Title: Cross-region neuron co-firing mediated by ripple oscillations supports distributed working memory representations.

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Abstract
High-frequency (~90Hz) ripple oscillations may promote integrative processing in mammalian brains. Previous work has demonstrated that the co-occurrence of these ripple oscillations is associated with enhanced temporal binding of neural activity between human cortical neurons separated by up to 12mm. However, it remains unclear whether co-ripple facilitation of neuronal coupling supports cognitive processing, or if it occurs at greater distances. Here, we analyze intracranial recordings from patients implanted with microwire electrodes in the hippocampus, amygdala, ventromedial prefrontal cortex, anterior cingulate cortex, and pre-supplementary motor area, bilaterally, during a working memory task. We demonstrate that ripple oscillations significantly increase in all recorded regions, during encoding, maintenance and retrieval. Furthermore, co-occurrence of ripples increases between brain regions, associated with ~30% increases in cross-region co-firing, both without decrement over distances up to 220 mm. These increases in cross-regional co-rippling and associated co-firing scale with memory load during maintenance and retrieval. Critically, during retrieval, co-ripples promote the reinstatement of the stimulus-specific long-distance co-firing patterns observed during encoding, especially during rapid recognition. Our findings reveal that ripple oscillations orchestrate long-range neural communication that supports distributed neural representations during human cognition.

INTRODUCTION

The ability of distant brain regions to communicate effectively is fundamental to cognition, but the mechanisms facilitating long-range neuronal integration during cognitive processing remain incompletely understood. High frequency synchronous oscillations have been proposed to assist in such integration during complex cognitive operations [1-4] such as working memory (WM), but their characteristics in humans have not been fully delineated [5], and their role remains the subject of considerable debate[6].

High-frequency ripple oscillations, first identified as components of hippocampal sharp wave-ripple complexes in rodents, have traditionally been associated with memory consolidation during sleep and quiet wakefulness[7, 8]. While initially discovered embedded within sharp waves, subsequent research has demonstrated that similar oscillations occur widely throughout the cortex, often independently of any sharp wave activity[9-14]. These brief (50-100 ms) bursts of oscillatory activity facilitate the replay of previously experienced sequences of neuronal firing in rodents[15, 16], suggesting a role in information transfer between hippocampus and neocortex[17, 18]. Furthermore, the widespread detection of these ripple oscillations across various cortical regions in both awake animals[19, 20] and humans[10, 12-14, 21-25] indicates they represent a general cortical phenomenon rather than a hippocampus-specific event, raising the possibility that ripples might coordinate neuronal activity across widely distributed brain networks during active cognition.

These recent investigations in humans have demonstrated that coupled rippled oscillations in human neocortex are associated with more coordinated local neural firing patterns during spontaneous behavior[25], memory retrieval[21] and visual categorization[14], but it remains unclear whether ripples coordinate neural firing across brain-wide spatial scales. To fill this gap, we analyzed an open-access dataset of simultaneous single-unit and local field potential (LFP) recordings from intracranial Behnke-Fried microwires implanted in patients undergoing diagnostic monitoring for intractable epilepsy. Specifically, we examined if co-occurring ripples across limbic and frontal areas facilitate the temporal coupling of neuronal firing during a Sternberg working memory task[26]. This large dataset, which contains widespread unit spike trains and LFP recordings, make it possible to directly examine whether synchronous ripples in different forebrain locations and hemispheres provide a physiological environment conducive to

greater interaction of neuronal firing between those locations, and whether those co-ripples and coordinated unit-firing are systematically related to task demands.

We found that ripple oscillations occur in both limbic and frontal brain regions during the working memory task, with rates of ripples and associated neural firing significantly increasing during the encoding, maintenance and probe phases relative to pre-trial baseline. The co-occurrence of ripples between distant brain regions showed selective enhancement during these same phases, particularly under high memory load conditions. This ripple co-occurrence demonstrated stronger modulation by memory demands compared to other high-frequency oscillations, suggesting a specific role for ripples in coordinating neural activity during cognitively demanding operations such as WM maintenance.

Co-occurring ripples were associated with increased neural co-firing between brain regions during all task stages, showing an increasing effect with higher memory load during maintenance and retrieval. Co-ripples promoted the repetition of stimulus-specific patterns of neural co-firing during faster WM-based decision making, potentially supporting information encoding and more efficient memory retrieval. These findings show that ripple-mediated neural synchronization serves as a general mechanism for information integration across the human brain.

RESULTS

Ripples and units are detected in all areas during a Sternberg working memory task

We analyzed intracranial recordings from a dataset previously published by Daume et al. [26]. In our study, we included data from 35 patients across 43 sessions (14 males, 21 females; age range 20-67 years) implanted with electrodes for evaluation of medically refractory epilepsy (Fig. 1A,B). Electrodes targeted the ventromedial prefrontal cortex (vmPFC), anterior cingulate cortex (ACC), pre-supplementary motor area (preSMA), amygdala (AMY), and hippocampus (HIP), bilaterally. Local field potentials (LFPs) and single-unit activity were simultaneously recorded and subsequently analyzed from each of the 8 microwires. Single units were identified based on previously reported methods [25, 27], and single units were merged if they were simultaneously detected on two microwires within the same bundle (see Methods). All patients provided informed consent, and procedures were approved by the Institutional Review Board at each participating institution.

Patients performed a modified Sternberg working memory task (Fig. 1B) with varying memory loads (1 or 3 items), during which they were instructed to remember a set of images presented sequentially (encoding phase), maintain the memory over a delay period (maintenance phase), and identify whether a probe image was part of the original set (probe phase). A total of 1373 single units were isolated and classified across 1927 microwire channels (Fig. 1C). Ripples were detected in each brain region (Fig. 1D,E) using established methods[19] (peak of 70-100 Hz band-filtered LFP exceeding 2.5 standard deviations above mean power, at least 3 oscillations, the absence of sharp transients; see Methods. Care was taken to exclude epileptiform periods or electrodes). Per-region ripple density, amplitude, duration, and frequency (Fig. 1F-I) were as follows: hippocampus (31.8 ± 5.2 min^{-1}, 9.89 ± 9.00 μV, 73 ± 14 ms, 90.7 ± 1.3 Hz), amygdala (31.2 ± 6.9 min^{-1}, 9.24 ± 8.51 μV, 75 ± 13 ms, 90.9 ± 1.1 Hz), vmPFC (31.8 ± 5.5 min^{-1}, 7.23 ± 5.14 μV, 72 ± 11 ms, 91.1 ± 0.9 Hz), ACC (30.8 ± 5.6 min^{-1}, 5.85 ± 3.39 μV, 69 ± 8 ms, 91.3 ± 0.7 Hz), and preSMA (32.7 ± 5.2 min^{-1}, 6.92 ± 3.93 μV, 72 ± 10 ms, 91.2 ± 0.9 Hz). Ripple characteristics showed consistent profiles across patients despite differences in precise electrode locations (Supplementary Fig. 1). When a ripple was detected on any microwire channel within a bundle, single units recorded from that bundle fired an average of 0.23 ± 0.26 action potentials during the ripple event. No comparisons were made between units recorded on the same microwire to avoid contamination due to imperfect sorting.

Ripples and unit-firing co-occur across long separations without significant decrease with distance

To characterize the spatial properties of ripple-mediated neural coordination, we analyzed how ripple co-occurrence (i.e., the percent of ripples on two contacts that overlap by 25ms or more) and associated unit co-firing varied with distance between recording sites. We first examined ripple co-occurrence within microwire bundles (intra-bundle, <~4 mm separation) and found rates comparable to previous reports at the microscale[25], with a median co-occurrence rate of 13% [IQR 7%, 22%] within a bundle.

When examining ripple co-occurrence across greater distances, we found that while co-occurrence rates were lower between bundles (5% [IQR 4% 6%]) compared to within bundles, there was minimal further reduction as a function of fiber tract length between microwire bundles. Between different microwire bundles within the same hemisphere (inter-bundle, 71-203 mm separation), median ripple co-occurrence rates were 5% [IQR 4% 6%] (Fig. 1J). Surprisingly, cross-hemispheric (35-223 mm separation) ripple co-occurrence rates were almost identical (Δ 0.1% co-occurrence rate between intra and cross hemisphere co-ripple rates), indicating that once ripples extend beyond the local bundle, their co-occurrence probability remains relatively constant regardless of distance.

To determine whether co-ripples facilitate neural communication between distant brain regions, we analyzed unit co-firing (spikes co-occurring within a 25 ms window) during periods with co-occurring ripples versus periods without ripples. Specifically, we examined whether co-ripple periods were associated with enhanced co-firing relative to no-ripple periods, and whether this enhancement varied across different anatomical connections and distances.

We compared co-firing rates between units detected on wires within the same bundle and co-firing between units detected on different bundles. For each cell-pair, co-ripple periods were defined as times when any microwire in both units' respective bundles contained detected ripples. Conversely, no-ripple periods were defined as times when no ripples were detected in either bundle.

Across all unit pairs (n = 31489), we observed significantly higher co-firing rates during co-ripple periods compared to no-ripple periods (median increase 37% [IQR -14 – 104%], p ~ 0, Wilcoxon signed-rank test). We also found that co-firing during co-ripples was comparable between units recorded from the same microwire bundle (median co-fire rate 0.17Hz [IQR 0.04–0.61Hz]) and units recorded from different bundles (median co-fire rate 0.16 Hz [IQR 0.02–0.53Hz]), p = 7.5e-28 for the difference). While the difference between local and distant co-firing was significant, the effect size was minimal, challenging the intuitive expectation that co-firing would be stronger for nearby neurons.

To further investigate how ripple-mediated coordination varies across specific anatomical pathways, we categorized across-bundle unit pairs into five connection types: amygdala-cortex (AMY-CTX, n = 1884 pairs), hippocampus-cortex (HIP-CTX, n = 236 pairs), amygdala-hippocampus (AMY-HIP, n = 4019 pairs), ipsilateral cortico-cortical (CTX ipsi, n = 3063 pairs), and contralateral cortico-cortical (CTX contra, n = 5004 pairs). All five connection types showed significant enhancement of co-firing during co-ripple periods (coR) compared to no-ripple periods (noR) (Fig. 1K). The magnitude of this enhancement was broadly similar across connection types, with median increases of 38% [IQR: -4 – 211%, p = 9.9e-116, Wilcoxon signed rank test, coR vs noR] for AMY-CTX, 20% [IQR: -1 – 109%, p = 3.4e-17] for HIP-CTX, 33% [IQR: -13 – 196%, p = 1.6e-162] for AMY-HIP, 22% [IQR: -7 – 157%, p = 4.6e-140] for CTX ipsi, and 29% [IQR: -6 – 170%, p = 2.3e-263] for CTX contra pairs.

