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Repetition dynamically and rapidly increases cortical, but not hippocampal, offline reactivation

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No sooner is an experience over than its neural representation begins to be transformed through memory reactivation during offline periods. The lion's share of prior research has focused on understanding offline reactivation within the hippocampus. However, it is hypothesized that consolidation processes involve offline reactivation in cortical regions as well as coordinated reactivation in the hippocampus and cortex. Using fMRI, we presented novel and repeated paired associates to participants during encoding and measured offline memory reactivation for those events during an immediate post-encoding rest period. post-encoding reactivation frequency of repeated and once-presented events did not differ in the hippocampus. However, offline reactivation in widespread cortical regions and hippocampal-cortical coordinated reactivation were significantly enhanced for repeated events. These results provide evidence that repetition might facilitate the distribution of memory representations across cortical networks, a hallmark of systems-level consolidation. Interestingly, we found that offline reactivation frequency in both hippocampus and cortex explained variance in behavioral success on an immediate associative recognition test for the once-presented information, potentially indicating a role of offline reactivation in maintaining these novel, weaker, memories. Together, our findings highlight that endogenous offline reactivation can be robustly and significantly modulated by study repetition.

hippocampus | offline reactivation | replay | human memory | medial prefrontal cortex

Significance

The distribution of memories across hippocampal-cortical networks is a hallmark of memory consolidation. While repeated study has been shown to improve retention, the mechanisms supporting these effects remain unknown. Here, we show that repetition increases post-encoding offline reactivation in the cortex and enhances the coordinated offline reactivation between the hippocampus and cortex, providing important evidence that memory consolidation in hippocampal-cortical networks can be robustly accelerated through repeated learning. Further, we demonstrate that offline reactivation in both the hippocampus and cortex explains variance in the memory outcomes of once-encoded memories. Therefore, while prioritizing repeated memories, offline reactivation may also compensate for inadequate encoding to achieve balanced consolidation across memories.

How and when our experiences become stabilized in long-term memory has been an intense topic of research. Ample evidence shows that this stabilization occurs amid the distribution of recent memories across hippocampal-cortical networks (1–3). The precise neural mechanisms that support memory distribution are still unknown, however, a leading observable mechanism that has been proposed to contribute to this process is offline reactivation, or replay (3–8). Offline reactivation refers to the phenomenon whereby neural activity that characterizes an experience is reinstated during offline states following encoding, including quiet wakefulness and sleep (9–18). Importantly, the frequency or magnitude of post-encoding offline reactivation has been closely associated with the ultimate fate of a memory (14, 17–22), providing a link between reactivation and memory accessibility. However, it has not been fully characterized which factors modulate offline reactivation dynamics in the hippocampus and the cortex following encoding.

One way to approach this question is to examine whether the nature of recent experiences can modulate or prioritize those events for offline reactivation. While a consensus is emerging that memory consolidation is a highly selective process, this work has mainly focused on emotion- or value-based prioritization (23–28). We argue that this cannot explain the full range of differences in memory consolidation for our everyday, more neutral experiences. Regarding this issue, some competing evidence has highlighted the role of memory "strength" in modulating hippocampal offline reactivation. Specifically, one study has found increases in hippocampal offline reactivation for weakly learned information (14), while another data point shows that stronger hippocampal neural patterns tend to reactivate during post-encoding rest (17). However, a large gap in this literature is that the majority of work examining offline reactivation focuses solely on reactivation in the hippocampus, with much less known about offline reactivation as a systems-level phenomenon. Specifically, if memory distribution across hippocampal-cortical networks is a hallmark of memory stability and consolidation (1–3), it is critical to ask what features of new learning modulate offline reactivation across hippocampal-cortical networks.

Foundational behavioral work in humans has shown that one of the ways in which memories can be rapidly and robustly strengthened is through repeated study; decades of research has demonstrated that the frequency of encoding repetition is closely associated

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with memory strength (29–31). However, the critical relationship between repeated encoding and post-encoding offline reactivation in the hippocampal-cortical networks is unknown. To address these open questions, in the current human fMRI study, we directly manipulated memory strength through repetition during encoding. We then examined offline reactivation of thrice-repeated (strong) and once-presented novel (weak) memories in the rest period right after encoding. Immediately following the post-encoding rest period, participants were then tested on their memory for the studied pairs. To fully characterize differences in offline reactivation between conditions, we measured offline reactivation in the hippocampus and in cortical regions known to contribute to episodic memory. Further, we examined the extent to which offline reactivation events appeared coordinated between the hippocampus and the cortex. Given that the encoding content was all visual in nature, we focused our cortical reactivation analyses on higher-level visual processing region: ventral temporal cortex (VTC). We also selected two other a priori cortical regions of interest in the midline default network: retrosplenial cortex (RSC), a region closely interconnected with the hippocampus (32–34), and medial prefrontal cortex (mPFC), which has been previously implicated in memory consolidation (35–39).

Our results show that repetition does not increase post-encoding offline reactivation frequency in a nonspecific manner. Instead, we find that repetition increases post-encoding offline reactivation in the cortical regions, but not in the hippocampus, compared to the once-presented events. Moreover, we find that repetition significantly increases the frequency of coordinated offline reactivation across certain hippocampal-cortical networks as compared to weak memories. These results might suggest that repetition accelerates consolidation processes by facilitating the distribution of memory representations to cortical networks immediately following encoding. Interestingly, however, we also show that offline reactivation in the hippocampus and RSC explains behavioral variance in memory outcomes for the weakly encoded information, indicating that while prioritizing repeated, strong memories, post-encoding offline reactivation may compensate for weaker encoding and contribute to the strengthening of weak memories.

Results

Cued-Recognition Memory Performance. As expected, cued recognition showed a significant memory benefit for thrice-presented over once-presented events. On average, participants selected the correct object given the word cue for an accuracy of 0.90 (SD=0.14) in the repetition condition and 0.69 (SD = 0.20) in the once-presented condition, both were significantly above chance [0.25; strong: $t(28) = 24.22$, $P < 0.001$, 95% CI(0.84, 0.95), Cohen's d = 4.50; weak: $t(28) = 11.98$, $P < 0.001$, 95% CI(0.62, 0.77), Cohen's d = 2.23; Fig. 1*C*]. Accuracy was significantly higher for the thrice-presented pairs as compared to the once-presented pairs [$t(28) = 5.82$, $P < 0.001$, 95% CI(0.13, 0.28), Cohen's d = 1.08; Fig. 1*C*], confirming that repeated encoding improved memory performance (*SI Appendix, Supplemental Data* for results of response times on correct trials and for supplemental analysis comparing memory performance across encoding conditions and image categories).

Post-encoding Offline Reactivation in the Cortex Prioritizes Strong Memories. Having confirmed that our manipulation led to a reliable difference in memory performance, we next measured post-encoding offline neural reactivation, for the strongly and weakly encoded memories. Specifically, for each subject and within each region of interest (ROI; Fig. 2*A*), we derived the multivoxel activity pattern associated with each encoding trial and with each time point (i.e., TR) in the subsequent post-encoding rest period. We then computed the neural pattern similarity between each encoding trial and all of the time points during the post-encoding rest periods (i.e., Pearson correlations; 40). This resulted in an encoding-rest similarity matrix that represented the extent of pattern overlap between each encoding event and every time point during the post-encoding rest period (Fig. 2 *B* and *C*). Next, a threshold was applied to the matrix values at each rest time point to isolate encoding trials with the highest pattern similarity scores (i.e., exceeded 1.5 SD above the mean across pattern similarity values between encoding and the pre-encoding rest) as reliable evidence of offline neural reactivation of these trials (Fig. 2*C*; *Methods* for

