B). (A) An increase in grey matter volume at S3 relative to S1 was associated with an increase in behavioural cueing benefit at S4. (B) Reduction in grey matter volume at S3 relative to S1 associated with cueing benefit at S3. S1-4: Session 1-4. n = 24 for (A), n = 29 for (B).

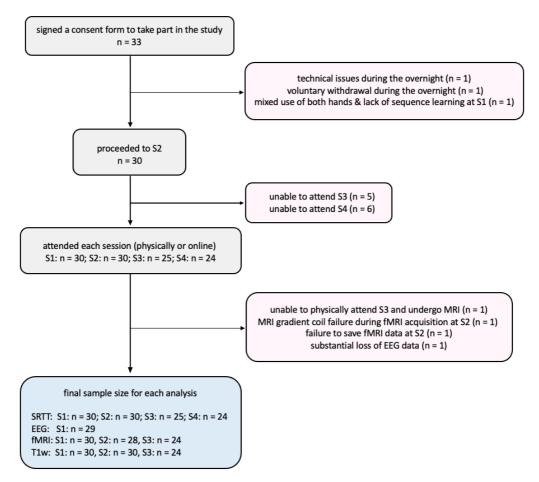


Fig.S3. A flowchart illustrating participants included and excluded from the analysis. On the left, the number of participants included in the study at different time points, with the final sample size shown at the bottom. On the right, the number of participants excluded (in red) together with a reason for the exclusion.

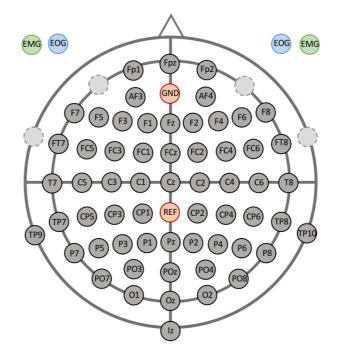


Fig. S4. EEG electrodes layout. Orange: ground (GND) and reference (REF) electrodes; light grey (dashed circles): original position of the electrodes used to record EMG and EOG; green: EMG electrodes; blue: EOG electrodes.

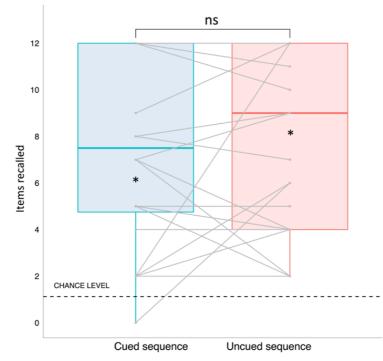


Fig. S5. Cueing memory reactivation during sleep does not affect explicit memory of the sequence. Explicit knowledge of both sequences was significantly above chance (significance denoted with *) 20 days post-encoding, although no effect of TMR was evident. Geoms represent median $\pm IQR$ for the cued (blue) and uncued (red) sequence, whiskers represent largest and lowest values within 1.5 IQR above and below the 75th and the 25th percentile, respectively. Grey dots represent performance of each subject. ns: non-significant. n = 24.

SUPPLEMENTARY REFERENCES:

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