

CO-OCCURRENCE OF SLEEP OSCILLATIONS IN HIPPOCAMPUS AND  
PREFRONTAL CORTEX IN A MEMORY CONSOLIDATION TASK

by

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## APPROVAL BY THESIS DIRECTOR

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## **Abstract**

The co-occurrence of hippocampal sharp-wave ripple oscillations (100-250 Hz) and cortical spindle oscillations (12-15 Hz) is thought to be a key mechanism for memory consolidation. The present study uses simultaneous recordings from dorsal hippocampal area CA1 and from the prelimbic area of the medial prefrontal cortex (mPFC) in adult male Brown Norway rats to test the hypothesis that learning-related increases in oscillation density enhance the cross-correlation between ripples and spindles. We first confirm that ripple and spindle density increase during sleep after learning in a spatial navigation task on an open field maze. Same-day recordings of learning and non-learning tasks show no significant enhancement of ripple-spindle cross-correlation during post-task sleep. The percentages of spindles with co-occurring ripples and of ripples occurring during or near spindles do not vary significantly with task. However, ripples appear to occur before, during, and after spindles at rates greater than chance. This suggests that the statistics of ripple and spindle co-occurrence may be fixed, with increased oscillation densities potentially enhancing single unit interactions between regions.

## **Introduction**

Memory consolidation is the process by which an encoded memory is integrated and stabilized for future retrieval. Rather than ending in a completely stable or static storage state, though, consolidation is an active process (Nadel et al., 2012), and consolidated memories may be returned to a labile state that requires their subsequent reconsolidation (Debiec et al., 2002).

Several models of memory consolidation have been proposed. In the two-stage theory of memory consolidation, information is transferred from the hippocampus to the cortex for long-term storage (McClelland et al., 1995; O'Reilly et al., 2014). Relational theory posits that the hippocampus functions as an index that binds features of a memory across multiple brain regions (Cohen and Eichenbaum, 1993; Cohen et al., 1997). Multiple trace theory goes further to acknowledge the dynamic nature of memory, proposing that intact memory traces are strengthened through repeated retrieval and consolidation that broaden the representation of a memory in the brain over time (Nadel and Moscovitch, 1997). Each of these theories features the common concept of the hippocampus and cortex as an axis of memory.

The precise effects of proposed mechanisms in memory consolidation remain to be determined, but different sleep stages and oscillations have been demonstrated to correlate with learning prior to sleep and even subsequent recall performance. Some known contributions of sleep stages, hippocampal sharp-wave ripple oscillations, and cortical spindle oscillations are outlined in the next paragraphs.

### *Role of Sleep Stage*

Across mammalian species, sleep can be divided into two major phases: rapid eye movement (REM) sleep, and non-rapid eye movement (NREM) sleep. In humans, NREM sleep

is further subdivided into stage 1 (light sleep), stage 2, and slow-wave sleep (SWS) (Iber et al., 2007); no agreed-upon method has yet been established to define homologous stages in rodents, although some criteria have been proposed (Lacroix et al., 2015).

Greater REM sleep times are correlated with improved consolidation of negatively valenced emotional memory (Wagner et al., 2001; Groch et al., 2013), with some caveats related to reproducibility and recent evidence supporting effects with positive emotional valence as well (Cellini et al., 2016). Time spent in NREM and slow wave sleep, on the other hand, is classically associated with declarative memory (Plihal and Born, 1997). NREM is of critical importance to the method of targeted memory reactivation, as cueing during NREM sleep has been shown to affect learning and memory—though it should be noted that the memory effects of NREM cueing may be related in part to subsequent REM (Blaskovich et al., 2017; Tamminen et al., 2017). In contrast to theories of the cortex as secondary to the hippocampus in memory, recent evidence has shown that the cortex is necessary to perceptual memory consolidation during NREM sleep (Miyamoto et al., 2016).

NREM sleep is typically of greater interest to active systems consolidation theory because the hippocampus and cortex both exhibit signature oscillations during this period: hippocampal sharp-wave ripples and cortical sleep spindles. In accordance with this, these two features of NREM sleep are the focus of this investigation.

### *Role of Sharp-Wave Ripples*

Sharp-wave ripples are high-frequency (110-200 Hz) oscillations observed in the mammalian hippocampus (Ylinen et al., 1995). They are known to occur during quiet wake and NREM sleep and have roles in memory consolidation and planning (Buzsaki, 2015). Ripples also appear to guide much of reactivation during NREM sleep (Buzsaki et al., 1992). Recent research

indicates that their frequency and density change with age, making ripples an important new subject of study in models of aging and disease (Kouvaros et al., 2015; Wiegand et al., 2016).

Ripple density, measured in ripples per second due to their frequent occurrence, increases during sleep immediately after learning (Eschenko et al., 2008). Electrical stimulation in the hippocampus produces memory interference when administered during ripples, suggesting that ripple events are necessary to memory consolidation (Girardeau et al., 2009). This effect may be related to disruption of their role in the coordination of hippocampal single units (Ego-Stengel and Wilson, 2010), an idea that has been strengthened in recent years by other studies establishing a relationship between ripples and single units not just in the hippocampus but also in the prefrontal cortex (Wang and Ikemoto, 2016).

### *Role of Spindles*

Spindles are 12-15 Hz oscillations observed in the neocortex. The thalamus serves as a central generator of spindle activity partly guided by cortical input (Contreras et al., 1997), and it has been suggested that multiple thalamic mechanisms exist for the generation of spindles (Piantoni et al., 2016). Spindles are not simultaneous across the entire cortex (Nir et al., 2011), although recent evidence shows relatively global patterns of spindle activity across the medial temporal lobe (Muller et al., 2016). Spindles exhibit different average frequencies in different areas, and have been characterized as “fast” and “slow” in, respectively, the parietal and prefrontal cortices in humans (Andrillon et al., 2011). In the rat, spindles may also be categorized as high-voltage and low-voltage spindles (Kandel and Buzsaki, 1997; Johnson et al., 2010). Though these may be differentially implicated in memory, their functional differences are not yet clear.

Spindle density—the number of spindles per minute—is known to increase in the prefrontal cortex during sleep immediately after learning in rats (Eschenko et al., 2006). In humans, greater frontal spindle density after learning word pairs with high and low rewards is associated with improved retention for highly rewarded stimuli (Studte et al., 2016). Spindle density in central and occipital, but not frontal, channels during targeted memory reactivation also correlates with improved memory for new information related to prior knowledge (Hennies et al., 2016). This developing body of evidence supports a role for spindles in memory consolidation.

Importantly, spindles are involved in several forms of memory. As memory is not monolithic, caution must be exercised in drawing conclusions about all spindles using data from only one area and studying only one memory task at a time. For example, tactile stimulation in NREM sleep during the consolidation of procedural memory increases sleep spindle and slow oscillation density without enhancing subsequent motor skill (Pereira et al., 2017). Exogenous spindle enhancement has yet to be tested in other regions, and the spatial task used in the present study is similar to others known to engage the prefrontal cortex (Bower et al., 2005; Euston et al., 2007; Peyrache et al., 2009). Spindles have a clear role in consolidation across tasks and across the cortex, but further investigations using direct manipulations of spindle incidence will be necessary to elucidate how this role may be specified for different memory processes.