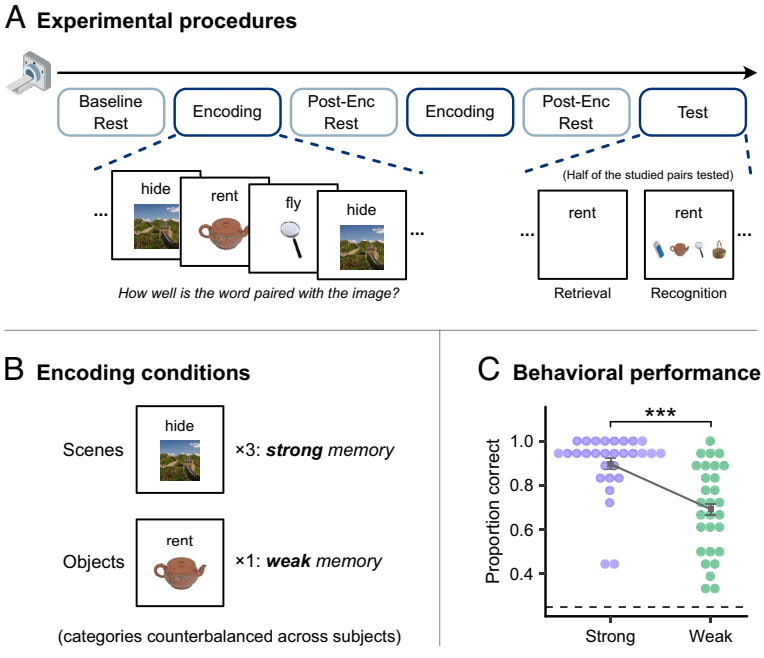

Fig. 1. Experimental design and behavioral performance. (*A*) Experimental Procedures. The fMRI study started with a baseline rest period followed by the associative encoding of word-image pairs across two separate encoding blocks, each followed by a post-encoding rest period. Immediately after the second rest period, memory was tested on an associative recognition test for pairs from one of the encoding blocks. (*B*) During encoding, word-image pairs from one visual category (counterbalanced across participants) were presented three times, which formed the strong memory condition, and word-image pairs with images from another visual category were presented only once, which formed the weak memory condition. (*C*) Memory performance on the immediate associative recognition test. Each dot represents a participant, squared dots indicate mean accuracy. The dashed line represents the chance level (0.25). Error bars show within-subject SE. ***$P < 0.001$.

details). This approach to identifying offline reactivation was adapted from prior published work (14, 18).

After computing the thresholded matrix (Fig. 2*C*, *Right*), the number of post-encoding rest time points that showed evidence for offline reactivation within the weak or strong condition was used to calculate the offline reactivation frequency for each encoding condition. Further, for the strong memory condition, reactivation frequency was computed separately for each of the three presentations: strong 1st presentation, strong 2nd presentation, and strong last (3rd) presentation. Importantly, our main comparison of interest was between the weak memory condition and the strong last presentation condition (see reasons discussed below; *Methods* for details).

Perhaps somewhat surprisingly, we found no evidence for significant differences in the rate of offline reactivation for strong and weak memories in the hippocampus [t(28) = 0.42, P = 0.68, 95% CI(−2.63, 3.98); Fig. 3, *Top* panel, highlighted]. Given that there is evidence for functional distinctions across the hippocampal long axis (43–46), we performed a follow-up analysis of offline reactivation within the anterior and the posterior thirds of the hippocampus. The results did not reveal any significant differences in reactivation frequency across conditions in the anterior [t(28) = −0.85, P = 0.40, 95% CI(−5.01, 2.08)] or the posterior hippocampus [t (28) = 1.25, P = 0.40, 95% CI(−1.05, 4.33)].

Next, we examined post-encoding offline reactivation in the preselected cortical regions. Offline reactivation frequency in these cortical regions was robustly and significantly enhanced for the repeated memories compared to once-presented events (Fig. 3, *Bottom* panel, highlighted). This was evident in all three a priori cortical ROIs examined: the ventral temporal cortex [VTC: t (28) = 2.94, P = 0.007, 95% CI(1.32, 7.40), Cohen's d = 0.55], the retrosplenial cortex [RSC: t (28) = 3.99, P < 0.001, 95% CI(4.03, 12.55), Cohen's d = 0.74], and the medial prefrontal cortex [mPFC: t(28) = 4.99, P < 0.001, 95% CI(5.76, 13.76), Cohen's d = 0.93]. The differences in offline reactivation between strong and weak memories in the cortical ROIs were also marginally or significantly greater as compared to that in the hippocampus (condition × region: all P < 0.056; *SI Appendix*, *Supplemental Data*). These results suggest that repeated encoding leads to substantially increased post-encoding offline reactivation specifically in cortical regions.

To further understand these differences in cortical offline reactivation between the repeated and once-encoded memories, we asked whether these effects might be simply because the encoding activity patterns were more similar between repeated trials in the strong (last presentation) condition compared to the weak memory condition. The logic behind encoding pattern similarity being a potential confound is that if trials within a condition share high representation similarity, they would lead to similar encoding-rest similarity values, and those values might then collectively pass (or not pass) the reactivation threshold at a single rest time point. Consequently, if some trials "ride on the coat tails" of the other trials from the same condition during thresholding, it might lead to an overestimation of reactivation frequency for that given condition. To examine this, we computed the pattern similarity between different trials within each condition during encoding (*SI Appendix*, *Supplemental Methods*). Interestingly, counter to our concerns, we found that encoding patterns were more similar among the weak memories than the strong (last presentation) memories in all cortical ROIs (all P < 0.001; *SI Appendix*, Fig. S1). In other words, repeated memories showed increased neural differentiation during encoding. Moreover, to minimize the impact of encoding pattern similarity on our reactivation count in each condition, in our previous reactivation analysis, we considered the reactivation frequency at each TR to be either 0 (when no trial passed the threshold) or 1 (when any trial, no matter how many, passed the threshold) for any given condition (see also *Methods*). This conservative way of counting condition-level reactivation frequency allowed us to further ensure that any difference in cortical reactivation across conditions would not be driven by memories sharing higher levels of encoding pattern similarity in one condition than the other.

Additionally, it is worth noting that the increase in cortical offline reactivation also cannot be explained by the difference in visual categories across encoding conditions, as (i) the assignments of categories to encoding conditions were counterbalanced, thus

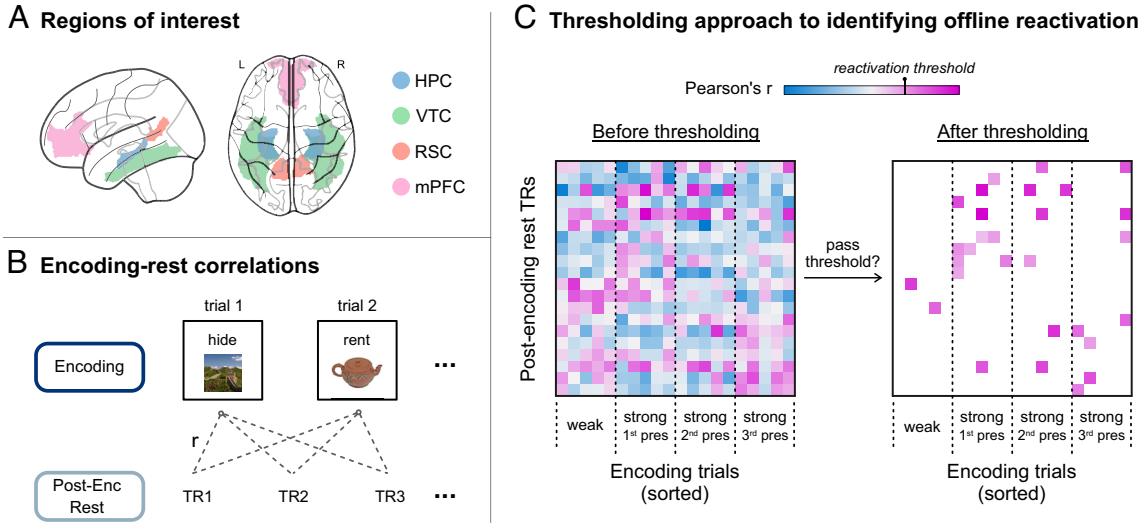

Fig. 2. Offline reactivation analysis. (*A*) Regions of interest (ROIs) were defined within each participant. ROIs included the hippocampus (HPC) and ventral temporal cortex (VTC) extracted from Freesurfer's volumetric segmentation (41), as well as retrosplenial cortex (RSC) and medial prefrontal cortex (mPFC) converted from the Schaefer atlas (42). (*B*) To measure offline reactivation in each ROI, the activation pattern of each encoding trial was extracted and used to compute its similarity score (Pearson correlation coefficient) with the activation pattern of each timepoint, or TR, during the corresponding post-encoding rest. We then applied a thresholding approach as in panel *C*. (*C*) Schematic diagram of the thresholding approach using example matrices. We created a correlation matrix for each pair of encoding block and post-encoding rest period in each ROI and for each participant. We then thresholded the entire post-encoding matrix to select only the high similarity scores (i.e., values greater than 1.5 SD above the mean score across all similarity values between the encoding block and the pre-encoding baseline rest period) as evidence for post-encoding offline reactivation of the encoding trials (14, 18).