Next, we examined how the magnitude of co-firing enhancement between co-ripple and no-ripple periods varied with fiber tract distances between recording sites. Surprisingly, we found that the difference between co-ripple and no-ripple co-firing increased slightly with distance (r = 0.04, p = 5.3e-14). Independently, co-firing increased slightly with distance during co-ripple (r = 0.03, p = 5.5e-8) and no-ripple periods (r = 0.013, p=0.018). To ensure these findings were not

driven by differences in baseline firing rates or recording quality across regions, we normalized co-firing rates by the geometric mean of the individual firing rates of each unit pair, which yielded similar results (Supplementary Fig. 2).

These findings reveal that ripple oscillations and neuronal firing are coordinated across widely distributed brain networks with minimal attenuation over anatomical distance. This distance-invariant enhancement of communication provides a potential mechanism for binding distributed neural representations during cognitive processing.

Ripple and unit-firing rates increase in all regions during all task phases

To characterize the dynamics of neural activity during different phases of the working memory task, we quantified ripple occurrence rates and associated unit firing across all recorded brain regions. We observed systematic modulation of ripple activity that varied across task phases, with distinct patterns in limbic (hippocampus, amygdala), preSMA, and frontal (vmPFC, ACC) regions. During the encoding and maintenance phases, ripple rates increased moderately compared to pre-trial baseline levels in all recorded regions (encoding: 13% increase, maintenance: 10% increase, Fig. 2A). The greatest increase in ripple rates relative to baseline during encoding was observed in the hippocampus (20% increase, $p < 1e-10$, Wilcoxon rank-sum) and amygdala (16% increase, $p < 1e-10$), while the greatest increase during maintenance was observed in the vmPFC (13% increase, $p < 1e-10$) and hippocampus (13% increase, $p < 1e-10$). The task-related modulation of ripple activity was most pronounced during the probe phase, where we observed substantial increases in ripple rates across hippocampus (13% increase from baseline, $p < 1e-10$), amygdala (12% increase, $p < 1e-10$), and preSMA (36% increase, $p < 1e-10$).

Importantly, mean activity across all detected single units activity demonstrated similar, although not identical, modulation patterns (Fig 2B), with neuronal firing rates showing significant positive correlations with ripple rates during encoding ($r = 0.21$, $p = 1.8e-115$), maintenance ($r = 0.21$, $p = 1.41e-59$) and probe phases ($r = 0.25$, $p = 1.9e-87$) in all regions. Overall firing increased by 6% during encoding, 4% during maintenance, and 6% during the probe. Within-ripple firing rates of neurons also increased significantly during these phases compared to ripples occurring during baseline periods (2.95 Hz vs 2.83 Hz, $p = 3.40e-4$, Wilcoxon signed rank test), suggesting enhanced neuronal recruitment during task-relevant ripples.

Within region co-ripple rates and unit firing rates were also differentially modulated by memory load. Co-ripple rates increased during maintenance according to memory demand across the entire set of microwires ($p = 0.038$, load 3 vs. load 1, FDR-corrected linear mixed effects) and within ACC ($p = 0.006$). Firing rates across all neurons did not increase significantly with memory demand during maintenance ($p=0.99$), but did increase within the subset of neurons located in the ACC ($p = 0.03$) and SMA ($p = 0.003$). During the probe phase, co-ripple rates also increased with memory load across all microwires ($p = 3.2e-6$) as well as within SMA ($p = 0.007$), AMY ($p=0.04$) and HIP ($p=7.8e-5$). Similarly, firing rates across all neurons increased with memory demand during probe ($p=0.001$), as well as for neurons located in the ACC ($p = 1e-4$), SMA ($p = 0.002$) and AMY ($p=0.03$).

We next investigated the relationship between ripple activity and behavioral performance, specifically whether there was any relationship between ripple rates during fast and slow response times (RTs). We only analyzed rippling in high memory load trials (i.e., load 3), defining fast and slow responses as above and below the median response time for load 3 trials. We found that overall ripple rates after the probe stimulus were significantly higher during fast response trials compared to slow response trials, specifically in the hippocampus ($p = 9.3e-4$, linear mixed effects) and amygdala ($p = 0.013$). Temporal analysis revealed that the peak hippocampal ripple response occurred earlier for fast response trials compared to slow response trials (peak at 408ms vs 565ms post-probe onset). Moreover, the hippocampal ripple peak consistently preceded the amygdala peak in both fast and slow response conditions (515ms vs 600ms)

suggesting a potential directional flow of ripple-mediated information from hippocampus to amygdala during memory retrieval.

These findings indicate that ripple oscillations and unit firing in both limbic and frontal regions are dynamically modulated during working memory processes, with robust activation during all phases of the task, especially when memory load is higher. These findings indicate that not only the magnitude but also the timing of ripple activity, particularly in limbic structures, is associated with efficient memory-guided decision making.

Beyond the stimulus-locked, load modulated and response timing effects that form the core of our findings, we examined multiple task-related parameters to comprehensively characterize ripple and unit dynamics during working memory processing. Specifically, we investigated ripple rates when probe stimuli matched versus mismatched the encoded stimulus set (Supplementary Fig. 4), as well as neural activity locked to the response key press timing (Supplementary Fig. 5). While all of these analyses revealed significant modulation of both ripple oscillations and unit firing, we found that neural responses were most robustly engaged by stimulus-related processing compared to motor response execution. Furthermore, memory load manipulations elicited stronger and more consistent differences in ripple and unit activity profiles across brain regions than other task parameters. Given the superior signal-to-noise ratio observed in stimulus-locked analyses under varying memory loads and response times, we focused much of our investigation on these conditions, which provided the clearest window into understanding how ripple oscillations coordinate distributed neural communication during working memory processing.

Long-range ripple co-occurrence selectively increases with memory load during maintenance and retrieval.

Having established that ripple rates increase locally within individual brain regions during working memory, we next examined whether ripples co-occur across distant brain regions, potentially serving as a mechanism for long-range information integration. We defined co-occurring ripples (co-ripples) as periods where at least one ripple was simultaneously detected across two different microwire bundles. Across most possible region pairs, we observed a significant increase in ripple co-occurrence during both maintenance and probe stages of the task (Fig. 3). This effect was most pronounced between HIP-SMA (48% change from baseline) during the probe, and OFC-HIP (21% change from baseline) during maintenance.

Critically, we also found that memory load significantly modulated the rate of co-ripples across brain regions. When participants maintained three items in working memory, the rate of co-ripples was substantially higher compared to trials with only one item (Fig. 3B,C). This load-dependent modulation was observed during both maintenance and probe phases, with stronger effects during probe (mean increase across all pairs: 2% increase from load 1 to load 3 during maintenance, $p= 0.036$, 6% increase during probe, $p = = 3.2e-6$). The greatest significant increase in load 3 vs load 1 co-rippling during the probe was observed between HIP-SMA (18% increase, $p = 0.001$, FDR-corrected linear mixed effects) and OFC-HIP (17% increase, $p = 3e-4$). In contrast, load-modulated maintenance co-rippling was greatest between ACC-SMA (8% increase, $p = 0.01$) and ACC-HIP (8% increase, $p=0.03$). Therefore, long distance co-rippling increases and is modulated by task load during maintenance and probe stages.

Ripple co-occurrence shows greater modulation by memory load than other high gamma signals

To determine whether this load-dependent coordination was specific to the ripple frequency band, we performed parallel analyses on oscillatory events detected in low gamma (30-55 Hz) and very high gamma (120-190 Hz) frequency bands (see Methods). While we observed task-related increases in co-occurrence of oscillations in these other frequency bands, the contrast-to-noise (CNR) for load modulation was smaller during probe (low gamma: CNR = 6.21 between 1 to 3

WM items, p = 3.4e-4; very high gamma: CNR = 10.13, p = 1.5e-5) and maintenance (low gamma: CNR = 4.71, p = 8.0e-4; very high gamma: CNR = 2.70, p = 0.14) compared to the ripple band (probe: CNR = 12.42, p = 1.4e-7; maintenance CNR = 4.81, p = 8.0e-3, Fig. 3B,C, Fig S4C,D).

During the probe stage, linear mixed effects modelling revealed significant load-dependent modulation across 12 site pairs during co-ripples, only 1 site pair during low gamma oscillations and 7 site pairs during very high gamma oscillations. Load-dependent oscillation co-occurrence during maintenance was also greatest for ripples (5 significant pairs) compared to low gamma (0 pairs) and very high gamma (2 pairs). These findings suggest that co-occurring ripples across limbic and frontal regions represent a specific neural mechanism for coordinating distributed brain activity during working memory, with the degree of coordination scaling with cognitive demand more than other high gamma signals. (Fig. 3B,C, Supplementary Fig. 6,7).

Ripple co-occurrence increases unit co-firing in each task stage

To investigate whether co-ripples facilitate neural communication between brain regions, we analyzed cross-regional unit co-firing during different task periods. We defined co-firing as the co-occurrence of spikes from units in separate brain regions within a 25 ms window. For each region pair, we calculated the percent change in co-firing rate relative to the median baseline co-firing rate during three conditions: 1) periods with co-occurring ripples (co-ripple periods), 2) periods without ripples in either region (no-ripple periods), and 3) across all periods regardless of ripple occurrence (all periods). In this analysis we only included unit pairs that co-fired at least once during co-ripples in any of the three task stages. This resulted in a 13901 out of a total 16274 cell pairs being included in the analysis.

Examination of unit activity revealed a robust increase in cross-regional unit co-firing specifically during co-ripple periods across all task stages (Fig. 4). During encoding, maintenance, and probe phases, co-ripple periods showed significant enhancement of median unit co-firing compared to baseline (encoding: 35% increase, p = 0, paired permutation test; maintenance: 50% increase, p = 0; probe: 36% increase, p = 0). This effect was most pronounced among cross-region pairs during maintenance between ACC-AMY (84% increase during co-ripples, p = 0) and SMA-ACC pairs (86% increase, p = 0) during probe, but was consistently observed across almost all region pairs (Fig. 4A,C).