### *Role of Ripple-Spindle Co-occurrence*

The individual importance of hippocampal ripples and cortical spindles to consolidation during NREM sleep has drawn speculation that their co-occurrence may represent a mechanism for active systems consolidation. This is compatible with most concepts of memory consolidation: whether information is transferred, shared, or reinforced, long-term memory

transformation is commonly thought to rely on coordinated interaction between the hippocampus and cortex.

Early evidence related to this subject shows a significant cross-correlation of ripples at spindle onset (Siapas and Wilson, 1998). Hippocampal firing significantly precedes prefrontal firing in correlated cell pairs from the two regions, and these cell pair correlations are associated with hippocampal ripples (Wierzynski et al., 2009). Prefrontal cells are also known to reorganize their firing in relation to coherent theta between the hippocampus and mPFC during learning in a Y-maze task, and these cells replay preferentially in conjunction with hippocampal sharp-wave ripples during subsequent sleep (Benchenane et al., 2010). The relationship between cortical cells, hippocampal cells, and hippocampal ripples has been characterized in several studies, providing evidence suggestive of a cortical-hippocampal-cortical loop (Eichenbaum, 2000; Wang and Morris, 2010; Schlichting and Preston, 2015; Wang and Ikemoto, 2016; Rothschild et al., 2017). It has also been proposed that this correlation may be modulated by slow oscillations, also known as downstates, which often occur immediately preceding spindles and represent periods of low population activity (Sirota and Buzsaki, 2005; Clemens et al., 2007).

However, the role of cortical spindles in these interactions is not as well understood. Studies reporting on spindles have often emphasized a relationship between ripple and spindle onset (Siapas and Wilson, 1998; Molle et al., 2009). Most compellingly, stimulating spindle activity in conjunction with online ripple detection enhances memory consolidation to the extent that otherwise insufficiently-encoded information becomes accessible for later recall (Maingret et al., 2016). Yet spindles may last seconds at a time (Luthi, 2014). This is long past the tens of milliseconds in which a ripple at onset might influence synaptic weights by a mechanism such as spike-timing dependent plasticity (Bi and Poo, 1998). If ripples are implicated as causal or

modulatory in the endogenous reactivation of cortical assemblies, it is important to consider any ripples that occur in concert with spindles—not only those at the time of spindle onset. Hippocampal single-unit activity and ripple activity are more likely at particular phases relative to spindle cycles in the rat somatosensory cortex (Sirota et al., 2003). Ripples are also statistically time-locked to spindle cycles at the scalp vertex (electrode Cz) and in parahippocampal, but not prefrontal, recordings in humans (Clemens et al., 2011; Staresina et al., 2015). These studies suggest a meaningful timing relationship between ripples and spindles not just at spindle onset but throughout spindle activity.

It is remarkable that the potential effect of learning on ripple-spindle relationships has not yet been tested. Learning increases ripple coordination with slow oscillations (Molle et al., 2009), but there are no reports that this is associated with an increased tendency for ripples to occur during spindles. The research presented here begins to address this critical omission in the memory consolidation literature by testing the hypotheses that ripple-spindle cross-correlation and their rates of co-occurrence should increase during post-learning sleep relative to sleep after a non-learning task. This may lead to a better understanding of the co-occurrence of ripples and spindles and their contribution to the mechanisms of hippocampo-cortical interactions during memory consolidation.

## Methods

### Animals

This study was approved by the Institutional Animal Care and Use Committee of the University of Arizona. Experimental protocols followed all relevant guidelines of the National Institutes of Health. Data were collected from 6 male Brown Norway rats housed on a reversed 12h/12h dark/light cycle in a temperature- and humidity-controlled room. Training and experiments took place in low-light conditions (0.5 lux) during the dark phase of the cycle. Rats weighed at least 85% of their ad libitum body weight throughout training and experiments.

### Surgery

Rats were implanted under 2-2.5% isoflurane anesthesia with a 14-tetrode hyperdrive directed to the right medial prefrontal cortex (AP: +3.1, L: +1.1, angle: 9.0°) and two staggered stainless steel recording electrodes in the dorsal CA1 region of the hippocampus (AP: -4.5, L: +3.0, DV: 2.2). Rats recovered for a minimum of 72h after surgery before training resumed.

### Task

Prior to implantation, rats were habituated to the behavioral room and trained on a 1.3m diameter maze with an octagonal wall featuring 8 sugar-water feeders equally spaced at the periphery. Sugar-water rewards were cued by a 2.5 Hz blinking light triggered in pseudo-random order at any of the 8 feeders. Consecutive cues occurred at different feeders. Rats rested on a towel-lined flowerpot before and after each training period and experimental task period.

Experiments consisted of five task periods, each flanked by 30-minute rest epochs (Appendix A). The first and fifth tasks were Random sessions in which rats retrieved 200 cued rewards as a same-day control. The second and third tasks were Learning sessions using different subsets of three non-adjacent feeders, Set 1 and Set 2. Each Learning session consisted of 75

cued rewards, immediately followed by a period with cues delayed by 15 seconds to assess free recall while rats searched for rewards. Rats typically cross the maze in less than 5 seconds. The session ended when rats reached the Learning criterion of 15 consecutive correct feeder visits with 2 or fewer cued rewards, or timed out at 45 minutes. An additional 2-hour Wait epoch followed the 30-minute sleep epoch after each Learning session. The fourth task was a Recall session using Set 1 feeders, with a cue delay of 15 seconds to assess recall. The Recall criterion was the same as for Learning phases. Recall time-out sessions were excluded from analysis.

Context manipulations included visual, tactile, and olfactory cues: maze wall color, local cues on the maze wall, configuration of curtains surrounding the maze, global cues attached to curtains, texture and color of the maze floor, and room fragrance. Each experiment used a novel context arranged before the rat entered the room that day. In the Same Context condition, the entire experiment took place in one context (Context A). In the Different Contexts condition, Context A changed to a second context (Context B) before Set2 Learning and reverted to Context A before Recall. Context changes took place during Wait epochs, with rats surrounded by a cylinder and blanket to prevent context exposure until the end of each Wait epoch. Context change artifacts (noise, flowerpot movement) were simulated in the Same Context condition.

Additional two-task experiments were run to test and control for potential context-related effects. These consisted of either two Random sessions or two Learning sessions, with a 30-minute rest epoch before and after each task. The first task took place with the experiment room in the training state, with no novel cues. In between the second and third sleep epochs, the rat was covered for a 20-minute period and the context was changed as described above.