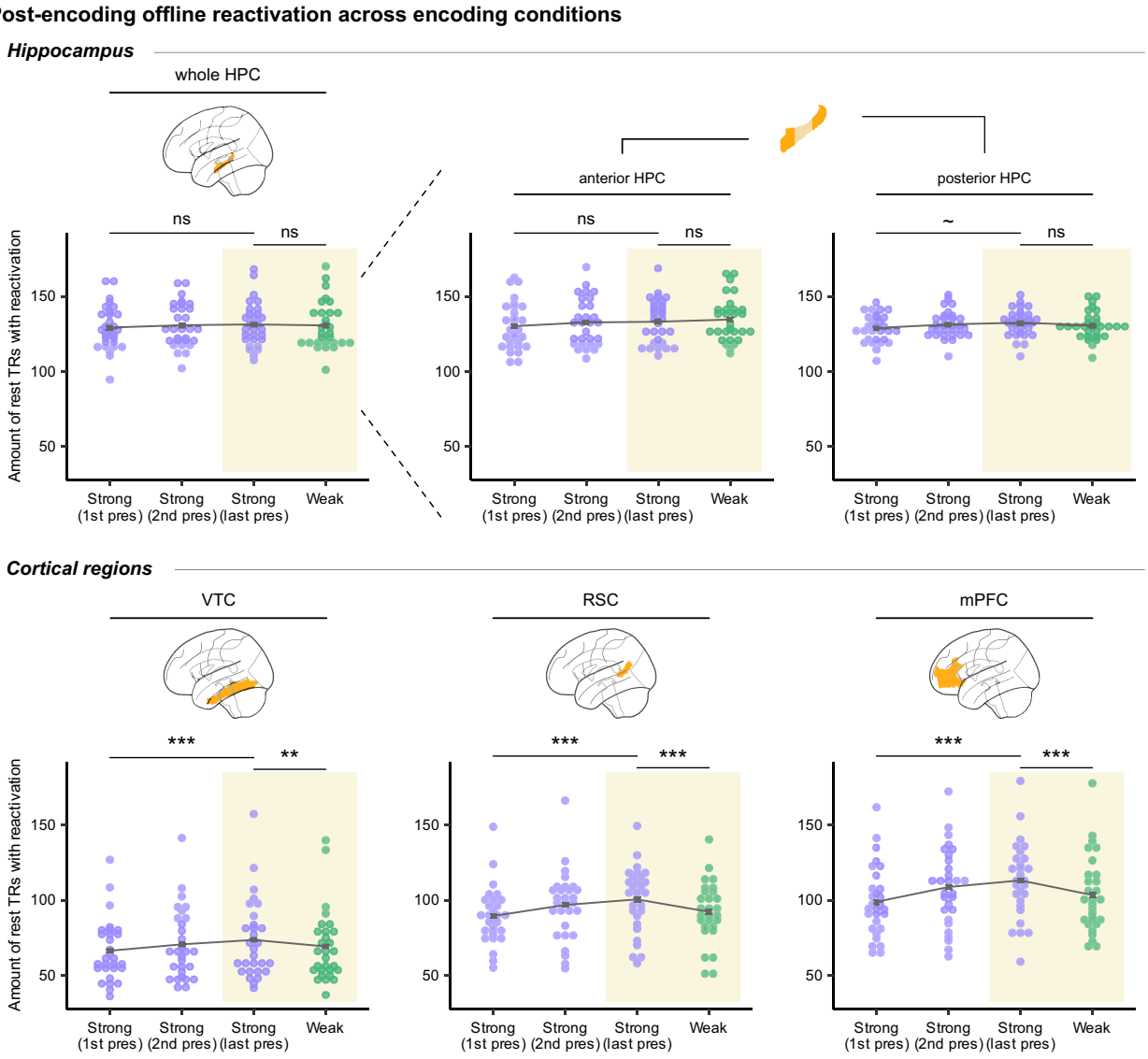

Fig. 3. Post-encoding offline reactivation of strong and weak memories. Post-encoding offline reactivation for each of the three presentations of the repeated trials (strong memories) and the once-presented trials (weak memories), in the hippocampus (*Top* panel) and cortical ROIs (*Bottom* panel). The main comparison of interest was between strong last presentation condition and weak memory condition, which is highlighted in yellow. Each dot represents a participant, squared dots indicate mean values. Error bars show within-subject SE. ns: not significant, ~$P < 0.1$, **$P < 0.01$, ***$P < 0.001$ (statistical significance adjusted with FDR correction).

the image category of the repeated events varied across participants, and (ii) we did not observe any significant interactions between encoding condition and image categories in any ROI across participants (all $P > 0.36$; *SI Appendix*, *Supplemental Data*). Together, our results provided strong evidence that it was repeated learning that immediately increased the frequency of post-encoding offline reactivation in cortical networks as compared to the once-encoded experiences, which was in contrast to the offline reactivation pattern in the hippocampus.

For the preceding reactivation analyses, we also focused on comparing offline reactivation frequency of once-presented items with the last presentation of the thrice-presented items. We selected the strong memory last presentation condition reasoning that memory representations for repeatedly presented pairs might change every time participants studied these pairs during encoding, and that this change in representation with repetition may be reflected in post-encoding offline reactivation. Specifically, we hypothesized that for the repeatedly studied pairs, offline reactivation may be more likely to prioritize the most recent presentation. To directly examine this hypothesis, we next computed the reactivation frequency across the three presentations (strong 1st, 2nd, and last presentations) of trials in strong memory condition, treating them as separate encoding conditions. In all cortical ROIs, there was a significant difference in offline reactivation frequency across the three repetitions of the strong memory conditions [VTC: $F(2, 56) = 10.04$, $P < 0.001$, $\eta^2 = 0.02$; RSC: $F(2, 56) = 16.05$, $P < 0.001$, $\eta^2 = 0.05$; mPFC: $F(2, 56) = 28.12$, $P < 0.001$, $\eta^2 = 0.06$; Fig. 3, *Bottom* panel]. Specifically, the last presentation of the thrice presented items was most frequently reactivated across the three repetitions, followed by strong 2nd presentation condition, while trials in the strong 1st presentation condition were least reactivated offline (all pairwise comparisons except one were significant with $P < 0.028$; the comparison between strong 2nd and strong last presentation conditions in VTC was marginal with $P = 0.083$). These results provided strong evidence that the most updated, or newest representations, of repeatedly encountered information are more likely to be reactivated during offline periods in cortical regions. By contrast, this prioritization of memory for the last presentation was not evident in the whole hippocampus [$F(2, 56) = 0.77$, $P = 0.47$]. Follow-up analyses of the hippocampal subregions

showed that there was also no difference in the anterior third of the hippocampus across repeated presentations [F(2, 56) = 1.82, P = 0.17], but a marginal difference was noted in the posterior hippocampus, such that there was a trend toward a significant increase in offline reactivation for the newest representations of the repeated trials [F(2, 56) = 3.42, P = 0.079, η^2= 0.03; Fig. 3, *Top* panel].

Thus far, our results show clear evidence for increased post-encoding offline reactivation of strongly encoded memories compared to the weak ones in midline cortical regions and VTC. Further, cortical offline reactivation of the repeated memories tends to reflect that the most updated memory representation, measured as the last presentation of the thrice-presented items, is most reactivated. By contrast, we found no significant differences between these conditions in the hippocampus.

To further characterize our findings, we performed two separate lines of supplementary control analyses. The first line of analyses examined whether the offline reactivation events identified with our approach reflected experience-dependent changes from pre- to post-encoding rest. Specifically, for each ROI, we applied the same reactivation threshold to the similarity matrix between encoding and the pre-encoding rest, and identified "pseudo-reactivation" events from the preencoding matrix. We then compared a) the number of rest time points that contained similarity values above the reactivation threshold between the pre- and post-encoding rests, and b) the mean similarity values of the pre-encoding "pseudo-reactivation" events with that of the post-encoding reactivation events (*SI Appendix*, *Supplemental Methods* for details). This latter dependent measure examined the continuous encoding-rest similarity values before and after the encoding experiences, allowing us to test whether post-encoding reactivation events showed significantly greater reinstatement of the encoding trials than "pseudo-reactivation" events.

Results revealed that in all of our ROIs, the number of rest TRs that showed "reactivation" significantly increased from pre- to post-encoding rest (all P < 0.032; *SI Appendix*, Fig. S2*A* for details), suggesting that the frequency of reactivation events in the rest period following encoding was significantly greater than that preceding encoding. Further, in the hippocampus, VTC, and mPFC, the continuous thresholded encoding-rest similarity values associated with these "reactivation" events also showed a significant increase from pre- to post-encoding rest (all P < 0.035; *SI Appendix*, Fig. S2*B*). Interestingly, in the RSC, while significantly more TRs in post-encoding rest showed evidence for reactivation than that in the pre-encoding rest, the similarity values associated with the post-encoding reactivation events were not statistically higher than the "pseudo-reactivation" events [t(28) = 0.55, P = 0.58, 95% CI(−0.001, 0.002); *SI Appendix*, Fig. S2*B*]. This finding might pose an open question of to what extent offline reactivation events identified in this region demonstrate reliable experience-dependent changes.