In contrast, no-ripple periods showed minimal to no change in co-firing rates compared to baseline across any task stage (encoding: 3% change, p = 0; maintenance: 1% change, p = 0.09; probe: 2% change, p = 1, Fig. 4E). When examining all periods together regardless of ripple occurrence, we observed a modest increase in co-firing (encoding: 12% increase, p = 0; maintenance: 12% increase, p = 0; probe: 12% increase, p = 0, Fig. 4B), likely driven by the contribution of co-ripple periods. Overall, the difference between co-ripple, no-ripple and all period co-firing was significantly different during encoding (p = 1.73e-11, Kruskal-Wallis test), maintenance (p = 6.95e-82) and probe (p = 3.22e-80) stages. These results indicate that co-occurring ripples across brain regions specifically enhance the temporal coordination of neural firing, potentially providing a mechanism for information transfer between distant brain areas during cognitive processing.

Unit co-firing is enhanced by memory load during co-ripples

Having observed that co-ripples enhance cross-regional unit co-firing throughout the task, we next examined whether this effect is modulated by memory load. For each region pair, we calculated the percent change in the amount of co-firing per trial between high memory load and low memory load conditions during co-ripple periods, no-ripple periods, and across all periods (see Methods).

Our analysis revealed a load-dependent enhancement of unit co-firing specifically during co-ripple periods (Fig. 5A). Across distributed region pairs, co-firing during co-ripples was substantially higher in the high load condition compared to the low load condition (maintenance: 13% increase from load 1 to load 3, p = 7.6e-39 paired Student's t-test; probe: 19% increase, p =

4.38e-39). In contrast, no-ripple periods showed minimal modulation by memory load, with minimal to no significant increase in co-firing rates between high and low load conditions (maintenance: -1% change, $p = 0.043$; probe: -0.2% change, $p = 0.77$). When examining all periods together, we observed a modest load-dependent increase in co-firing (maintenance: 1.43% increase, $p = 0.003$; probe: 5% increase, $p = 1e-15$), substantially smaller than the effect observed during co-ripple periods.

The differential effect of memory load on co-firing during maintenance was significant in 7 site pairs during co-ripple periods, 3 site pairs during no-ripple periods and 6 site pairs during all periods of the recording. The effect was even greater during the probe, with 13 significant site pairs during co-ripple periods, 3 site pairs during no-ripple periods and 11 site pairs during all periods (Fig. 5B). These findings suggest that co-ripples are specifically associated with the temporal window during which inter-regional communication is enhanced and dynamically modulated by cognitive demand, potentially serving as a mechanism for load-dependent scaling of information transfer during working memory processing.

Co-ripples increase the repetition of stimulus-specific co-firing during response probes

We next investigated whether co-ripples support the reinstatement of stimulus-specific neural representations across distant structures during memory retrieval. To this end, we examined the repetition of co-firing patterns between encoding and probe phases. For each cell pair recorded from different brain regions, we identified co-firing events (spikes within 25 ms) during the encoding phase when a specific stimulus was presented and during the probe phase when the same stimulus was presented (Fig 6A).

We calculated the percentage of cell-pair trials (# cell-pairs × # total trials) that exhibited repeated co-firing patterns between encoding and probe phases. When we separated our analysis into co-ripple periods and no-ripple periods (duration-matched across 25 shuffles per cell-pair trial), we found that pattern repetition was substantially enhanced during co-ripple periods compared to no-ripple periods. During co-ripples, the repetition rate was 0.29%, while during no-ripple periods, this rate dropped to 0.14% ($p = 0$, $\chi^2 = 213.9$). This enhancement during co-ripples exceeded what would be expected from the general increase in co-firing rates alone (Supplemental Fig. 8).

The effect during co-ripples was present across different regional connections but was particularly pronounced for hippocampus-cortical pairs (0.28% during co-ripples vs 0.11% during no-ripples, $p = 1.1e-16$, $\chi^2 = 23.5$) and amygdala-cortical pairs (0.31% during co-ripples vs 0.16% during no-ripples, $p = 1.1e-16$, $\chi^2 = 69.5$). Effects were similar for cell pairs between ipsilateral (0.26% during co-ripples vs 0.14% during no-ripples, $p = 0.002$, $\chi^2 = 69.1$) and contralateral regions (0.31% during co-ripples vs 0.14% during no-ripples, $p = 0$, $\chi^2 = 145.6$).

Critically, we also found that co-ripple-mediated co-firing repetition was associated with behavioral performance (i.e., patient response time). We focused our analysis on high memory load (3 items) trials where the probe stimulus matched one of the encoded stimuli. This design ensured that the sensory input was identical during both encoding and probe phases, allowing us to isolate the contribution of behavioral variability while controlling for stimulus-related factors. The high-load condition also represented a more challenging task and provided the widest range of response latencies. During these high-load trials, co-firing repetition during co-ripples was significantly greater for trials with fast response times compared to slow response times (0.36% for fast responses vs 0.23% for slow responses, $p = 3.3e-14$, $\chi^2 = 57.5$, Fig. 6B). These findings demonstrate that co-ripple-mediated repetition of neural patterns is associated with more efficient responses to the working memory probe.

We next determined the time-course of the reinstatement of stimulus-specific co-firing patterns during co-ripple activity, by plotting their occurrence relative to the centers of detected co-ripples (ripples occurring simultaneously in both recorded regions). We then tested the significance of co-firing enrichment around co-ripple centers compared to their enrichment around

ripples that occur in only one of the two bundle locations. As an additional control, we created surrogate data by shuffling spike times within each trial while preserving the overall firing rate profiles and recomputed the enrichment of matching co-fire events around the original co-ripple centers. We found a marked increase in co-firing events around the centers of co-ripples compared to single ripple and shuffled spike controls (Fig. 6C,D). This increase was seen within hemispheres ($p = 6.1e-8$, one-way ANOVA) and across hemispheres ($p = 7.2e-15$) during the encoding stage, and within hemispheres ($p = 4e-4$) and across hemispheres ($p = 3.2e-10$) during the probe stage.

These findings demonstrate that co-ripples provide privileged temporal windows for repeating stimulus-specific neural patterns across distant brain regions during memory retrieval. Our results reveal an association between co-ripple-mediated neuronal firing repetition and behavioral performance, highlighting their functional importance in supporting efficient recognition memory. By facilitating the coordinated reactivation of encoding-related activity patterns, co-ripples facilitate the neural processes necessary for successful working memory performance.

Discussion

Our findings provide strong evidence that co-occurrence of transient bursts of high-frequency oscillations ('co-ripples') serve as a fundamental mechanism for enhancing long-range neural communication during cognitive processing. Previous studies have demonstrated that co-ripples between widespread cortical areas occur spontaneously in NREM sleep and waking [9, 10], and are strongly modulated by task conditions [24]. Furthermore, unit-interactions between cortical areas are modulated by co-ripples [25], and hippocampo-cortical co-ripples are correlated with enhanced recall[10] and replay of local cortical firing [14, 21, 28] during memory tasks. Our results extend these observations to show that co-ripples between distant limbic and cortical structures enhance the integration of unit co-firing between those structures. We show that such cross-structure coordination occurs during active cognitive processing, with specific task modulation and firing patterns that support a role in facilitating inter-regional integration.

A key finding from our study is that enhanced long-distance neural coordination occurs across multiple region pairs spanning the limbic system and neocortex. Previous research in rodents and humans has documented ripple coordination between hippocampus-cortex [17, 19] and between cortical regions [10, 11, 25], but primarily at the level of local field potentials, and without a systematic examination of the effects of task and co-ripples on the co-firing of neurons between different cortical and subcortical areas. Our study confirms and extends these prior ripple studies to include unit co-firing during co-ripples between all combinations of hippocampus, amygdala, limbic, and frontal neocortex. While the effects were qualitatively similar across region pairs, we observed some quantitative differences (e.g., stronger effects in preSMA during the response period) that warrant further investigation in additional paradigms.

Remarkably, the enhanced neural coordination we observed spans considerable physical distances, occurring between brain regions separated by white matter fiber tracts up to 220 mm, across different lobes, and even between hemispheres. Most striking was our observation that the strength of ripple-mediated co-firing showed minimal decay with increasing fiber tract distance between locations, extending upon the growing evidence in rodents that neural coding is widely distributed [29]. This distance-invariance suggests an emergent network phenomenon rather than simple point-to-point transmission of activity[1, 30]. While our sampling was necessarily limited to specific recording sites, the fact that robust co-ripples and associated co-firing were observed across diverse, unselected locations implies that this coordination mechanism may operate generally throughout the cortex and closely-associated structures.

To our knowledge, co-firing of human neurons at long distances during cognitive tasks has not previously been studied. We found that neither co-firing, nor the co-ripple induced enhancement in co-firing, decrements with distance, even between lobes or hemispheres, despite

the well-established exponential decrease in cortico-cortical connectivity with distance[31, 32]. The lack of decrement is consistent with other data showing that co-rippling during a similar task is characterized by zero-lag phase-locking across the left hemisphere[24] and suggests that co-rippling reflects a distributed state of enhanced cortico-cortical communication[25].

The specificity of these coordination effects to the ripple frequency band is also noteworthy. Our comparative analysis across frequency bands revealed that ripple oscillations (70-100 Hz) exhibited significantly stronger enhancement of long-range coordination than oscillations in either low gamma (30-55 Hz) or very high gamma (120-190 Hz) ranges. Similarly, across widespread cortical areas, ripple band co-oscillations showed much stronger modulation by a semantic judgement task than those in the very high gamma range[24]. This frequency specificity suggests that ripples occupy an optimal range for long-distance communication, potentially balancing the time needed for effective local processing with that required for propagation between co-rippling sites.

Co-ripples appear to provide temporal windows of heightened excitability that enable spike transmission between distant regions with sufficient temporal precision to drive plasticity and information transfer, by acting as coupled oscillators, a common mechanism in biology and physics[33]. Previous work has shown that co-ripples are characterized by zero-lag task-modulated phase-locking across large cortical areas during tasks[24] and spontaneous waking[9]. The center frequency of ripples is very reliably 90Hz, with greater phase-locking associated with smaller frequency differences as would be expected from coupled oscillators[24]. Even small firing correlations can have large effects on network function[34-37].