## Histology

Electrode position was verified using electrolytic lesions (20  $\mu$ A; 24 hours and 30 minutes before perfusion). To assess shrinkage during the subsequent staining procedure, one tetrode was retracted during lesioning and current was passed every two turns (634  $\mu$ m). Rats were perfused with 4% paraformaldehyde following ketamine-xylazine administration under deep isoflurane anesthesia. Brains were transferred to 30% sucrose solution 72 hours after extraction and allowed to sink before sectioning. 50  $\mu$ m sections were Nissl stained using 0.5% cresyl violet solution (Appendix A, panel B, top right: coronal section of right prefrontal cortex, bottom left: coronal section of left hippocampus).

## Recordings

Single unit and local field potential recordings were obtained. Single unit recordings are not described here because this analysis focuses on local field potentials. Local field potentials were sampled from the prefrontal cortex and hippocampus at 2.4 kHz with 0.1 Hz (high pass) and 500 Hz (low pass) digital filtering using Cheetah data acquisition software (Neuralynx, Inc., Bozeman, MT). The electromyogram (EMG) signal was sampled at the same rate with 200 Hz (high pass) and 2 kHz (low pass) digital filtering. Data were transmitted from the hyperdrive via a buffered headstage. Spindles were recorded from the medial prefrontal cortex (mPFC) at depths of 1400 to 4500  $\mu$ m to include the anterior cingulate (ACC), prelimbic (PL), and infralimbic (IL) regions.

## Data Processing

Local field potential data were analyzed offline in MATLAB (Mathworks, Natick, MA) using software written in-house (Harper et al., 2016). Spindles were extracted from three simultaneously recorded channels using 8-20 Hz bandpass filtering followed by a Hilbert

transform with a threshold of 1.2 standard deviations (STD) above the mean signal strength across three channels. Artifacts were automatically rejected using criteria of amplitude 5.5 STD above mean signal strength and voltage differential 3 STD above that of the channel. Extraction thresholding accuracy was assessed by eye and electrophysiology data from one rat were excluded from analysis due to failure of artifact rejection. SPW-Rs were extracted using a 100-240 Hz bandpass filter followed by a Hilbert transform with a threshold of 3 STD above the mean signal strength across one or two channels.

Extractions excluded wake periods on the basis of electromyogram (EMG). Comparing EMG- and tracker-based immobility detection methods, there is a high rate of agreement but EMG-detected stops often exclude periods detected as immobility using the tracker (Appendix B). This appears to be consistent with distinction between sleep and quiet wake, and seems to give EMG-based filtering an advantage over tracker-based filtering. EMG-based detection was used for all data presented here. Thresholds for active EMG were set separately for each recording session using task periods as a marker for wake.

In addition to the automatic extraction, spindles from one data set were manually scored using an in house written MATLAB graphic user interface. Scoring categories included quality judgments and, importantly, the feature category “k-less” for spindles with no apparent downstate (Appendix F). Spindles not marked as “k-less” include an associated downstate.

### Behavioral Analysis

Behavioral analysis considered the number of feeders visited until Recall criterion, Set 1 recall rate, and Set 2 intrusion rate using the same methods as in (Jones et al., 2012).

## Co-Occurrence and Cross-Correlation

Driven by the theory that co-occurrence or lack thereof may give important insights into the neural mechanisms of memory consolidation, some analyses categorize spindles using a binary classification of “solo” spindles (with no co-occurring ripples) and “tandem” spindles (with at least one co-occurring ripple).

To shuffle data, inter-ripple intervals (IRIs) were indexed from each sleep epoch independently, and redistributed within the epoch using the *randperm* function for random number generation in MATLAB (Mathworks, Natick, MA). This method preserves the ripple count and IRI distribution while removing any pattern related to ripple-spindle co-occurrence. Shuffling was performed ten times and the results were averaged for comparison with the data shown here.

Cross-correlations are shown with a 15 second window surrounding spindle onset times. Each spindle cross-correlation considers only ripples from the offset of the previous spindle to onset of the following spindle to reduce multiple inclusions of the same ripples. The post-spindle window is also adjusted for the duration of the spindle to keep data symmetrical about the time of spindle onset.

## Statistics

Statistics were calculated using SPSS (IBM, Armonk, NY) and MATLAB (Mathworks, Natick, MA). A table summarizing the tests used for each result is available in Appendix C. Nonparametric tests were used where data could not fulfill parametric assumptions. Error bars represent standard error of the mean.