We next examined whether the differences in cortical offline reactivation across the memory conditions were driven by the temporal proximity between encoding and rest (i.e., a recency effect). In other words, on average, the last presentation of the thrice-presented items occurred closer in time to the post-encoding rest period where we measured offline reactivation events, while the once-presented items often occurred evenly across the whole encoding blocks. To examine whether a recency effect existed in post-encoding offline reactivation measures, we performed two control analyses. In the first analysis, we compared cortical offline reactivation of a subset of trials from each of the strong (last presentation) and weak memory conditions that were matched in terms of their temporal proximity to the post-encoding rest period. For each participant, we selected weak memory trials presented in the latter part of an encoding block; then for each selected weak memory trial, we identified a strong memory (last presentation) trial that was presented closest in time and prior to the given weak memory trial, ensuring that overall the selected weak memory trials were in fact encoded more recently (i.e., closer in time to the post-encoding rest period) than the selected strong memory trials (*SI Appendix*, *Supplemental Methods*).

Results revealed that in the RSC and mPFC, the frequency of reactivation in the strong (last presentation) condition remained greater than weak memory condition after controlling for the temporal lag between item encoding and post-encoding rest. This was significant in the RSC [t (24) = 2.23, P = 0.035, 95% CI(0.40, 10.24), Cohen's d = 0.45; *SI Appendix*, Fig. S3*A*] and marginally significant in the mPFC [t (24) = 1.83, P = 0.079, 95% CI(−0.83,13.95), Cohen's d = 0.37; *SI Appendix*, Fig. S3*A*]. It is worth noting that a decrease in the effect sizes of the results from this analysis as compared to the main reactivation analysis was expected due to the reduction of power, as only around a third of all the encoding data were included in this control analysis (*SI Appendix*, *Supplemental Methods*). However, in contrast to results in the two midline cortical regions, there was no longer a significant difference in offline reactivation frequency in the VTC between strong and weak memories when we restricted the analysis to the trials matched in recency [t (24) = 0.46, P = 0.65, 95% CI(−3.45, 5.41); *SI Appendix*, Fig. S3*A*]. This result in the VTC could be driven by a recency effect but it also may simply be due to a loss of power. To adjudicate between these two possibilities, we directly tested for a recency effect by measuring whether the temporal position of a once-presented pair in the encoding block predicted the corresponding trial-specific subsequent offline reactivation frequency (*SI Appendix*, *Supplemental Methods*). Results did not show a recency effect in any cortical ROI. Interestingly, while there was no significant association between encoding temporal position and offline reactivation in the RSC or the mPFC (both P > 0.19), we found a significant primacy effect in the VTC (b = −0.032, P = 0.004; *SI Appendix*, Fig. S3*B* and *SI Appendix*, Table S3 for full model outputs). Together, our results suggest that offline reactivation in the VTC might be sensitive to the temporal lag between encoding and post-encoding rest. Overall, given that we did not find evidence indicating a recency effect in cortical post-encoding offline reactivation, the prioritization of strong memory in offline reactivation in the cortex is a phenomenon not fully explained by the temporal proximity between encoding and rest.

Coordinated Hippocampal-Cortical Offline Reactivation. After assessing offline reactivation in the hippocampus and cortical regions separately, we next examined whether offline reactivation in the hippocampus and cortex was differentially synchronized for strong and weak memories. This was inspired by prior work showing that memories learned in a distributed fashion are associated with significantly enhanced hippocampal-cortical connectivity during their reactivation but not increased hippocampal or cortical activation locally (47). Therefore, while the hippocampus did not prioritize strong over weak memories for offline reactivation, we hypothesized that repetition may facilitate hippocampal-cortical coordinated reactivation in addition to local cortical reactivation frequency.

Extensive literature has suggested that different hippocampal subregions are not only functionally distinct from each other (43–46), but importantly, they also show differently weighted connectivity with cortical regions (33, 48–51). Therefore, in examining coordinated offline reactivation with cortical regions, we separately examined each of the anterior and posterior thirds

of the hippocampus. Our approach to assessing coordinated hippocampal-cortical offline reactivation was adapted from prior work (52). For each rest TR and within each memory condition, we took the binary offline reactivation patterns across the encoding trials (marking each trial as reactivated or not reactivated) from the thresholded similarity matrix of each brain region (Fig. 2*C*, *Right* for an example matrix). We then computed the Jaccard similarity (53) between the patterns for each pair of hippocampal and cortical regions (e.g. anterior hippocampus and VTC) during that TR, which represented the proportion of trials that were reactivated simultaneously in both regions. We then obtained an averaged Jaccard similarity index across all rest time points for each condition and for each participant, as a metric of coordinated offline reactivation (*Methods* for details). Additionally, for each pair of hippocampal-cortical ROIs, we also computed a chance-level Jaccard similarity index between the offline reactivation patterns using a permutation test and compared the observed levels of coordinated reactivation with the chance levels (see Methods for details).

We first confirmed that the observed levels of coordinated reactivation in all pairs of ROIs were significantly above the corresponding chance levels (all $t > 2.25$, all $P < 0.032$; Fig. 4). This result provides strong evidence for hippocampal-cortical coordinated reactivation with multiple cortical regions immediately following encoding. We next compared coordinated offline reactivation across the strong (last presentation) and weak memory conditions. Results revealed significantly greater coordinated reactivation between the posterior hippocampus and the mPFC for strong versus weak memories [$t(28) = 2.19$, $P = 0.037$, 95% CI(0.0002, 0.006), Cohen's $d = 0.41$; Fig. 4], suggesting that repetition increases the proportion of coordinated offline reactivation events between these two regions. This was not the case for other pairs of hippocampal-cortical ROIs [all $P > 0.12$; although a numerically similar trend was noted for coordinated reactivation between the posterior hippocampus and the RSC, $t(28) = -1.57$, $P = 0.127$].

To confirm that the difference in posterior HPC-mPFC coordinated reactivation between strong and weak memory conditions was driven by the encoding experience, we also performed a supplemental analysis examining the level of synchronization between the "pseudo-reactivation" in these two regions during the pre-encoding rest, and there we did not observe a significant difference between conditions [$t(28) = 0.81$, $P = 0.43$; *SI Appendix*, Fig. S4 and *SI Appendix*, *Supplemental Methods*]. Therefore, the increased coordinated reactivation for the repeated memories was a phenomenon that emerged only following encoding.

Taken together, our results show that while repeated encoding leads to a more general increase in reactivation events across cortical regions, coordinated reactivation increases due to repeated encoding are evident only in specific hippocampal-cortical networks, in this case, between the posterior hippocampus and mPFC.

Posten-coding Memory Offline Reactivation and Behavior. Offline post-encoding connectivity and reactivation have previously been shown to be correlated with later memory retrieval success (17, 18, 22, 23, 25, 54–58). Thus, we next examined whether post-encoding reactivation frequency is related to associative memory retrieval success for weakly and strongly encoded memories. To investigate this, we ran a trial-level mixed-effects linear model in each ROI, predicting retrieval success of each tested pair (remembered or forgotten on the immediate memory test) with the offline reactivation frequency of the corresponding encoding trial in the strong (last presentation) or weak memory conditions during the post-encoding rest period, while also controlling for the trial-specific univariate activity during encoding (*Methods* for details; *SI Appendix*, Tables S1 and S2 for full model outputs). It is noteworthy that our experimental design only allowed for the clean assessment of immediate associative memory in half of the studied pairs, because the other half were restudied in a separate task block before being tested the next day (see also *SI Appendix*, *Supplemental Methods*).

We found a significantly positive association between trial-specific hippocampal reactivation frequency and retrieval success probability for weakly encoded pairs ($b = 0.045$, $P = 0.004$; Fig. 5, *Top* panel; *SI Appendix*, Table S1-1). This result suggests that post-encoding hippocampal reactivation explains significant variance in memory outcomes seen at the time of the immediate associative recognition test for once-presented, or weakly encoded, information. In the strong memory condition, we did not see a significant effect of offline reactivation but, instead, found a trending interaction between hippocampal reactivation and encoding univariate activity strength in predicting memory performance ($b = 0.002$, $P = 0.091$), such that the association between offline reactivation and memory was marginally more positive for pairs that elicited greater univariate encoding activity. Follow-up analyses subdividing the hippocampus into anterior and posterior portions revealed a marginally positive association between reactivation and memory in the anterior hippocampus in the weak memory condition ($b = 0.028$, $P = 0.084$; Fig. 5, *Top* panel; *SI Appendix*, Table S1-1), and no significant associations between offline reactivation in the posterior hippocampus and memory outcomes in either weak or strong conditions (both $P > 0.41$; Fig. 5, *Top* panel; *SI Appendix*, Table S1-1 and S1-2). Together, these results suggest hippocampal offline reactivation, potentially driven by the anterior portion, predicts the successful retention of novel, once-presented information. In a follow-up exploratory analysis focused on individual differences (*SI Appendix, Supplemental Methods* for details), we also found that post-encoding reactivation in the whole hippocampus (as well as the anterior hippocampus alone) was positively associated with memory for the once-presented trials in "poor performers" whose accuracy fell below the median level of the group, but not in "good performers" whose performance was closer to ceiling (*SI Appendix*, Fig. S5 and Table S4).