We found that the degree of ripple-enhanced co-firing increases systematically with cognitive demand, with stronger coordination observed under high memory load conditions. This load-dependent modulation suggests that ripples provide a flexible mechanism for adjusting the strength of inter-regional communication according to task requirements. However, future work is needed to understand how ripples may coordinate with slower oscillations according to cognitive demand. An analysis of the same dataset included in this study by Daume and colleagues [26] demonstrated that theta-gamma phase-amplitude coupling (PAC) in human hippocampal neurons similarly scales with cognitive control demands, with PAC neurons showing enhanced phase-locking to frontal theta specifically during high-load conditions. This suggests that multiple oscillatory mechanisms—both ripples and cross-frequency coupling—work in concert (or perhaps in opposition [38]) to support flexible inter-regional communication during demanding cognitive tasks.

Importantly, the mechanisms supporting short-term working memory may be associated with broader memory related functions. In another study by Daume et al. [39] also examining the present dataset, persistent activity of hippocampal neurons during working memory maintenance predicted successful long-term memory formation in a separate task., demonstrating a direct neuronal-to-neuronal link between working memory maintenance and long-term memory encoding across distributed networks. The convergent evidence from ripple-mediated coordination, phase-amplitude coupling mechanisms[26], and the shared neural substrates linking working and long-term memory systems[39] collectively support widespread-interactive models of cortical function, revealing multiple, complementary mechanisms through which the brain achieves flexible integration of information across its distributed networks.

Beyond enhancing co-firing rates, co-ripples specifically facilitated the repetition of stimulus-specific co-firing patterns across distant regions during memory retrieval, expanding on previous work correlating gamma bursting with specific encoding of stimulus identity by local neural populations[14, 38, 39]. This finding suggests that ripples not only increase overall communication but also support the replay of specific firing-patterns widely within and between hemispheres[40]. Behavioral data from the category of memory test used in this study, the Sternberg Paradigm, was originally taken to support serial scanning of items being held in working memory, with subsequent work suggesting that in some circumstances a parallel interrogation of

‘memory strength’ is operative[41, 42]. Our finding that rapid reaction times are associated with repetition of a specific distributed firing-pattern evoked by a stimulus from its Encoding to its Probe may provide a mechanism whereby memory strength could be available on some trials. Specifically, multiple processes may be engaged by the Probe, and if reinstatement of firing patterns is successful, then a rapid response is made; otherwise slower (possibly sequential) processes support longer latency responses. Neurophysiological models of recognition judgments also often posit two parallel mechanisms: hippocampo-cortical firing-pattern reinstatement and facilitated processing from recent cortico-cortical activation[43]. Our observation of the former on faster trials suggests that one of the two processes active in working memory tasks is hippocampal-dependent, and that it may sometimes be responsible for quicker trials. Yonelinas[44] reviews the hippocampal contribution to working memory but proposes that it assists in familiarity rather than recollection.

Our findings also have implications for theories of cortical integration, which may be broadly classified as focal-sequential versus distributed-interactive, continuing the longstanding debate between localizationist and equipotentiality views of brain function[45]. While the focal-sequential framework has been successful in describing early sensory processing, such as the hierarchical organization of the ventral visual stream[46], it faces challenges in accounting for higher cognitive functions in association cortex. Approximately 75% of human cortex lies beyond the clearly hierarchical early sensory and late motor areas[47], and about 75% of reaction time in a simple semantic judgment task occurs after visual wordform encoding[24].Many theories of cognitive processing posit a division of cortex into sequential-hierarchical and interactive-associative areas, with the later integrating various sensory, semantic, and executive information in guiding a response, and termed NeuroCognitive Networks [48, 49], Cognits [50], or the Global Neuronal Workspace[51]. A serious challenge to such theories has been the general lack of evidence for integration of neuronal firing over wide expanses of association cortex and closely-linked subcortical areas. Our study provides such evidence, demonstrating task-related enhancement of specific neuronal firing between widely distributed cortical and subcortical structures in both hemispheres.

Limitations

Importantly, electrode placement was determined entirely by clinical necessity, targeting regions most relevant for seizure monitoring rather than areas traditionally associated with working memory processing. Despite this constraint—which resulted in uneven regional coverage and prevented systematic testing of areas like posterior parietal cortex—we observed task-related modulation across all recorded sites. This widespread modulation, even in regions not classically implicated in working memory, supports theories of distributed cortical processing and suggests that cognitive functions engage broad neural networks rather than isolated specialized regions. While our sparse sampling likely captures only a subset of the broader coordination occurring during working memory, the consistent effects across diverse recording locations strengthen the evidence for ripples as a general mechanism of cortical communication.

Finally, our investigation was limited to the Sternberg working memory task with a narrow load manipulation (1 versus 3 items). Future studies employing diverse paradigms and broader task difficulty ranges would help establish whether ripple coordination represents a general cognitive mechanism or is specific to particular memory operations.

Conclusion

In conclusion, our findings establish ripple oscillations as a key mechanism for facilitating long-range neural communication during human cognition. By providing temporal windows that enhance unit co-firing between distant brain regions with minimal distance-dependent attenuation, ripples could coordinate distributed neural representations necessary for complex

cognitive processes. This mechanism for long-range neural coordination may represent a fundamental organizational principle of brain function underlying our capacity for integrated cognition with distributed neural processing.

Methods:

Participants and Data Collection. In this study, we analyzed data previously described by Daume et al. [26], which consists of intracranial recordings from 36 patients (44 sessions; 21 female, 15 male; age range 20-67 years) implanted with depth electrodes with embedded microwires for evaluation of medically refractory epilepsy. All patients and sessions from the original dataset were included in our study except for one patient (P88T), which was removed due to a lack of clean LFP microwire data in any of the implanted sites. Each patient was implanted with up to ten electrodes, comprised of a clinical macro-electrode with 8 microwires protruding ~2-5 mm from the tip [52]. Electrodes considered for analysis targeted the ventromedial prefrontal cortex (vmPFC), anterior cingulate cortex (ACC), pre-supplementary motor area (preSMA), amygdala (AMY), and hippocampus (HIP), bilaterally.

Local field potentials (LFPs) and single-unit activity were simultaneously recorded at 32 kHz (ATLAS system, Neuralynx; Cedars-Sinai Medical Center and Toronto Western Hospital) or 30 kHz (Blackrock Neurotech; Johns Hopkins Hospital) from the implanted microwires. All recordings were locally referenced within each recording site using either one of the available microwire channels or a dedicated reference channel with lower impedance. The dataset included a total of 2253 microwire channels from which LFPs were recorded. All patients provided informed consent, and procedures were approved by the Institutional Review Board at each participating institution.

Sternberg Memory Task. The task analyzed has been described in detail previously [26]. Briefly, patients performed a modified Sternberg working memory task with varying memory loads (1 or 3 items). In each trial (see Fig. 1c), they were instructed to remember a set of images presented sequentially (encoding phase), maintain the memory over a delay period (maintenance phase), and identify whether a probe image was part of the original set (probe phase). Each session consists of 140 trials.

The 280 images were chosen from five semantic categories (human faces, animals, landscapes, fruits, and either cars or tools depending on the version). Each trial sequence began with a variable-duration baseline point (900-1200ms), followed by a stimulus presentation phase. In the low-load condition (70 trials), participants viewed a single image for 2 seconds. In the high-load condition (70 trials), participants viewed a sequence of three images from different categories, each displayed for 2 seconds with brief interstimulus intervals ranging from 17-200ms. Following stimulus presentation, a retention interval commenced, signaled by the word "HOLD" appearing centrally for 2.5-2.8 seconds. This maintenance period was identical for both load conditions.

The trial concluded with a memory probe showing a single image, during which participants indicated whether the probe image matched one of the images shown in the current trial's encoding phase. To prevent strategies based on image familiarity, non-matching probes were always selected from earlier trials rather than introducing entirely novel images. These non-matching probes were deliberately chosen from categories not represented in the current trial's encoding set. Responses were collected using a Cedrus response pad, with response mapping reversed midway through the session following a brief rest period. For participants completing multiple sessions, entirely new image sets were used.

Data Curation and Quality Control. To ensure high data quality and reliable analyses, we implemented a rigorous data curation protocol. All recordings were manually reviewed to identify and exclude channels contaminated with epileptiform activity, electrical artifacts, or excessive noise. For each patient, recordings were excluded from time periods containing interictal epileptiform discharges spreading across multiple channels, or periods of increased noise due to patient movement. Electrodes substantially contaminated by epileptiform activity were not used for analysis.
Artifact and interictal discharge detection was performed on a per-wire basis using a semiautomated algorithm together with subsequent visual inspection. To detect high-amplitude noise events and inter-ictal discharges, we z-scored the amplitude in each channel across all trials. To avoid amplitude biasing from extreme values, we first capped the data at 6 standard deviations from the mean and then re-performed z-scoring on the capped data. Trials in which any time sample exceeded a threshold of 4 standard deviations were removed from the analysis for that wire. Signal jumps were detected by z-scoring the difference between every fourth sample of the capped signal, with trials containing jumps exceeding 10 standard deviations being excluded.

Electrodes and Visualization. Electrode locations were used as stated in the dataset (Fig 1.A). Electrode locations were distributed across frontal and temporal regions, allowing for the analysis of both local and long-range interactions. We estimated cross bundle fiber tract distances according to probabilistic diffusion MRI white matter tractography [53], computed from population averages [54]. To determine the appropriate streamline lengths, each bundle was associated with a parcel from the HCP-MMP1.0 atlas [55]. Specifically, bundles implanted in the OFC, ACC, SMA, AMY and HIP sites were assigned to the s32, p24, 8BM, TGd and H parcels, respectively.

Detection of Ripples and Oscillations in Other Gamma Ranges. Ripple detection was performed on local field potential data from each microwire electrode, downsampled to 1000 Hz, and using established methods adapted from previous studies[24, 25]. Data were bandpass filtered using a sixth-order Butterworth filter in the ripple frequency range (70-100 Hz, applied forward and reverse for zero-phase distortion), then z-scored relative to the entire recording session. Initial candidate events were identified as containing at least three consecutive ripple cycles with z-scores exceeding 1.

Candidate events underwent further selection criteria, requiring the maximum z-score of the analytic amplitude in the ripple band to exceed 2.5 standard deviations. Ripple events occurring within 25 ms of each other were merged into single events. Ripple centers were defined as the timepoint of maximum positive deflection in the ripple-band filtered signal.