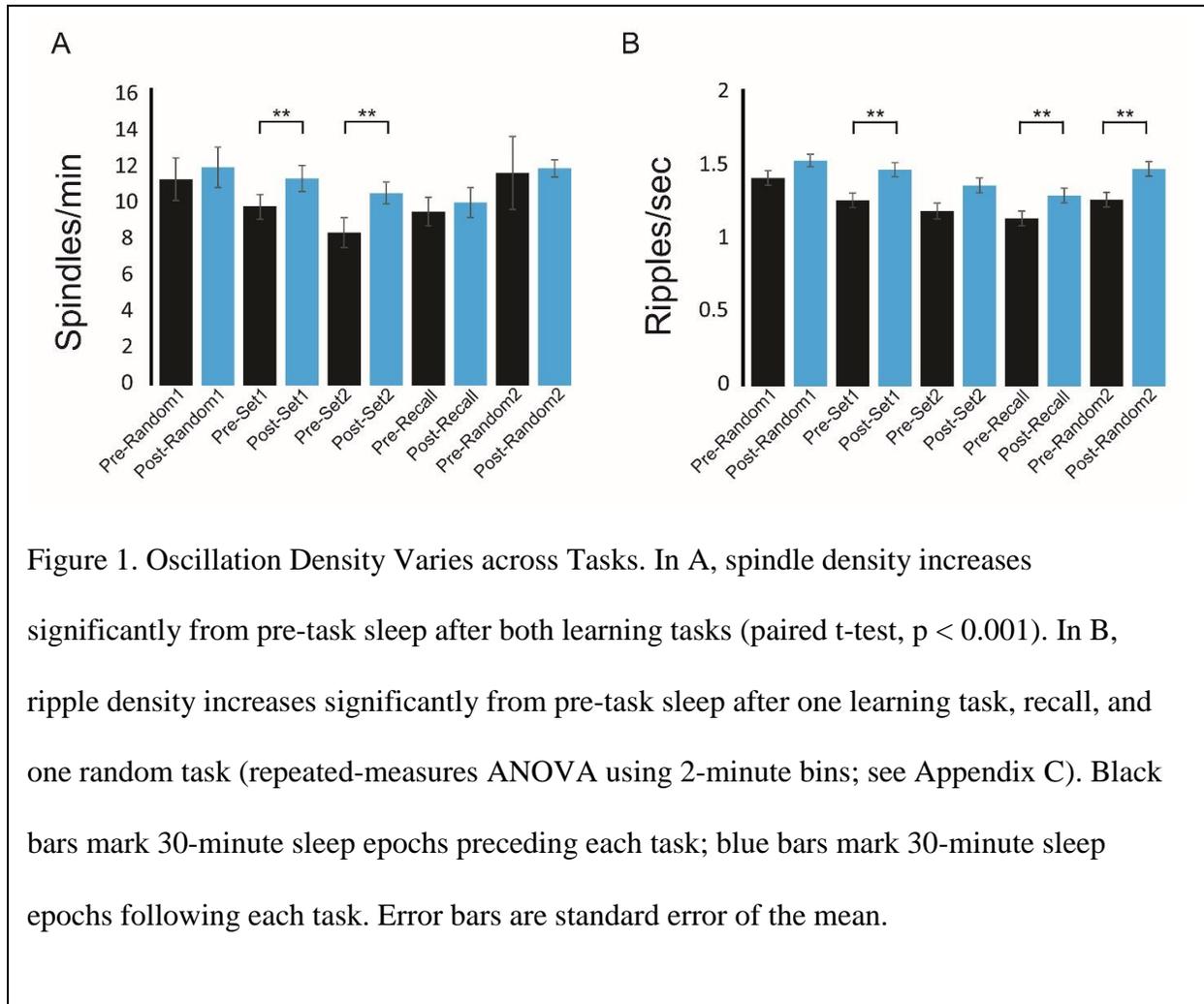
## Results

The data presented here were collected as part of a memory reconsolidation experiment using context manipulations to influence behavioral performance in a spatial navigation task. This analysis uses recordings surrounding two types of same-day task in the experiment: Random tasks using pseudorandomly cued rewards at any of eight feeders on the open field maze, and Learning tasks using pseudorandom cues at subsets of three locations followed by a delayed-cue period testing free recall (additional details in Methods). In some experiment sessions, context cues were changed before the second Learning task. The influence of context was not studied here and is left for further work.

### *Ripple and Spindle Densities Increase after a Spatial Learning Task*

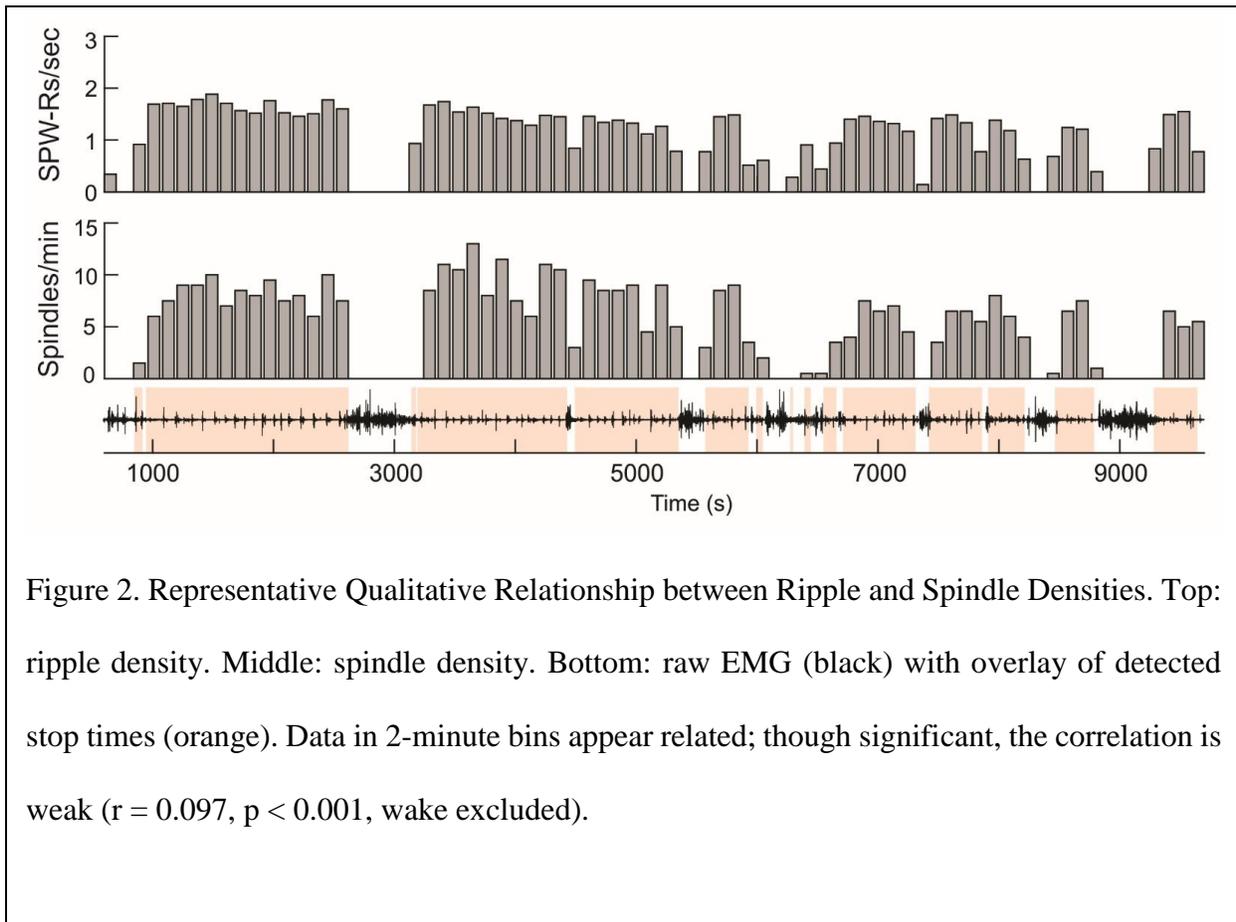
The motivation for this study relies in part on established findings that both prefrontal spindle density and hippocampal sharp-wave ripple density increase during sleep immediately after learning in an olfactory paired-associates task (Eschenko et al., 2006; Eschenko et al., 2008). In the present experiments, spindle density was significantly greater after learning, comparing 30-minute sleep epochs before and after the first Learning session (Pre-Learning 1,  $M = 9.91$ ,  $SD = 3.21$ ; Post-Learning 1,  $M = 11.46$ ,  $SD = 3.53$ ),  $t(23) = -2.894$ ,  $p = 0.008$ , and second Learning session (Pre-Learning 2,  $M = 8.46$ ,  $SD = 3.85$ ; Post-Learning 2,  $M = 10.73$ ,  $SD = 2.90$ ),  $t(21) = -2.955$ ,  $p = 0.008$  (Figure 1A). Ripple density across 2-minute bins also increased after the first Learning session ( $F(1, 230) = 18.51$ ,  $p < 0.001$ ), and showed a trend for an increase after the second same-day Learning session ( $F(1, 213) = 3.224$ ,  $p = 0.074$ ) (Figure 1B). The observed increases are consistent with previous reports and confirm that the phenomenon is conserved in the spatial navigation task used here. These increases are therefore

hypothesized to contribute to the potential effect of learning on the co-occurrence and cross-correlation of these two oscillations.