We did not see a significant relationship between cortical offline reactivation in the VTC or mPFC and memory outcomes (all $P > 0.14$; Fig. 5, *Bottom* panel; *SI Appendix*, Table S2-1 and S2-2). However, in the RSC, the association between offline reactivation frequency and memory for the once-encoded pairs depended on the levels of univariate activity during the encoding of that pair (offline reactivation frequency × univariate activity: $b = -0.0007$, $P = 0.013$; Fig. 5, *Bottom* panel; *SI Appendix*, Table S2-1). Specifically, reactivation frequency in the RSC more positively predicted retrieval success of the once-presented items associated with weaker versus stronger univariate encoding activity. This interaction effect between offline reactivation and encoding activity strength was also significantly greater in the weak memory condition than in the strong memory condition (offline reactivation frequency × univariate activity × memory condition: $b = 0.001$, $P = 0.028$; *SI Appendix*, Table S2-1). In line with our finding that hippocampal offline reactivation is significantly associated with once-presented memories, cortical offline reactivation in the RSC differentially predicts the weakest of the weak memories, that is, those once-presented items associated with low univariate activity during encoding.

We did not find that offline reactivation in hippocampus or any preselected cortical ROIs significantly predicted immediate recognition success of the repeatedly encoded memories (all $P > 0.13$;

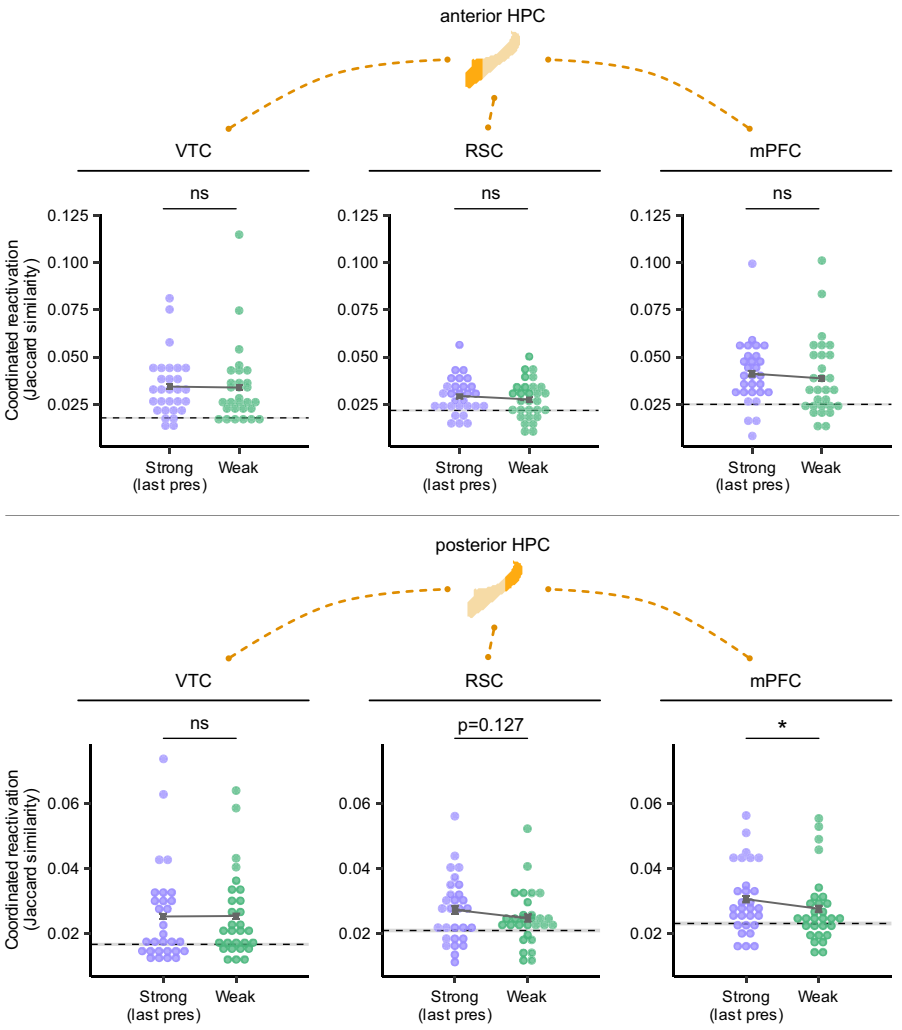

Fig. 4. Hippocampal-cortical coordinated offline reactivation for strong and weak memories. The Jaccard similarity index between offline reactivation patterns across anterior or posterior hippocampus and each of the cortical ROIs—our metric of coordinated offline reactivation—represents the proportion of trials that were simultaneously reactivated in both regions. Each dot represents a participant. Squared dots indicate mean values. Error bars show within-subject SE. Each shaded dashed line represents the ROIs-specific chance level of coordinated reactivation and its corresponding 95% CI. ns: not significant, $*P < 0.05$.

SI Appendix, Table S1-2 and S2-2). However, given that participants' memory accuracy for the repeatedly encoded pairs was close to ceiling, it is likely that the lack of reactivation-memory association resulted from the limited variance in the behavioral performance in the strong memory condition. To formally examine whether the near-ceiling performance would completely mask any potential relationship between offline reactivation and strong memory, we explored whether reactivation in cortical regions outside our preselected ROIs might be associated with the memory behaviors for the repeated pairs. To do this, we performed an exploratory parcel-based cortical searchlight analysis and repeated the offline reactivation analysis in each of the 400 cortical parcels from the Schaefer 17-network atlas (42). We identified the top five cortical regions showing strong evidence for prioritized reactivation of the repeated memories and then, using a similar mixed-effects model as in the previous analyses, we assessed the association between offline reactivation and retrieval success for the strong memories in each of these top five parcels. This searchlight analysis first revealed that beyond our a priori ROIs, the prioritization of strong over weak memories during offline reactivation was in fact a widespread effect in the cortex, predominantly observed in regions within the default mode networks, the executive control networks, and the dorsal attention networks (*SI Appendix*, Fig. S6*A* for details). Notably, a parcel within the mPFC, one of our preselected ROIs, demonstrated the second-strongest effect size among all 400 cortical parcels (see also *SI Appendix*, Table S5 and Fig. S6*B* for details of the top five parcels). Examining the reactivation-memory relationships in the identified top parcels, we found that in two of the top five parcels, one within the left lateral temporal cortex and the other within the left ventrolateral prefrontal cortex, offline reactivation significantly predicted subsequent memory of a repeatedly encoded pair (left lateral temporal cortex: b = 0.041, $P = 0.039$; left ventrolateral prefrontal cortex: b = 0.045, $P = 0.043$; *SI Appendix*, Fig. S6*C* and *SI Appendix*, Table S6 for complete model outputs of all five parcels). These results suggest that it is statistically possible to detect a significant association between offline reactivation and behavior in the strong memory condition despite the limited variance in accuracy in that condition. Nevertheless, it is still possible that the near-ceiling memory performance for the repeated pairs was a key factor that limited the sensitivity of our analysis in detecting a reactivation-memory association in our ROIs.