For determining event boundaries, the z-scored ripple-band analytic amplitude was smoothed using a 100 ms sliding Gaussian kernel. Event onsets and offsets were marked when this smoothed amplitude envelope dropped below a z-score threshold of 0.75. Ripples were excluded if the 100-Hz high-pass z-score exceeded 7 in absolute value or if they occurred within 2 seconds of large voltage deflections (≥3 mV/ms). Events were also rejected if they fell within ±500 ms of detected interictal spikes [10, 25].

Additional quality control measures included excluding events with single prominent cycles or those where the largest valley-to-peak amplitude in the broadband signal exceeded 2.5 times the third-largest amplitude. For each recording channel, the average ripple-triggered LFP was visually inspected to verify multiple distinct cycles at ripple frequency, and time-frequency spectrograms were examined to confirm discrete power increases within the ripple band. Individual ripple events and co-occurring ripples across channels were manually reviewed to ensure multiple ripple-

frequency cycles without contamination from artifacts, unit activity bleed-through, or epileptiform discharges.

For comparison, we also detected oscillatory events in low gamma (30-55 Hz) and very high gamma (120-190 Hz) frequency bands using the same thresholding methodology, adjusting the filter bands accordingly. We characterized the reliability of these signals to differentiate between memory load conditions using the contrast-to-noise ratio (CNR):

$$CNR = \frac{S_3 - S_1}{SE_b},$$

where S_3 and S_1 are the oscillation occurrence rates in load 3 and load 1 respectively, and SE_b is the standard error across all subjects and trials during the 1s baseline period.

Time Frequency Analysis. Time-frequency analysis of ripple-associated spectral dynamics was performed using broadband local field potential recordings processed through EEGLAB [56]. We computed event-related spectral perturbations (ERSP) across frequencies ranging from 1 to 500 Hz at 1 Hz intervals, with ripple onset aligned to time zero. The analysis employed windowed fast Fourier transforms using Hanning tapers, which were subsequently averaged to generate mean time-frequency representations.

Baseline normalization was applied to each frequency bin by dividing spectral power values by the average power recorded during the pre-event period (-2000 to -1500 ms). Statistical significance testing utilized two-tailed bootstrap procedures (200 iterations) with multiple comparison correction via false discovery rate (FDR) control at $\alpha=0.05$.

Detection and Selection of Single Units. Isolated single neurons were used as provided in the dataset [26]. We applied additional exclusion criteria:

Units were excluded if the mean and distribution of spike waveforms exhibited low signal-to-noise characteristics, defined as a peak signal-to-noise ratio of 2.5 or less. Additionally, we excluded units whose auto-correlograms indicated contamination from multiple neurons, specifically those with greater than 3% of spikes occurring within 3 ms of each other. This 3 ms criterion corresponds to the typical refractory period of neuronal firing, during which a single neuron cannot generate consecutive action potentials. Units violating this criterion likely represent multi-unit activity, which could confound analyses of precise spike timing relationships essential for co-firing analyses. These conservative selection criteria ensured that our dataset comprised well-isolated single neurons with reliable spike timing, critical for examining the temporal relationships between unit activity and ripple oscillations.

The flexible positioning of microwires within each electrode bundle can occasionally result in multiple wires detecting signals from the same neuron, especially when slight wire movements bring them into closer proximity. To prevent this from confounding our analyses, we systematically identified and merged units across different wires within the same bundle that likely originated from a single neuronal source. Units exhibiting greater than 30% of spikes occurring within 1 ms of each other were merged, as this exceptionally high temporal correlation indicates recording from the same neuron rather than genuine co-firing between distinct cells. This merging procedure was essential for preventing artificial inflation of co-firing rates and ensuring that all observed neural coordination reflected authentic interactions between separate neuronal units.

Following these quality control procedures, we successfully isolated 1,373 high-quality single units distributed across all recorded brain regions: 360 units in the hippocampus, 496 in the amygdala, 206 in the ventromedial prefrontal cortex, 188 in the dorsal anterior cingulate cortex, and 204 in the pre-supplementary motor area.

Spike Removal from LFP. While local field potentials reflect slower network oscillations, the high-amplitude nature of action potentials can introduce significant artifacts into LFP recordings, particularly in higher frequency bands where spike-LFP coupling analyses are performed. This contamination occurs because the large voltage deflections associated with individual spikes can influence the filtered LFP signal across a broad frequency spectrum, creating spurious correlations between unit activity and oscillatory patterns [57].

To address this potential confound, we implemented a spike removal procedure prior to LFP processing. For each identified unit, we calculated the average spike waveform and subtracted this template from the raw 30 kHz signal at every detected spike time from – 1ms to + 2ms around a the peak of the spike. This preprocessing step was applied before down-sampling and frequency filtering operations, ensuring that subsequent LFP analyses reflected genuine network oscillations rather than artifacts from unit activity. This approach preserves the integrity of ripple-unit relationships while eliminating the influence of spike waveform contamination on oscillatory measurements, allowing for more accurate assessment of true physiological relationships between neural firing and local field oscillations.

Co-ripple Rates and Co-firing Calculation. Co-occurring ripples (co-ripples) were defined as ripple events that temporally overlapped by at least 25ms across two different microwire bundles. We quantified co-ripple rates as the percentage of ripples on one electrode that co-occurred with ripples on another electrode. We examined ripple co-occurrence within microwire bundles (intra-bundle, <~5 mm separation) and across different microwire bundles within the same hemisphere (inter-bundle, 71-203 mm separation) and across hemispheres (35-223 mm separation).

To measure neural co-firing between distant brain regions, we defined co-firing as the occurrence of spikes from units in separate brain regions within a 25ms window. Co-ripple periods were defined as times when ripples were simultaneously detected on microwires in both units' respective bundles. Conversely, no-ripple periods were defined as times when no ripples were detected in either bundle. For across-bundle unit pairs, we categorized them into five connection types: amygdala-cortex (AMY-CTX), hippocampus-cortex (HIP-CTX), amygdala-hippocampus (AMY-HIP), ipsilateral cortico-cortical (CTX ipsi), and contralateral cortico-cortical (CTX contra).

For each cell-pair, we calculated the co-firing rate during co-ripple periods and no-ripple periods by counting the number of co-occurring spikes and dividing by the total duration of the respective periods. To account for potential bias from differences in baseline firing rates across brain regions, we also computed normalized co-firing rates by dividing the observed co-firing rate by the geometric mean of the individual firing rates of each unit pair (Supplemental Fig. S2).

Within-Bundle and Across-Bundle Ripple Response Curves. To examine how ripple activity varies with cognitive demand and behavioral performance, we computed ripple overlap rates both within individual microwire bundles and across different bundles under various task conditions.

For within-bundle ripple rate analysis (Fig. 2), we calculated the ripple density as the number of ripples across all microwires within each bundle that overlapped in 1 millisecond bins and averaged across all trials and participants, yielding the mean total ripples occurring within each bundle per trial (ie., ripples trial^{-1}). These response curves were computed separately for high

memory load (3 items) and low memory load (1 item) trials. To assess the relationship between ripple activity and behavioral performance, we further subdivided load 3 trials based on response time. For each session, we calculated the median response time for all load 3 trials, then classified trials as "fast" (below median) or "slow" (above median). Unlike memory load conditions which were experimentally balanced, fast and slow response trials varied naturally across participants, and each participant had different numbers of useable microwires per bundle. Therefore, when comparing response time effects, we normalized ripple rates by the number of viable microwires per bundle (ie., ripples trial^{-1} wire^{-1}) to prevent bias from unequal sampling across participants.

For across-bundle co-ripple analysis by memory load (Fig. 3), we identified periods of the recording where at least one ripple was simultaneously detected across two different microwire bundles. In contrast to the within-bundle analysis, any periods where only one bundle was rippling were considered as non-co-rippling periods. To quantify co-ripple activity, we counted the number of microwires in each bundle that contained overlapping ripples, binned this count in 1 ms windows, and then averaged across all trials and participants to obtain a co-ripple rate (co-ripples trial^{-1}). This metric was computed separately for each bundle pair and memory load condition to assess how cognitive demand modulates inter-regional ripple coordination.

Calculation of Task Phase and Load Modulation of Co-firing. We examined how ripple occurrence and associated co-firing varied across different cognitive phases by measuring percentage changes relative to a pre-trial baseline period (spanning -0.9 to -0.3 seconds before the first stimulus appeared). This baseline comparison allowed us to identify which task phases—encoding, maintenance, or probe—showed the greatest enhancement of synchronized neural activity, thereby revealing how memory processes selectively recruit coordinated activity across distributed brain networks during distinct cognitive operations.

To assess how memory demands influence neural coordination between brain regions, we analyzed co-firing rates under different conditions: during co-ripple periods (when ripples occurred simultaneously in both regions), during no-ripple periods (when no ripples were detected in either region), and across all time periods combined. For each analysis, we compared co-firing between high memory load (load 3) and low memory load (load 1) trials.

For every unit pair, we calculated the percentage change in co-firing from load 1 to load 3 trials using the formula:

$$\frac{[X^c_{load\ 3} - X^c_{load\ 1}]}{X^c_{load\ 1}}\ x\ 100\ ,$$

where X is the number of co-firing events during a given load and during the ripple condition c (i.e., co-ripple, no-ripple and all periods). To ensure statistical reliability, we restricted our analysis to unit pairs that exhibited co-firing during co-ripples in at least one task phase (encoding, maintenance, or probe), yielding 13,901 analyzable pairs from the total pool of 16,274 recorded unit pairs.

Repeated Stimulus Co-firing. To investigate whether co-ripples support the reinstatement of stimulus-specific neural representations during memory retrieval, we analyzed the repetition of co-firing patterns between encoding and probe phases. For each cell pair recorded from different brain regions, we identified co-firing events, defined as spikes occurring within a 25 ms temporal window between the two units.

We tracked co-firing patterns separately for each stimulus presented during the task. During the encoding phase, we recorded which cell pairs exhibited co-firing when each specific stimulus was presented. During the probe phase, we then examined whether these same cell pairs showed co-firing when either the same stimulus (match trials) or a different stimulus (mismatch trials) was presented.