### *Weak Relationship between Ripple and Spindle Densities over Time*

When discretized into 2-minute bins, ripple and spindle density demonstrate a qualitative tendency to vary together through time (Figure 2). A Pearson correlation was conducted to test the relationship between ripple and spindle density, revealing a significant, but weak correlation across 2-minute bins excluding wake periods detected by EMG ( $r = 0.097$ ,  $p < 0.001$ ). This coarse measure runs counter to expectation, possibly due to the far greater incidence of ripples relative to spindles. Analysis on a finer timescale is necessary to show detailed interactions between the two oscillations.



### *Number of Ripples per Spindle Varies with Spindle Duration*

Spindle duration is correlated with co-occurring ripple count (Figure 3A, Spearman  $r = 0.658$ ,  $p < 0.001$ ). There is no significant difference in this relationship based on learning (Kruskal-Wallis test,  $p > 0.05$ ) (Appendix D). The average spindle duration for each ripple count suggests that the relationship is linear (Figure 3A, red dots). The strong tendency for spindle duration to vary with the number of co-occurring ripples creates an impression that co-occurrence may be explained in part by chance. To test this, a shuffling procedure was introduced (see Methods) and correlation was calculated for shuffled sleep, showing a similar relationship (Spearman  $r = 0.611$ ,  $p < 0.001$ ). This reiterates the importance of the shuffling procedure in subsequent analyses.

### *Tandem Spindles Occur at Levels Greater than Chance*

There are no significant differences in the proportion of spindles with at least one co-occurring ripple across all sleep epochs (repeated-measures ANOVA,  $p > 0.05$ ) (Figure 3B). However, there is a significant overall difference between sleep and shuffled data ( $F(1, 34) = 1774.13$ ,  $p < 0.001$ ). Post-hoc testing showed that all epochs were significantly different from chance (paired t-test, all  $p < 0.001$ , see Appendix C), showing a higher rate of co-occurrence.

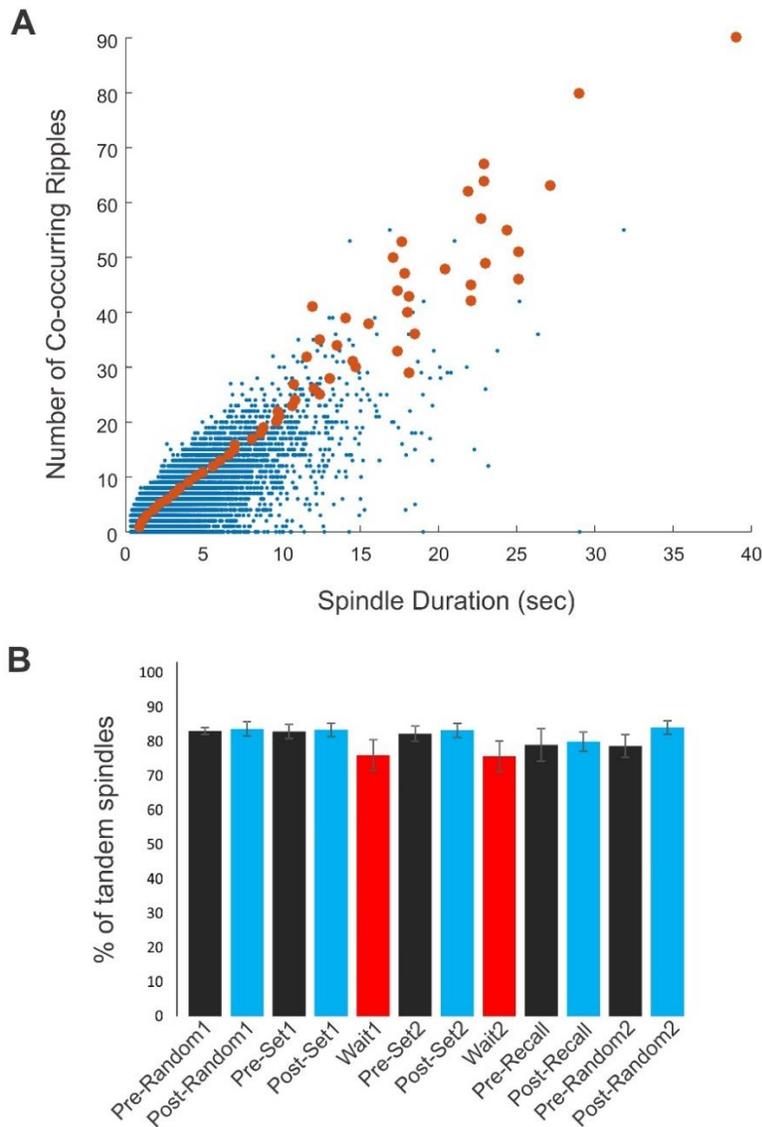


Figure 3. Spindle Co-occurrence with Ripples. In A, spindle duration significantly predicts the number of co-occurring ripples. Average duration for each ripple count shown in red. In B, the percentage of tandem spindles (with at least one co-occurring ripple) is not significantly different across tasks. Black = pre-task; blue = post-task; red = 30-minute rest period not adjacent to task. Error bars are standard error of the mean.

### *Ripples Occur During and Near Spindles above Chance Levels*

To evaluate whether a similar relationship might exist for ripples, ripples were categorized in relation to spindles as Before (ripples 0-200 milliseconds before spindles), During (co-occurring with spindles), After (0-200 milliseconds after spindles), and Out (outside of relationship to spindles), as well as a category to disambiguate any ripples that met criteria for “Before” and “After” classification (Figure 4A). Ripple proportions were calculated from each sleep epoch across all recordings and normalized to the shuffled mean (10 shuffles). One-sample t-tests were conducted to compare each of the distributions of ripples Before, During, and After spindles to the normalized chance mean. There was a significant difference for ripples Before spindles ( $M = 1.95$ ,  $SD = 2.44$ ;  $MD = 1.95$  (95% CI, 1.61 to 2.29)),  $t(204) = 11.44$ ,  $p < 0.001$ , ripples During spindles ( $M = 4.77$ ,  $SD = 2.43$ ;  $MD = 4.77$  (95% CI, 4.44 to 5.11)),  $t(205) = 28.13$ ,  $p < 0.001$ , and ripples After spindles ( $M = 1.55$ ,  $SD = 1.65$ ;  $MD = 1.55$  (95% CI, 1.32 to 1.77)),  $t(205) = 13.51$ ,  $p < 0.001$  (Figure 4B-D). This indicates that ripples disproportionately occur in conjunction with spindles, both during and immediately surrounding them.