Discussion

In the current human fMRI study, we examined how study repetition, a commonly used tool in myriad contexts of learning, modulates the frequency and nature of post-encoding offline memory reactivation, in both the hippocampus and cortical regions. Specifically, we directly manipulated memory strength during encoding by presenting study events either once or three times. We then computed the frequency of offline reactivation events within

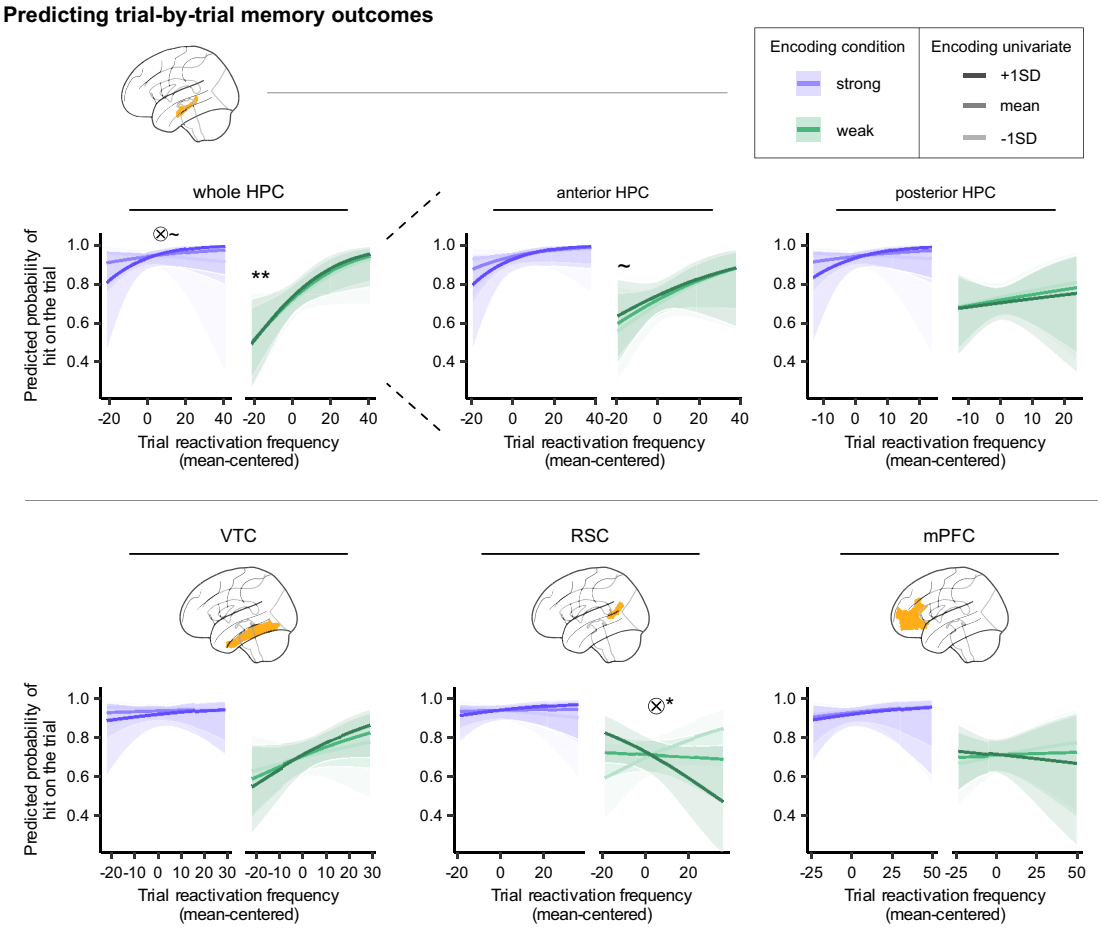

Fig. 5. Predicting memory outcome with post-encoding offline reactivation. Mixed-effects linear models predicting memory outcome of each pair with the trial-specific offline reactivation frequency and univariate encoding activity in weak and strong (last presentation) memory conditions. Saturation of lines indicates the strength of encoding univariate activity (included as a continuous variable in the models). Ribbons represent 95% CI. ⊗ interaction, ~$P < 0.1$, *$P < 0.05$, **$P < 0.01$.

each ROI right after learning. Interestingly, repeated encoding did not increase reactivation frequency in the hippocampus, which was statistically equivalent for once- and thrice-encoded memories. However, we found a striking increase in offline reactivation of repeated events in the VTC, RSC, and mPFC. Furthermore, we show evidence for significantly increased coordinated reactivation of repeated memories between the posterior hippocampus and mPFC compared to the once-encoded memories. Taken together, these results suggest that repetition during learning may augment the representation and distribution of memory traces across cortical networks immediately after learning, a strong biomarker of systems-level memory consolidation (1–3).

One of our main findings in this study is that the frequency of offline reactivation of strongly encoded memories, compared to once-presented associations, is significantly enhanced in cortical regions. This is consistent with prior work using principal components analyses to show that the strongest components of a learning event tend to be the ones that persist into post-encoding rest (17). Existing evidence has also revealed that memories associated with high emotional valence (59), high reward (12, 23, 25), or high utility (60) are prioritized during memory consolidation. Extending these findings, we show that neutral memories—with little motivational components or demands—strengthened through repeated encoding are also more extensively reactivated during offline periods in the cortex compared to events with more limited encoding opportunity. Importantly, the increased cortical reactivation of these events observed in our study cannot be simply explained by content differences across the once- and thrice-presentation conditions, as the assignment of image categories to encoding conditions was counterbalanced across participants. Further, for each event, the same content was presented on each repetition but the enhanced offline reactivation for the repeated events was restricted to the reactivation of the last presentation, suggesting that it was repetition, rather than the study content, that caused the change in cortical offline reactivation dynamics. Additionally, inconsistent with the possibility that encoding pattern similarity between items within a condition might have inflated our measures of offline reactivation, we found that repetition during encoding resulted in decreased interitem pattern similarity in cortical regions, indicating interitem increased cortical neural differentiation. Moreover, we examined whether the difference in cortical offline reactivation between encoding conditions was caused by encoding proximity to the rest period (i.e., a recency effect). However, we did not find evidence for a recency effect in offline reactivation in any of our cortical ROIs (although note that a primacy effect was revealed in the VTC). Therefore, these additional analyses rule out the influence of other experimental factors but point to repetition, and perhaps cortical differentiation, as being instrumental in augmenting post-encoding cortical reactivation.

Offline reactivation is a leading mechanism supporting consolidation (3, 9, 11, 13). Accordingly, our results showing increased reactivation for the repeated memories in the cortex align with the notion that repetition may accelerate or augment consolidation mechanisms (61–63). Importantly, the encoding repetition manipulation in our experimental design allowed us to examine offline reactivation for separate presentations of each repeated event. Existing work has shown that when the same information is repeatedly studied, a new memory trace may be established with each repetition, evidenced by people's ability to discriminate between repeated occurrences (64, 65). Here, we demonstrate that different memory traces linked to the same repeated experience also show varying levels of offline reactivation. Specifically, offline cortical reactivation of repeated events prioritizes the most updated, or the newest, memory representations, as

compared to the earlier presentations of the same content, which may suggest that they undergo diverging consolidation processes. On the one hand, the prioritization of the most updated encounters during consolidation may be because these memories will be more relevant in the future (24, c.f., 66). On the other hand, we show that with repetition, cortical memory representations become increasingly differentiated across experiences, in line with a sharpening effect (67, 68). It is possible that the observed reactivation differences across repetitions reflect ongoing fine-tuning of event representations in cortical networks during repeated encoding. Future work is required to further test these hypotheses.

Perhaps surprisingly, in the current study, we do not find convincing evidence in the hippocampus for differential offline reactivation between strong and weak memories. By contrast, and as mentioned above, some prior work in humans has demonstrated that the hippocampus selectively reactivates the strongest components of the neural encoding activity patterns during post-encoding rest (17), while others have shown that hippocampal offline reactivation prioritizes information that was weakly learned (14). One possible reason for the different findings within the hippocampus may be the inconsistent definitions or manipulations of memory strength. While these two aforementioned studies have distinguished between strong and weak memories based on the explanatory power of encoding activity principal components (17) and levels of memory performance (14), respectively, the current study directly manipulated memory strength via repeated presentations during encoding. Our repetition manipulation more strongly impacted offline reactivation in the cortex than in the hippocampus, such that repeated encoding solely increased cortical, but not hippocampal, reactivation. This begs the question of whether and how reactivation of a specific event in the hippocampus and certain cortical regions differentially impacts its memory representations in the brain, and how reactivation in distinct brain regions contributes to different forms of memory (69).

The current results also highlight the importance of studying offline reactivation across hippocampal-cortical networks as a systems-level phenomenon. By broadening the scope of investigation beyond the hippocampus, we not only reveal that the hippocampus and cortex show differences in their local offline reactivation dynamics, but importantly, we also demonstrate above-chance-level coordinated reactivation between the hippocampus and the cortical networks immediately following encoding for both strong and weak memory conditions. Our results further show that repeated encoding significantly increases the level of coordinated reactivation between the posterior hippocampus and mPFC. This result, along with the increased frequency of reactivation within the mPFC for the repeated events, may suggest accelerated distribution of repeated memories from the hippocampus to the cortex during ongoing consolidation. This hypothesis also aligns with findings from a recent study in rodents, showing active hippocampal offline replay following single experiences but decreased hippocampal replay rate for repeated experiences (70).