The analysis calculated the percentage of cell-pair trials (# cell-pairs × # total trials) that exhibited repeated co-firing patterns between encoding and probe phases as:

$$R = \frac{N_{repeat}}{N_{total}} \times 100$$

Where, R = repetition rate (percentage), N_{repeat} = number of cell-pair trials with co-firing at both encoding and probe phases, and N_{total} = total number of cell pair trials. A cell-pair trial was defined as a unique combination of a specific cell pair during a given task trial (i.e., recording from 100 cell pairs across 50 trials, would yield 5,000 cell-pair trials). R was computed during load 3 trials separately for fast responses and slow response, defined as the top and bottom 50% of response times.

To determine the specific contribution of co-ripples to repetition of co-firing patterns, we separated our analysis into co-ripple periods and no-ripple periods. Co-ripple periods were defined as times when ripples were simultaneously detected in both brain regions containing the analyzed unit pair. No-ripple periods were duration-matched to co-ripple periods through 25 random shuffles per cell-pair trial to ensure equivalent temporal sampling.

Temporal Relationship Between Co-rippling and Repeated co-firing. To examine the temporal relationship between stimulus-specific co-firing and co-ripples, we constructed peri-ripple time histograms to visualize when matching co-fire events occurred relative to co-ripple timing. This temporal analysis was conducted separately for unit pairs within the same hemisphere and those across different hemispheres, allowing us to assess whether the temporal coupling of stimulus-specific firing differed based on anatomical distance. For each detected co-ripple (defined as ripples occurring simultaneously in both recorded regions), we identified the temporal center of the overlapping period and created a time window extending ±200 ms around this center. We then counted all instances where the same cell pairs that co-fired during encoding of a specific stimulus also co-fired during the probe phase presentation of that same stimulus, binning these events by their temporal offset from the co-ripple center.

To establish the specificity of this temporal relationship, we implemented two control analyses. First, we repeated the same procedure for single ripples that occurred in only one of the two bundle locations while no ripple was detected in the other location, allowing us to determine whether the temporal coupling required simultaneous rippling in both regions. Second, we created surrogate data by randomly shuffling spike times within each trial while preserving the overall firing rate profiles and inter-spike interval distributions. Importantly, spike times were shuffled separately within the encoding and probe stages for each trial, maintaining the temporal structure within each task phase. We then recomputed the enrichment of matching co-fire events around the original co-ripple centers using these shuffled spike trains.

Statistical Methods. All statistical analyses were performed on data from 36 patients across 44 recording sessions during a modified Sternberg working memory task. Single units were identified

and classified across 1927 microwire channels, yielding 1373 isolated units. For analyses examining unit co-firing between brain regions, we included only unit pairs that co-fired at least once during co-ripple periods in any of the three task stages, resulting in 13,901 out of 16,274 total cell pairs being analyzed.

To assess task-related modulation of ripple rates and unit firing, we used Wilcoxon rank-sum tests to compare activity during encoding, maintenance, and probe phases against pre-trial baseline levels. The relationship between ripple rates and neuronal firing rates was evaluated using Pearson correlation coefficients across all task phases. For comparing co-firing rates between co-ripple and no-ripple periods across all unit pairs (n = 31,489), we employed Wilcoxon signed-rank tests. Differences in co-firing between anatomical connection types (amygdala-cortex, hippocampus-cortex, amygdala-hippocampus, ipsilateral cortico-cortical, and contralateral cortico-cortical) were also assessed using Wilcoxon signed-rank tests.

Memory load effects on ripple occurrence/co-occurrence and unit activity were analyzed using linear mixed-effects models with FDR correction for multiple comparisons. We examined how each neural signal was load-modulated by modelling the load condition as a fixed effect and the patient as a random effect according to the following:

$$rate \sim load + (1|patient)$$

These models tested whether ripple rates, co-ripple rates, and firing rates differed between high memory load (3 items) and low memory load (1 item) conditions during maintenance and probe phases. The same approach was used to identify region pairs showing significant load-dependent modulation of oscillatory co-occurrence across different frequency bands (ripples, low gamma, and very high gamma).

The same model was used to test for significance in the relationship between ripple occurrence during the probe stimulus and response timing. Instead of using memory load, we categorized each trial as fast RT or slow RT (bottom and top 50% of response times, respectively).

For analyses of unit co-firing modulation by task phase and memory load, we used paired permutation tests to compare median co-firing rates during different task stages relative to baseline. For all permutation-based testing, 10000 iterations were performed. Kruskal-Wallis tests were employed to assess overall differences between co-ripple, no-ripple, and all-period conditions across task stages. Percent change in load-dependent co-firing were evaluated using paired one-sided Student's t-tests comparing high versus low memory load conditions.

The analysis of stimulus-specific co-firing pattern repetition utilized chi-squared tests to compare the proportion of cell-pair trials exhibiting repeated co-firing patterns between encoding and probe phases for match trials (same stimulus) versus mismatch trials (different stimulus). This analysis was performed separately for co-ripple and no-ripple periods, with no-ripple periods duration-matched across 25 shuffles per cell-pair trial.

The statistical significance of co-firing enrichment around ripple events was assessed using one-way ANOVA across the three conditions (co-ripples, single ripples, shuffled controls). This analysis was performed separately for within-hemisphere and across-hemisphere connections during both encoding and probe stages, allowing us to determine whether co-ripples provide privileged temporal windows for reinstating stimulus-specific firing patterns across different anatomical pathways and task phases.

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Figures

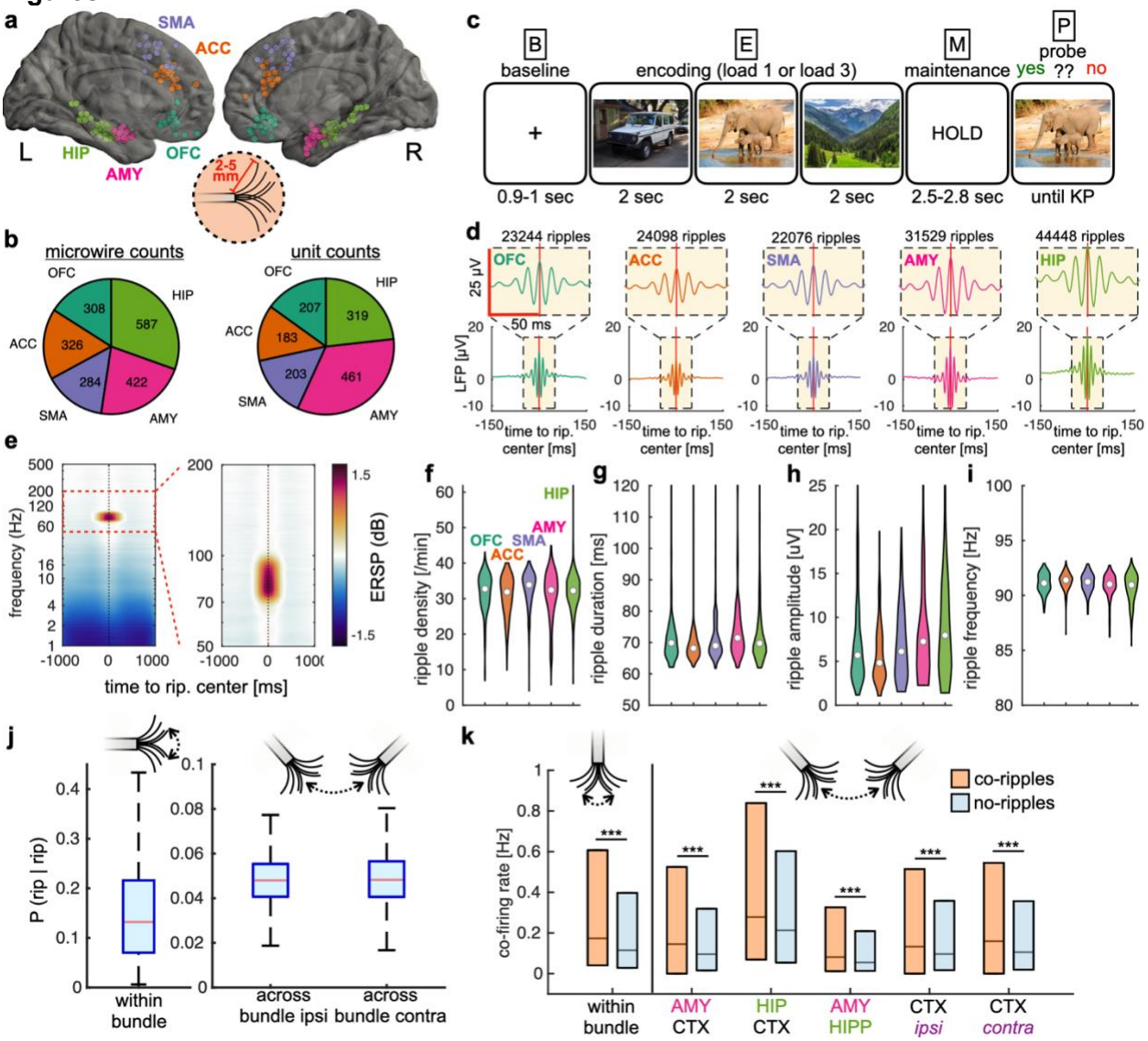

Figure 1 –Microwire locations, task, ripple characteristics, and increased co-firing during co-ripples at all recording separations. (a) Electrode locations in MNI152 coordinates overlaid on a template brain. Both left (L) and right (R) hemispheres are shown. Inset shows a schematic of typical microwire electrode spray. **(b)** Number of electrodes and single units recorded from each region. **(c)** Overview of the Sternberg task. After a baseline period (B), either one or three (load 1 / load 3) images (E1,E2,E3) are shown separated out by a variable blank screen for 0.17-200 ms, followed by a 2.5s maintenance period (M). The participant is then shown a probe image (P) and responds with a keypress (KP) to indicate if they recognize the photo from the previous set. Images shown here are from the same categories as the set used in the task, but are not identical due to copyright. (**d**) Average broadband (0.1 – 1000 Hz) LFP locked to ripple centers in all 5 regions. **(e)** Average time-frequency plot across all microelectrodes locked to ripple centers (left), and zoomed into the high gamma range (right). **(f-i)** Distribution of per-channel ripple characteristics separated by recording location. Data is plotted for **(f)** event density, **(g)** duration, **(h)** amplitude and **(i)** oscillation frequency. Circle shows median and violin plots show the underlying distribution**. (j)** Conditional probabilities of ripple co-occurrences (> 25 ms overlap)

between channels. Data is shown across microwires within the same bundle (left) and between different bundles within and across hemispheres (right) **(k)** Co-firing rate during co-ripples and no-ripples. Boxes denote median and inter quartile intervals. Data is shown for unit pairs within bundle (left) and across bundle (right). Between bundle data is separated across amygdala-cortex (AMY-CTX), hippocampus-cortex (HIP-CTX), amygdala-hippocampus (AMY-HIP), ipsilateral cortico-cortical (CTX ipsi) and contralateral cortico-cortical (CTX contra) bundle pairs.