### *Ripples Occur More Before than After Spindles*

An additional paired-samples t-test found a significant difference between the mean raw proportions of ripples Before and After spindles (Before:  $M = 3.08$ ,  $SD = 1.19$ ; After:  $M = 2.83$ ,  $SD = 0.98$ ),  $t(239) = 5.20$ ,  $p < 0.001$ , suggesting a higher rate of occurrence for ripples Before spindles than After. This may relate to previous findings of a memory consolidation benefit when ripples are used to prompt artificial cortical stimulation generating spindles, as the reported effect occurred with a ripple-spindle delay of approximately 130 milliseconds (Maingret et al., 2016). Post-spindle ripple enhancement has not yet been studied, but may be an interesting test period or active control for similar manipulations in the future.

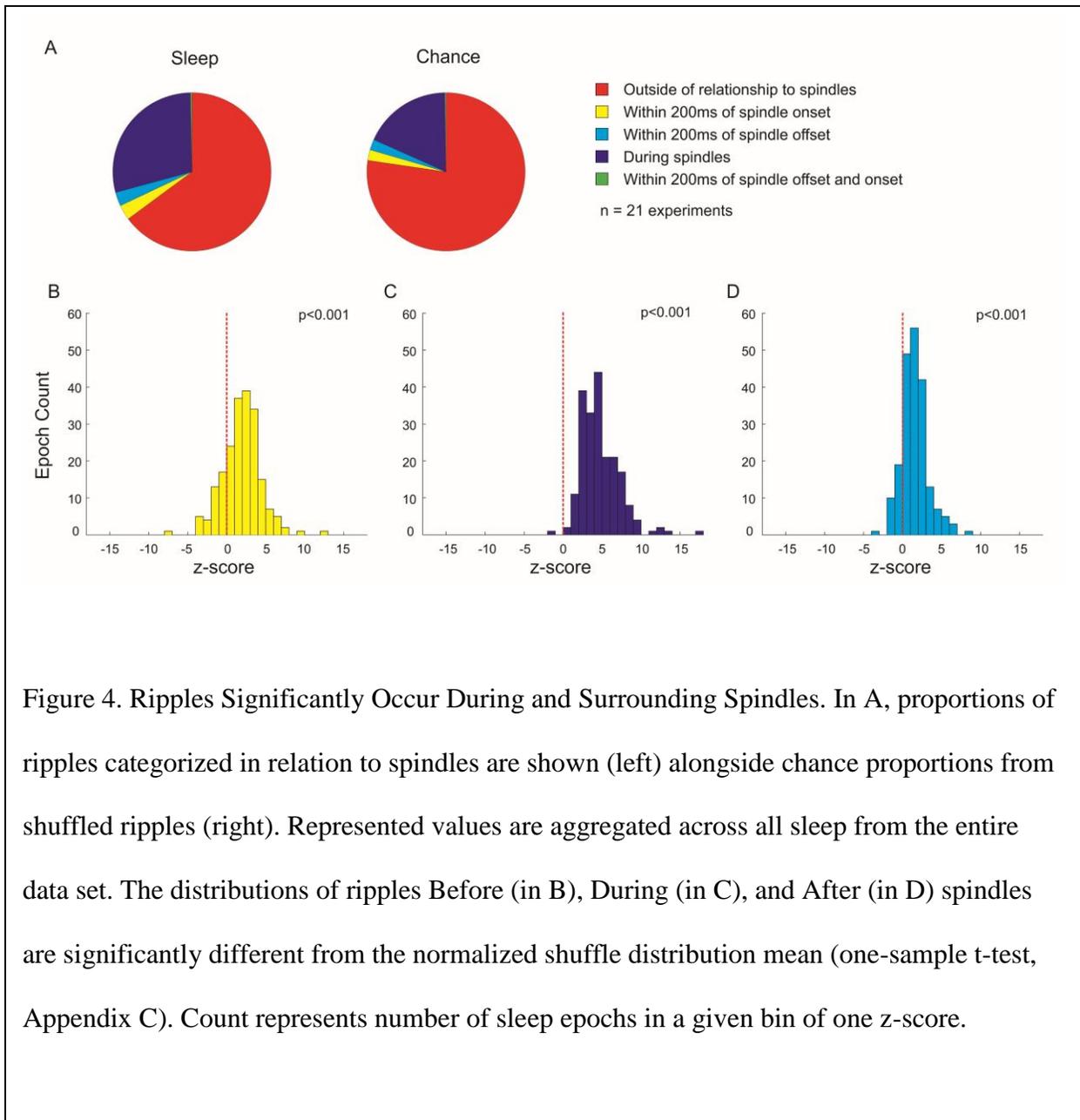


Figure 4. Ripples Significantly Occur During and Surrounding Spindles. In A, proportions of ripples categorized in relation to spindles are shown (left) alongside chance proportions from shuffled ripples (right). Represented values are aggregated across all sleep from the entire data set. The distributions of ripples Before (in B), During (in C), and After (in D) spindles are significantly different from the normalized shuffle distribution mean (one-sample t-test, Appendix C). Count represents number of sleep epochs in a given bin of one z-score.

### *Tandem Spindles Drive Ripple-Spindle Cross-Correlation*

Aggregate ripple-spindle cross-correlation shows a clear peak around the time of spindle occurrence (group data not shown; see Figure 5A for representative shape of distribution).

Separate plots for tandem and solo spindles demonstrate that tandem spindles drive this effect (Figure 5). Both spindle types exhibit a peak in ripple occurrence around spindle onset, but this peak occurs after spindle onset in tandem spindles (Figure 5A). Preliminary data from manual spindle scoring suggest that this may be related to the occurrence of downstates within automatically-detected spindle times (Appendix F). Ripple cross-correlation appears to fall to or below chance levels within the 15-second window for both tandem and solo spindles.

### *Ripple-Spindle Cross-Correlation does not vary with Learning*

Ripple-spindle cross-correlation for the sleep epochs before and after the first non-learning experimental task, as well as the sleep epochs after each learning task, are qualitatively similar (Figure 6). Two-sample Kolmogorov-Smirnov tests show that the post-task distributions are all significantly different ( $p < 0.001$ , Appendix C); however, this result is affected by bin-to-bin variability that does not reflect true differences between distributions. The bin heights across distributions are highly correlated (Non-Learning/Learning 1:  $r = 0.960$ ; Learning 1/Learning 2:  $r = 0.955$ ; Non-Learning/Learning 2:  $r = 0.944$ ; all  $p < 0.001$ ), which indicates that a measure using continuous data may better clarify the relationship.