Notably, this "acceleration" idea is built upon emerging evidence highlighting different routes to facilitating memory consolidation (71). For example, it has been demonstrated that with one-shot learning, novel memories that are schema-consistent can rapidly become hippocampal-independent, suggesting that schema promotes systems consolidation (72). Further, theoretical perspectives have suggested that memory retrieval quickly engages interactions between the hippocampus and the cortex, increasing neural plasticity in the cortex, which closely resembles mechanisms underlying consolidation (73). Consistent with this framework, rapid emergence of cortical memory engram has been observed following repeated encoding-retrieval cycles, providing evidence for facilitated consolidation (61–63). Extending beyond prior work, we highlight that repeated encoding alone may also show a similar facilitation effect on consolidation, evidenced by increases cortical offline reactivation and hippocampal-cortical coordinated reactivation in the post-encoding rest period immediately after learning. Taken together with previous work, it is clear that a more robust understanding is warranted regarding how the structure of learning influences plasticity in hippocampal-cortical systems, and the extent to which these accelerated consolidation processes might contribute to long-term memory distribution or reorganization of recent experiences. However, it should be mentioned that while existing work, including the current study, has largely focused on examining offline interactions between the hippocampus and the cortex during consolidation, it is possible that accelerated consolidation processes involve different forms of cross-region interactions, including both hippocampal-cortical and likely also cortical–cortical communications, which can be further explored in future studies.

In contrast to the repeated experiences, memories for the once-encoded events may be lagging behind in cortical offline reactivation and thus still highly hippocampal-dependent. In line with this hypothesis, we show that hippocampal reactivation frequency of once-presented memories positively predicts the probability of successful retrieval. One interpretation of this result can be that offline reactivation in the hippocampus may compensate for inadequate encoding and rescue weak memories (14). Consistent with this idea, we also show that, across participants, hippocampal offline reactivation predicts memory for once-encoded associations specifically in "poor" rather than "good" performers. Taken together, these results align with prior work in rodents showing that hippocampal offline reactivation is more essential for the memory of novel, rather than familiar, experiences (74).

Beyond the hippocampus, this "rescue" account may also help explain the relationship between offline reactivation and subsequent memory in the RSC, where we see an interaction between reactivation frequency and univariate encoding activity strength in predicting memory. Specifically, we find a more positive association between reactivation and later memory for once-presented events that elicited low, rather than high, levels of univariate activity during encoding, potentially suggesting a role of reactivation in the RSC in maintaining the weakest of the weak memories. Interestingly, some existing literature has suggested that low encoding activity in the broader posteromedial cortex—consisting of the RSC, precuneus, and posterior cingulate cortex—may be indicative of successful encoding (i.e., a negative encoding effect; 75, 76), which would point to a different interpretation of the aforementioned interaction effect, such that the most successfully encoded events are more benefitted from offline reactivation. However, it is important to mention that such negative encoding effects (where low encoding activity predicts *successful* encoding) have been more consistently observed in the precuneus and posterior cingulate cortex (75–78). By contrast, other studies have demonstrated the opposite in the RSC: successful encoding is associated with high, rather than low, encoding activity [i.e., a positive encoding effect; (69, 79, 80)]. In our data, there was no significant association between encoding univariate activity in the RSC and later memory (although the effect was numerically positive for weakly encoded events; *SI Appendix*, Table S2), so we refrain from making strong conclusions based on our results alone.

Interestingly, we did not find a significant association between offline reactivation frequency and immediate memory outcomes for the repeated events in our a priori ROIs. This lack of association could be due to several reasons. First, in the current study, memory was tested immediately following the rest period and memory performance was close to ceiling levels in the strong memory condition in many participants (mean accuracy = 0.90). Therefore, the limited variance in

the trial-to-trial performance in the strong memory condition likely prevented us from effectively capturing a relationship between post-encoding offline reactivation and retrieval success. However, results from our exploratory searchlight analysis show that offline reactivation in the lateral temporal cortex and the ventrolateral prefrontal cortex, two cortical regions that extensively reactivated the repeatedly encoded memories, significantly predict subsequent retrieval success of the repeated pairs. These results simply highlight that there are cortical regions whose reactivation is related to memory for the strongly encoded pairs, even with the near-ceiling performance. Future work will be needed to better characterize relationships between reactivation and memory for well-encoded experiences in experiments with longer study-test intervals and, perhaps, with more detailed probes of memory content. For example, facilitated consolidation of the repeated memories may lead to memory integration with prior knowledge (39, 81), or memory transformation toward semanticized, gist-like representations (82, 83). Further, we would like to note that in the current experiment, only pairs from one of the two encoding blocks were tested in the immediate recognition test (*SI Appendix*, Supplemental Methods), which may introduce additional factors affecting our results. Therefore, future investigations are needed to more carefully implement the optimal behavioral tests in order to better detect potential reactivation-memory associations and other possible consequences of post-encoding activities.

From the exploratory searchlight analysis, we also demonstrate that the prioritization of strong over weak memories during offline reactivation is a widespread effect that can be found in cortical regions including, but not limited to, our a priori ROIs. However, a main takeaway from our results is that the extent and implications of reactivation vary across regions. Even our three preselected cortical ROIs exhibit important distinctive characteristics of offline reactivation. For example, reactivation frequency in the VTC, but not RSC or mPFC, is sensitive to the temporal interval between encoding and rest. Coordinated reactivation is only significantly increased with repetition between the posterior hippocampus and mPFC. Additionally, the RSC is the only ROI that exhibits quite high levels of pattern similarity with encoding activities in its pre-encoding "pseudo-reactivation" events. Specifically, as part of our control analyses, we found that all ROIs showed evidence for an increase in the frequency of reactivation during rest after, as compared to before, the encoding experiences. However, when measured with continuous encoding-rest similarity above threshold, a metric precisely indexing the magnitude of reinstatement, the RSC did not show a significant increase from pre- to post-encoding rest. Therefore, one possibility is that compared to the other cortical ROIs, the RSC is more involved in establishing the backbone activity patterns that will be allocated to become the core representation structure for the to-be-encoded memories (84–87). This is considered on balance, however, with the finding that retrosplenial cortical reactivation frequency (and not that in VTC or mPFC) explains variance in later memory for once-presented events. Together, future work is needed to explore the shared features in rest activities before and after encoding, as well as to further characterize how experience-dependent changes in post-encoding reactivation might differ across regions.

In conclusion, the current study provides important evidence that repeated study leads to significantly increased levels of cortical offline reactivation and hippocampal-cortical coordinated reactivation right after encoding. This was not the case for hippocampal offline reactivation, which did not statistically differ for once and repeatedly encoded memories. These findings suggest that repetition during encoding facilitates the distribution of memory across hippocampal-cortical networks, potentially accelerating memory consolidation. We also reveal that post-encoding offline reactivation in the hippocampus and the RSC explains variance in the subsequent behavioral outcomes of weakly encoded memory, potentially suggesting that offline reactivation in certain regions plays a role in the stabilization of weak memories. Together, our findings provide insights into the hippocampal-cortical dynamics during offline memory processes. Future work can further investigate other aspects of our learning experiences to assess how and when we study materials might impact memory consolidation by examining post-encoding offline reactivation.

Methods

Participants and Procedures. Thirty-two participants completed the full experiment. Three participants were excluded from all analyses (two due to excessive head motion; one due to substantial signal dropouts in cortical regions). The final sample consisted of 29 participants. The current study included a pre-encoding baseline post-encoding rest period, two encoding blocks and their corresponding rest periods, as well as a test block; all parts were conducted in an fMRI scanner (Fig. 1*A*; see also *SI Appendix*, *Supplemental Methods* for details regarding full experimental procedures, study materials, MRI data acquisition and preprocessing procedures).

Encoding. Each participant studied 72 words paired with images from two visual categories. The word-image pairs were equally split across two encoding blocks, which had an identical structure. Within each encoding block, pairs with images from one visual category were presented only once, which formed the weak memory condition; whereas pairs with images from the other category were presented three times, which formed the strong memory condition (Fig. 1*B*). Each encoding block included a total of 72 trials (36 different pairs, 18 pairs in each memory condition), presented in a randomized order. Each encoding trial was on screen for 4 s, during which participants were asked to rate how well the word was associated with the image using a button box. Each trial was then followed by a 6 s intertrial interval (ITI), during which a fixation cross was presented.

Test. Participants completed an associative recognition test immediately following the encoding phase. They were tested on memory for pairs studied from one of the two encoding blocks (pairs from another encoding block were studied again in a restudy block and tested the next day, which was irrelevant to the current study; see also *SI Appendix*, *Supplemental Methods*). The temporal interval between the memory test and the corresponding encoding block was controlled for across all participants. The test included a total of 36 trials. Each test trial started with a 4-s retrieval phase, in which a studied word was presented on screen and participants were asked to mentally recall the image paired with the word. Following the retrieval phase, participants were asked to make an associative recognition judgment about the pair. Four image choices appeared on the screen together with the word, and participants were asked to choose the image that was paired with the word by pressing the corresponding button using a button box. The word and the image choices were presented on screen for 2 s, but the response window continued into a 6-s ITI following each trial.