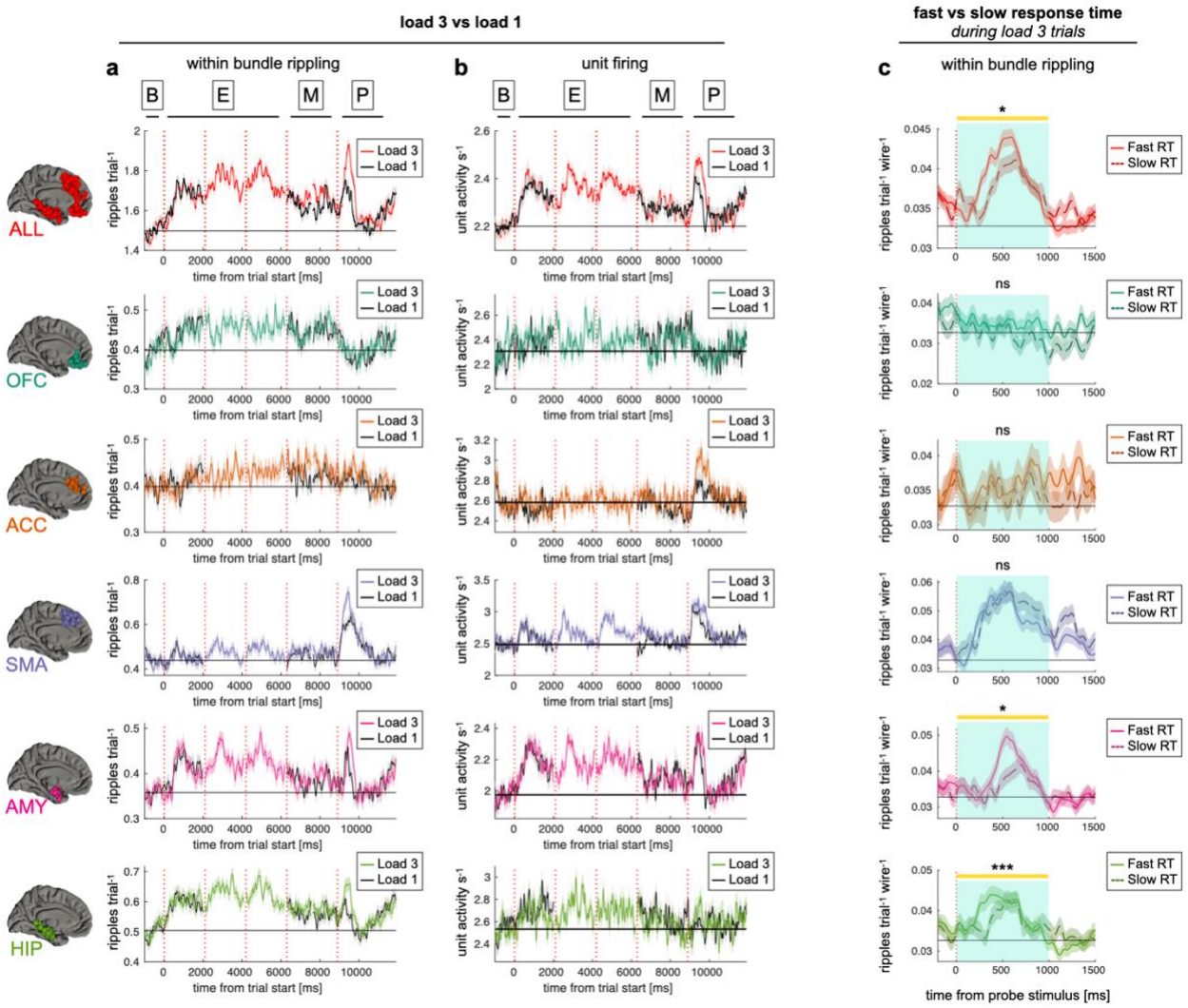

Figure 2 – Task modulation of ripples and units within regions according to task variables. **(a)** Ripple oscillation rates within each sampled region. Data is shown for mean number of rippling electrodes within a given bundle across all trials. Colored curve shows mean ± sem for all load 3 trials, while black curve shows load 1 trials. A gap is shown in the maintenance period because its duration is variable. For plotting purposes, data are smoothed with a 100 ms sliding gaussian window. **(b)** Same as **(a)** but for mean firing rate across all detected single neurons within each region. **(c)** Ripple oscillation rates for fast (solid line) and slow (dashed line) responses after the probe stimulus is shown in load 3 trials. To control for the uneven distribution of microwire yield across each patient, data is shown as mean number of ripples within each bundle *per microwire* per trial. Statistics were performed during the turquoise shaded 0- to 1000-ms interval following probe stimulus presentations. Statistics for load 1 vs load 3 in **(a)** and **(b)** are shown in Supplementary Figure 3. * $p < 0.01$ *** $p < 0.0001$, linear mixed-effects models with patient as random effect. ns, not statistically significant

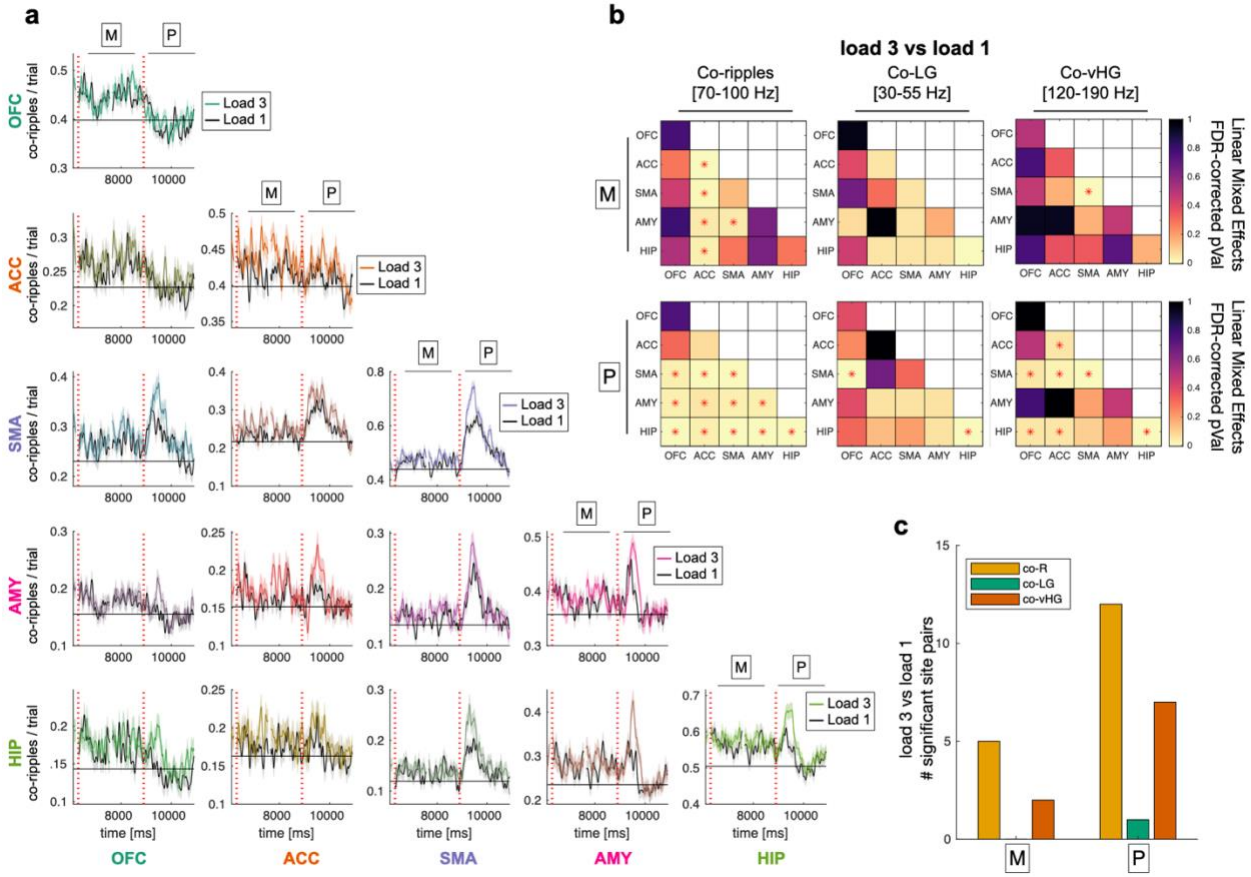

Figure 3 – Widespread ripple co-occurrence increases with greater memory load. (a) Co-ripple rates between all recorded regions during maintenance (M) and probe (P) periods. Data is plotted as mean±SEM for each pair of locations with load 3 trials in color and load 1 in black. Diagonal shows within bundle data. Black horizontal line shows mean baseline co-ripple rate for each region pair. **(b)** Results of linear mixed effects analysis of pairwise load 3 vs load 1 co-rippling across regions during maintenance and probe. Red stars indicate FDR-corrected p-values < 0.05 for detected co-ripples, low gamma (LG) co-oscillations and very high gamma (vHG) co-oscillations. **(c)** Summary of statistical analyses. Number of site pairs that show significant modulation during load 3 trials. Data is shown for co-ripples, co-LG and co-vHG. Note heightened response during maintenance and probe in the ripple band.