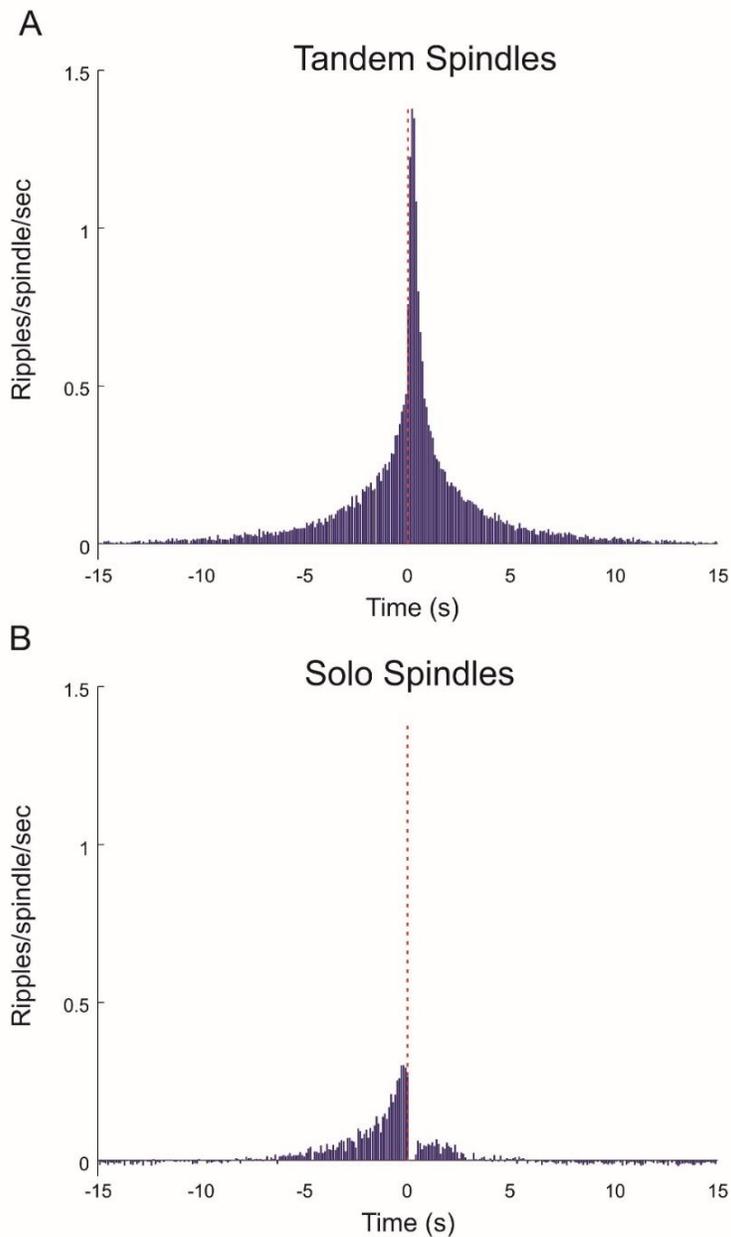


Figure 5. Ripple-Spindle Cross-Correlations. In A, ripples associated with tandem spindles ( $n = 58045$  spindles). In B, ripples associated with solo spindles ( $n = 13785$  spindles). Values shown are net cross-correlation (subtracting chance) in 100ms bins. Data is aggregated across all epochs and sessions.

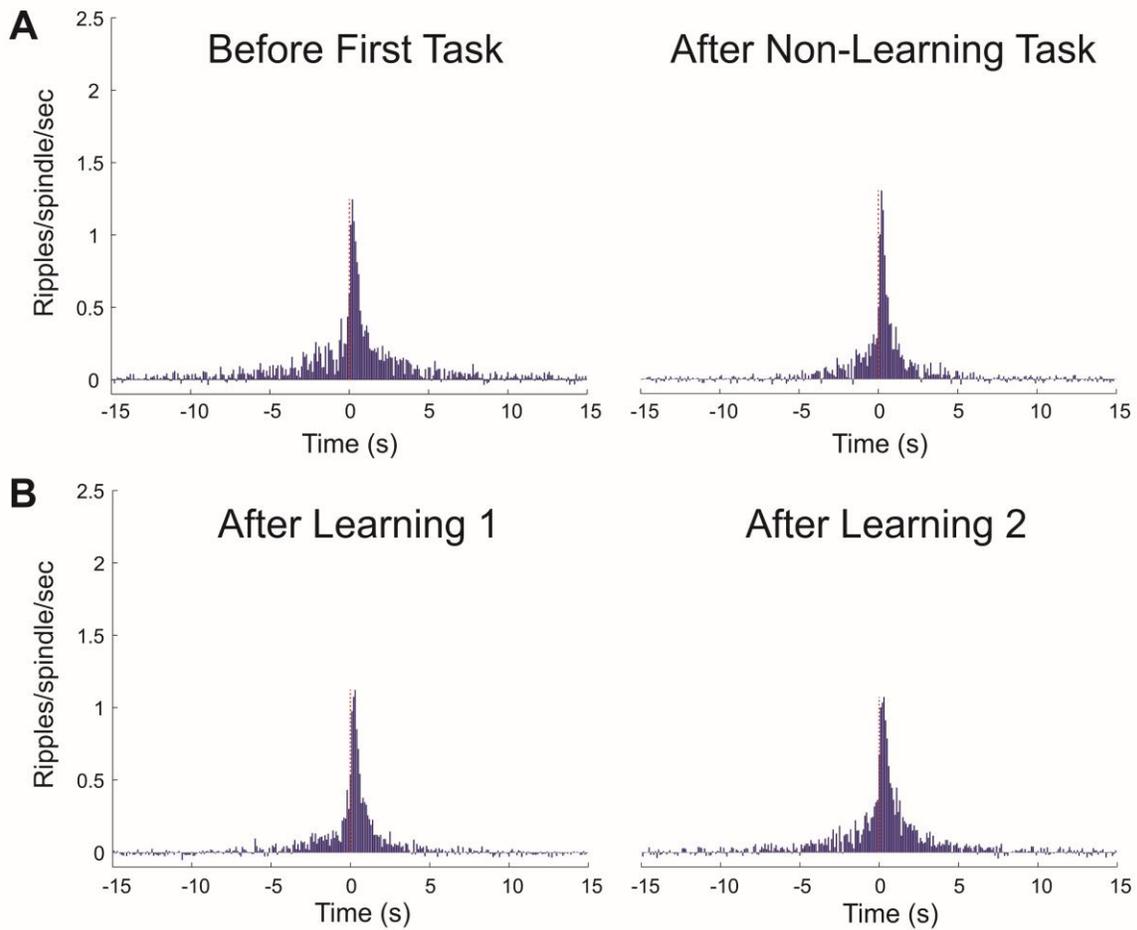


Figure 6. Ripple-Spindle Cross-Correlation Does Not Vary with Learning. In A, two non-learning periods are shown: an epoch before any tasks (top row, left) and the post-task sleep epoch of the first, non-learning task (top row, right). In B, the two post-learning epochs are shown (after first learning task, bottom left; after second learning task, bottom right). All epochs are 30 minutes long. Data shown here uses the corresponding epoch aggregated across all experiments.

## Discussion

The results presented above support and extend previous findings on ripple-spindle coordination. Despite a weak general relationship between ripple and spindle densities, ripples and spindles co-occur at levels significantly higher than chance. This finding strengthens reports from previous studies that may not have controlled for this effect, while simultaneously indicating the need for this measure to be incorporated as a standard procedure in analyses of ripple-spindle co-occurrence.