Rest Periods. The study began with a 7-min post-encoding rest scan (baseline rest period; 210 TRs), followed by the first encoding block. Each of the two encoding blocks was also immediately followed by another 7-min rest period (rest period; 210 TRs each). Participants were asked to keep their eyes closed while remaining awake during all rest periods.

Statistical Analyses. We performed statistical analyses using a combination of two-tailed *t* tests, ANOVA, and mixed-effects linear models. (For results presented in *SI Appendix*, Supplemental Figures, see *SI Appendix*, *Supplemental Methods* for analysis details.)

Behavioral Performance. We computed each participant's proportion correct (mean accuracy) on the associative recognition test for strong and weak memory conditions. We first compared the mean accuracy in each condition to the chance level (0.25) using a one-sample *t* test, and then compared accuracy across conditions using a paired *t* test.

Least Squares Separate (LSS) Modeling of Encoding Trials. To capture offline reactivation of the encoded information during post-encoding rest periods, we first measured the multivoxel activity pattern associated with each encoding trial. Specifically, we used the LSS modeling approach and conducted a separate general linear model (GLM) for each encoding trial (88, 89), implemented using FEAT (90). Each model included a single encoding trial as the regressor of interest, and four regressors of no interest grouping all other trials by condition within the given encoding block (trials of the once present pairs; trials of the first, second, or third presentation of the thrice

presented pairs). All trials were modeled as 4 s boxcars convolved with hemodynamic response function (HRF). Each GLM also included six motion parameters, their 1st temporal derivatives, and the top five anatomical CompCor separately from WM and CSF voxels ("fMRI data preprocessing" in *SI Appendix, Supplemental Methods*) as nuisance regressors. In this way, we obtained voxelwise parameter estimates of activation that were specific to each encoding trial.

Offline Reactivation Analysis. We adapted the analyses from recent work (14, 18) to assess offline reactivation of the encoding trials during post-encoding rest periods in each ROI (Fig. 2*A*; see also *SI Appendix, Supplemental Methods* for detailed ROI definition). First, we further cleaned the signals in the preprocessed rest scans by removing confounds including six motion parameters, their 1st temporal derivatives, and the top five anatomical CompCor separately from WM and CSF voxels (see "fMRI data preprocessing" in *SI Appendix, Supplemental Methods*). Next, for each encoding block from each participant and within each of their ROIs, we computed pattern similarity scores (i.e., Pearson correlations) between the activation patterns of each encoding trial and each TR during both the corresponding post-encoding rest period and the pre-encoding baseline rest period. We thus obtained two encoding-rest pattern similarity matrices associated with each encoding block, pre- and pre-encoding matrices, and each matrix contained similarity values for 72 encoding trials by 210 rest TRs (Fig. 2 *B* and *C*). We then derived a reactivation threshold from the pre-encoding matrix (1.5 SDs above the mean across all similarity values) and identified the high similarity values from the corresponding pre-encoding matrix that surpassed the threshold. We then treated rest TRs associated with these high pre-encoding similarity values as evidence for offline reactivation of specific encoding trials (Fig. 2*C*). Here, the similarity scores from the pre-encoding matrix provided a reference distribution for chance level reinstatement of the encoded information during a rest period (18).

After identifying the offline reactivation events during pre-encoding rest, we then compared the offline reactivation frequency across strong and weak conditions within each ROI. Here, we first divided the encoding trials in the strong memory condition into three more fine-grained conditions according to the iteration of presentations (strong 1st presentation, strong 2nd presentation, strong last presentation). Each of the three fine-grained strong memory conditions contained the same number of trials as in the weak memory condition (18 trials). We then counted the number of TRs in each pre-encoding rest period that showed offline reactivation for any encoding trial within each condition. Note that in this analysis comparing offline reactivation at the condition level, we assigned either 0 or 1 point to each encoding condition at each rest TR. That is, even when multiple similarity values within a condition passed the reactivation threshold at a rest TR, we still only considered a frequency of 1 for the given condition at that TR. This was to avoid the confound that the encoding pattern similarity across trials might differ between conditions (see also *SI Appendix*, Fig. S1).

We then averaged the offline reactivation frequency across the two pre-encoding rest periods and used a paired *t* test to compare reactivation frequency between the strong memory (last presentation) condition and the weak memory condition, which was our main comparison of interest (see Results for reasoning). Importantly, we also compared the amount of offline reactivation across the three presentations in the strong memory condition in each ROI (averaged across two rests), using a one-way repeated-measures ANOVA followed up with paired *t* tests. This analysis was conducted in order to test the hypothesis that the representations of the last presentation of the repeated pairs would be more reactivated than the earlier presentations. For the comparisons both between the strong (last presentation) and weak memory conditions, and between the three fine-grained strong conditions, we separately applied FDR corrections to adjust P-values of the statistical tests across the two hippocampal subregions (anterior, posterior), and across the three cortical ROIs (VTC, RSC, mPFC). Note that we did not apply FDR corrections for multiple comparisons across regions in the other reported analyses (unless otherwise specified), which either followed up on our primary findings regarding memory prioritization during offline reactivation, or addressed secondary research questions after we revealed distinct properties of offline reactivation in different ROIs (see Results).

Coordinated Offline Reactivation Between the Hippocampus and the Cortex. To examine coordinated hippocampal-cortical offline reactivation of strong and weak memories, we assessed the proportion of encoding trials in each condition that were simultaneously reactivated across regions. Given the segregation of connectivity between different HPC subregions to the cortex (33, 48–51), this analysis was separately conducted for the anterior and posterior portions of the hippocampus. For each TR during rest and for each memory condition, we first extracted the binary vector of offline reactivation patterns across the encoding trials (1 = trial reactivated, 0 = trial not reactivated) from the thresholded similarity matrix of each brain region (Fig. 2*C*, *Right*). We then computed a Jaccard similarity index between the patterns of each pair of the hippocampal subregion and the cortical region of interest (e.g., anterior HPC and VTC). We averaged the Jaccard similarity indices across all rest TRs in each condition as the measure of coordinated offline reactivation. We compared the mean Jaccard similarity index across the strong (last presentation) and weak memory conditions using a paired *t* test. Note that when comparing levels of coordinated reactivation for strong and weak memories, we did not control for encoding-rest temporal proximity across conditions. Given the limited number of trials coreactivated across regions at each TR, all these trials were included in the analysis.

To confirm whether the observed levels of coordinated offline reactivation as measured with the Jaccard index were greater than chance, we performed a permutation analysis to compute the chance-level coordinated reactivation for each pair of hippocampal-cortical ROIs. Specifically, in each ROI at each rest TR, we first sampled from the possible range of the total number of reactivated encoding trials within a TR based on the actual data. We then randomly shuffled the pair labels (i.e., the specific encoding content) of these trials and computed the Jaccard index across the simulated reactivated trials between a pair of hippocampal and cortical ROIs. We repeated this procedure for a total of 100,000 simulated rest TRs to obtain a distribution of chance-level coordinated reactivation and computed the corresponding mean and CI for each pair of ROIs. We then compared the observed levels of coordinated offline reactivation in each condition with the ROIs-specific chance levels using two-sample *t* tests.

Relating Offline Reactivation and Behavioral Performance. We performed trial-level mixed-effects linear models to examine the association between post-encoding offline reactivation and subsequent memory outcomes on the recognition test. Here, we counted the number of TRs each encoding trial was reactivated across the entire post-encoding rest period. Note that for pairs in the strong memory condition, we only included trials within the strong memory last presentation condition. In each ROI, we predicted whether a pair was remembered or forgotten on the recognition test (i.e., retrieval success: correct vs. incorrect) with the reactivation frequency of the given trial during post-encoding rest (mean-centered), the encoding condition of the pair (strong vs. weak; we separately treated each condition as the baseline in parallel models with identical terms), the univariate activity associated with the encoding trial (mean-centered), as well as their interactions. Univariate encoding activity for each trial was measured by computing the average of the trial-specific parameter estimates obtained from LSS modeling across all voxels within each ROI. All fixed effects (including the intercept) were included as random effects, grouped by participant. Models used a logistic linking function.

Data, Materials, and Software Availability. Anonymized fMRI raw scans have been deposited in OpenNeuro, available at https://doi.org/10.18112/openneuro.ds005464.v1.0.0 (91). Processed/cleaned data and analysis code necessary to recreate results and plots have been shared on the Open Science Framework at https://osf.io/vwdu3/ (92).

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