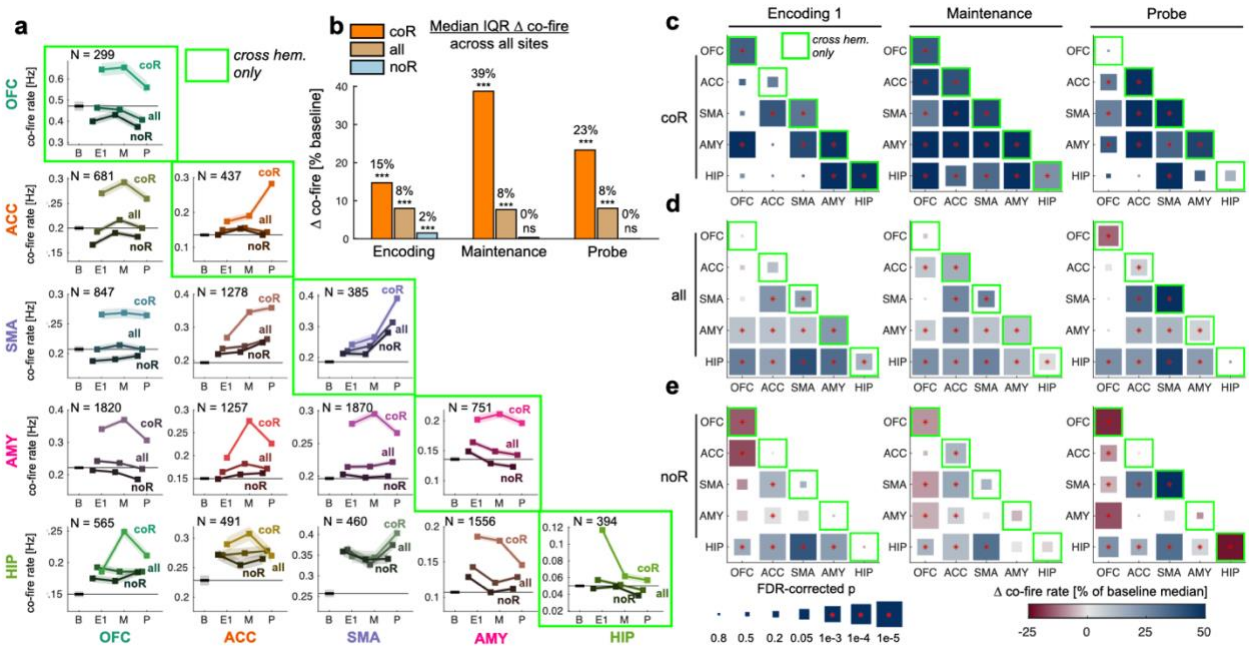

Figure 4 - Task related modulation of cross-region neuron co-firing is mediated by ripple oscillations. (a) Inter-area co-firing rates during baseline (B), first image presentation (E1), maintenance period (M), and response probe (P). Co-firing is shown for all conditions (all), co-ripple periods (coR) and periods where neither site has a detected ripple (noR). Plots show median ± mean absolute deviation. Number of cell pairs is shown in the top left of each plot. Only cell pairs with one co-fire during co-ripples are included in this analysis. **(b)** Distribution of inter-area co-firing across all recorded sites. Statistics are shown for each condition compared with baseline. Red stars indicate $p < 0.05$ FDR-corrected permutation-based test of difference between medians. **(c-e)** Co-firing rates vs baseline statistics during all task stages for coR **(c)**, all **(d)**, and noR **(e)** conditions. The color of each box indicates the magnitude of the difference from baseline, and the size of the box indicates the FDR-corrected significance (permutations test). The diagonal for **(a, c-e)** shows cross hemisphere co-firing between the same structure.

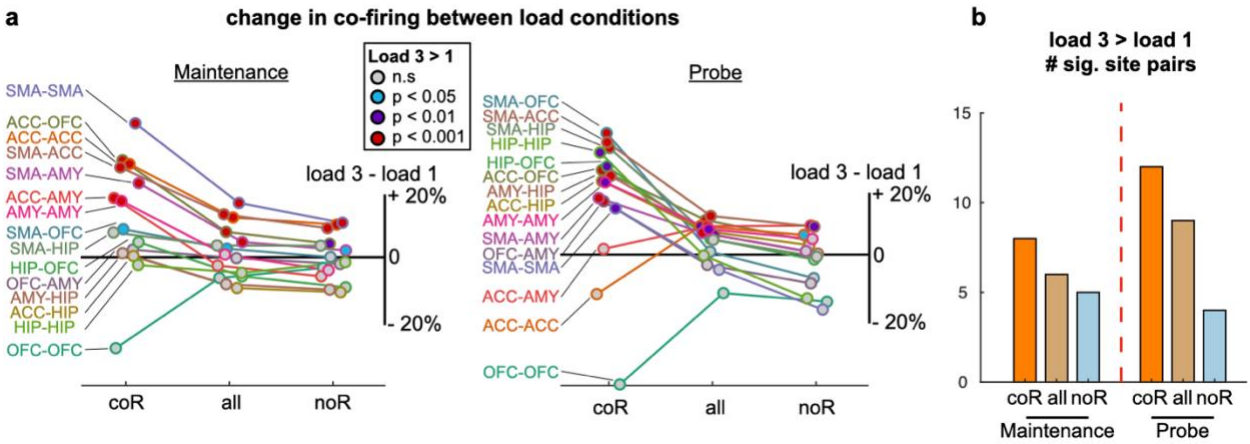

Figure 5 - Ripple oscillations increase co-firing according to memory load. (a) Change in inter-area total co-firing for load 3 vs load 1 conditions in the maintenance, and response probe periods, as a function of co-ripples between those areas. The percent change in co-firing is shown for all conditions (all), co-ripple periods (coR) and periods where neither site has a detected ripple (noR). Plots show mean percent change in co-firing for each site pair across all conditions. As in Fig 4, site pairs within the same structure (e.g., SMA-SMA) exclusively contain contralateral hemispheric microwires. Only cell pairs with one co-fire event during co-ripples are analyzed. The color of the dots represents the significance of the load 3 > load 1 modulation, with $p < 0.05$ colored blue, $p<1e-2$ colored purple, and $p < 1e-3$ colored red, one-sided Student's t-test, FDR-corrected. **(b)** Summary of statistics indicating the number of site pairs (out of 15 possible) whose co-firing is significantly elevated for load 3 compared to load 1 in Maintenance and Probe phases of the task.

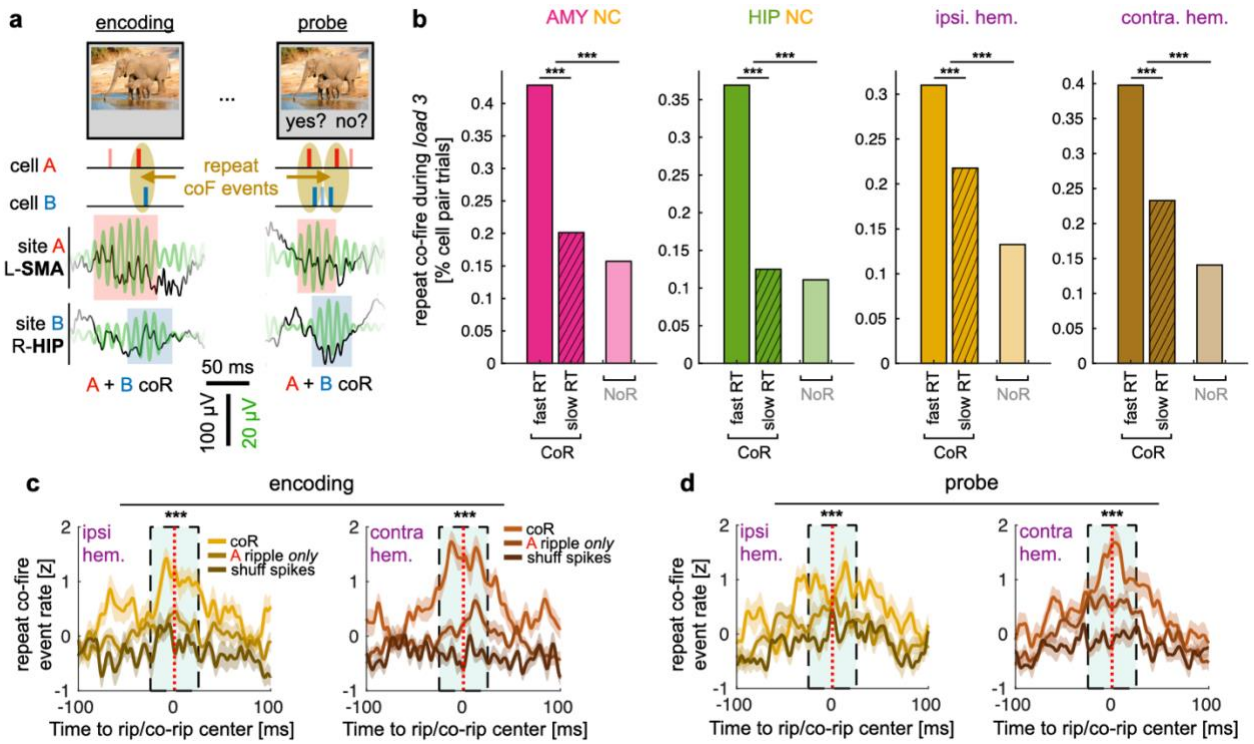

Figure 6 – Increased repetition of stimulus-specific cross-structure firing during coR. (a) Example of repeated coF (co-firing) between encoding and probe phases on a trial when both sites co-rippled. For each cell pair across structures, we assessed whether they co-fired during encoding, and if so whether their co-firing was repeated during the probe when the same stimulus was presented. **(b)** Comparison of co-firing repetition during fast and slow response times in load 3 trials. Bars indicate the percentage of repeated co-firing across cell pairs and trials during the probe period when that co-firing pattern was previously observed for the same stimulus during the encoding period. Analysis shows co-firing repetition during co-ripples (**coR**) for trials with fast response times (below median RT) and slow response times (above median RT). Additionally, co-firing repetition rates are shown for all load 3 trials during duration-matched no-ripple periods (**noR**). Co-firing repetition during coR is significantly greater for fast responses compared to slow responses. All effects are replicated using amygdala-neocortical (AMY NC), hippocampus-neocortical (HIP NC) pairs, and when using all within hemisphere (ipsi hem.) or cross hemisphere (contra hem.) cell pairs. **(c,d)** Time-course of repeated Encoding→Probe co-fire events relative to co-ripple centers. This is compared to when ripples occurred in site A only, and to shuffled spike times. Cyan box outlines the ± 25 ms window where the three conditions were compared. Repeated Encoding→Probe co-fire events increase during coR during both the **encoding (c) and probe stages (d)**, and again, these effects are replicated within hemisphere and cross hemisphere. * $p < 0.05$ *** $p < 0.0001$