The greater-than-chance interactions described here are remarkable in the context of evidence that the majority of ripples exhibit no apparent timing relationship with spindles, and even surprising given the known and largely independent mechanisms of ripple and spindle generation. Yet coarse-grained and fine-timescale relationships cannot be equated. Their apparent difference here may relate to our current understanding that memory consolidation, though facilitated by sleep, is not complete after a single night. From this it may seem reasonable that data from a few hours of sleep demonstrate only a statistical effect. It remains unresolved how many times such a process may need to occur in order to produce a consolidated memory, and the characteristics of consolidated memory require further definition. At the same time, “complete” consolidation is a theoretical memory state with limited range for associated testable hypotheses, and there is rich opportunity for studies of memory consolidation to consider the possibility that consolidating memories may have different timelines of susceptibility to different forms of interference.

In this regard, it should be noted that behavioral effects are observed during this experiment, which takes place within a 24-hour period (Appendix G). There is a maximum total sleep time of 6 hours between the first learning session and recall testing. The interaction

between ripples and spindles therefore cannot be interpreted as only contributing to memory over long timescales. Further analysis of the ripple-spindle relationship over the course of these experiments may find a relationship to behavior, although the results discussed here suggest that the measure of cross-correlation may not be well-suited to demonstrate such a relationship.

The lack of significant difference in ripple-spindle interactions across learning and non-learning conditions may be explained by the fact that rates of spindle co-occurrence with ripples, ripple co-occurrence with spindles, and ripple-spindle cross-correlation are already greater than chance at baseline. The evidence favors an interpretation that the ripple-spindle relationship may be relatively fixed regardless of behavioral circumstances. Consequently, the increase in spindle and ripple density observed by many others may be the result of a coordinated system-wide increase in the inputs to the generators of sharp-wave ripple and spindle events in, respectively, CA3 and thalamocortical circuits. Such a conjecture should, of course, be studied at other developmental stages and in the context of a variety of tasks and memory modalities. To date, there is no evidence for an internal mechanism by which the baseline rate of interaction itself can be increased. However, if there is no endogenous variability in this interaction, it may be possible to alter it pharmacologically or optogenetically to determine its function more closely and study the potential benefits of its enhancement. Individual differences in baseline interactions may also correlate with measures of memory performance. Follow-up studies will be necessary to test hypotheses related to these questions.

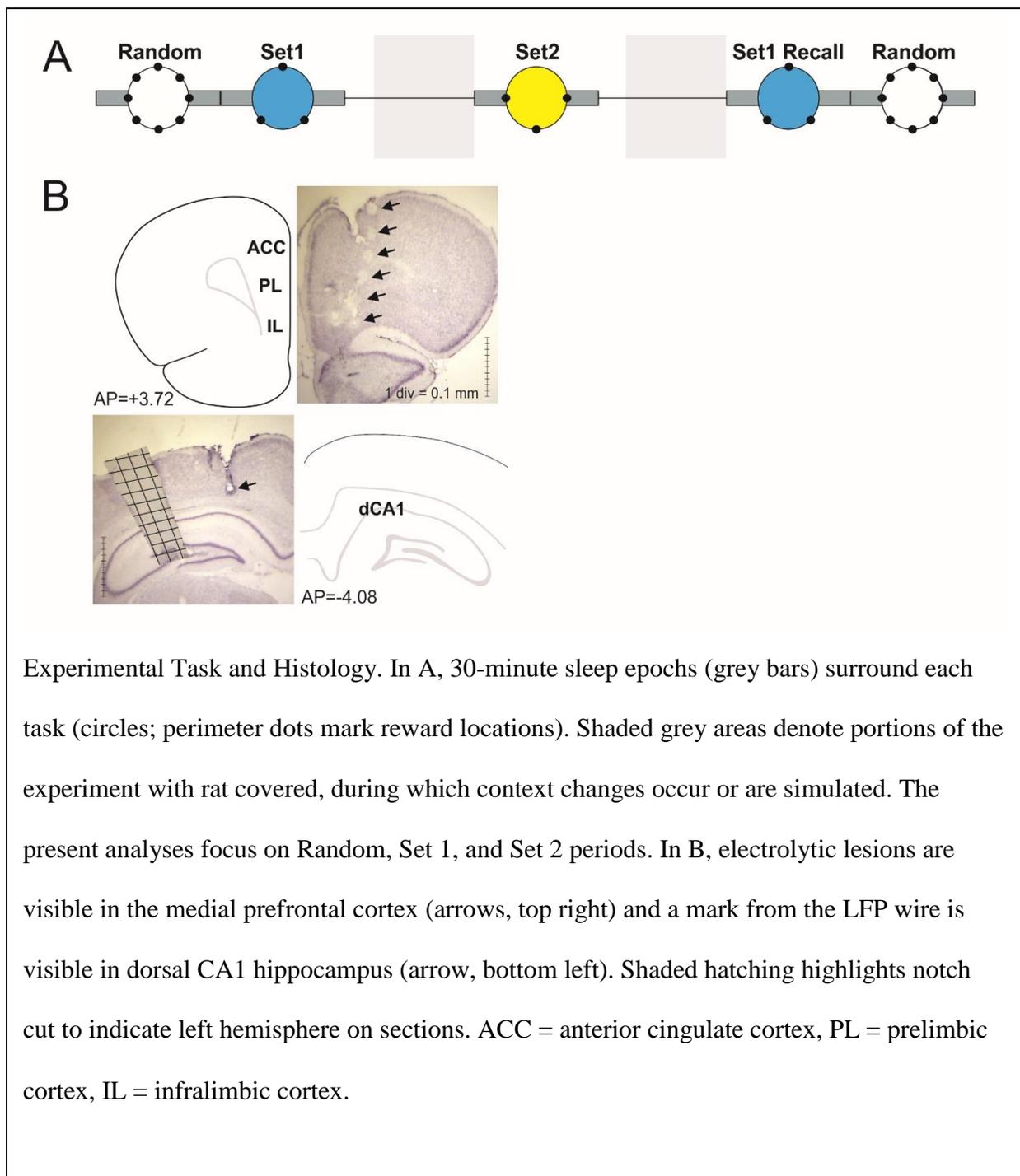
Another aspect that may contribute to the lack of significant difference based on learning is that interregional systems consolidation is not the only purpose of these oscillations. Local ripple and spindle relevance within the hippocampus and cortex, respectively, may explain the seeming constancy of spindle-ripple interaction across learning conditions: if there is cause for

more interregional interaction supporting consolidation, it may follow that there is cause for more consolidation-related activity within regions as well. The concepts of quality and quantity are often evoked in relation to sleep and memory consolidation, and the evidence presented here supports the idea that learning-based upregulations in spindle and ripple density may critically modulate quantity. A net increase of ripple-spindle interactions across tasks was not tested here, but this question will be addressed in continuing analyses.

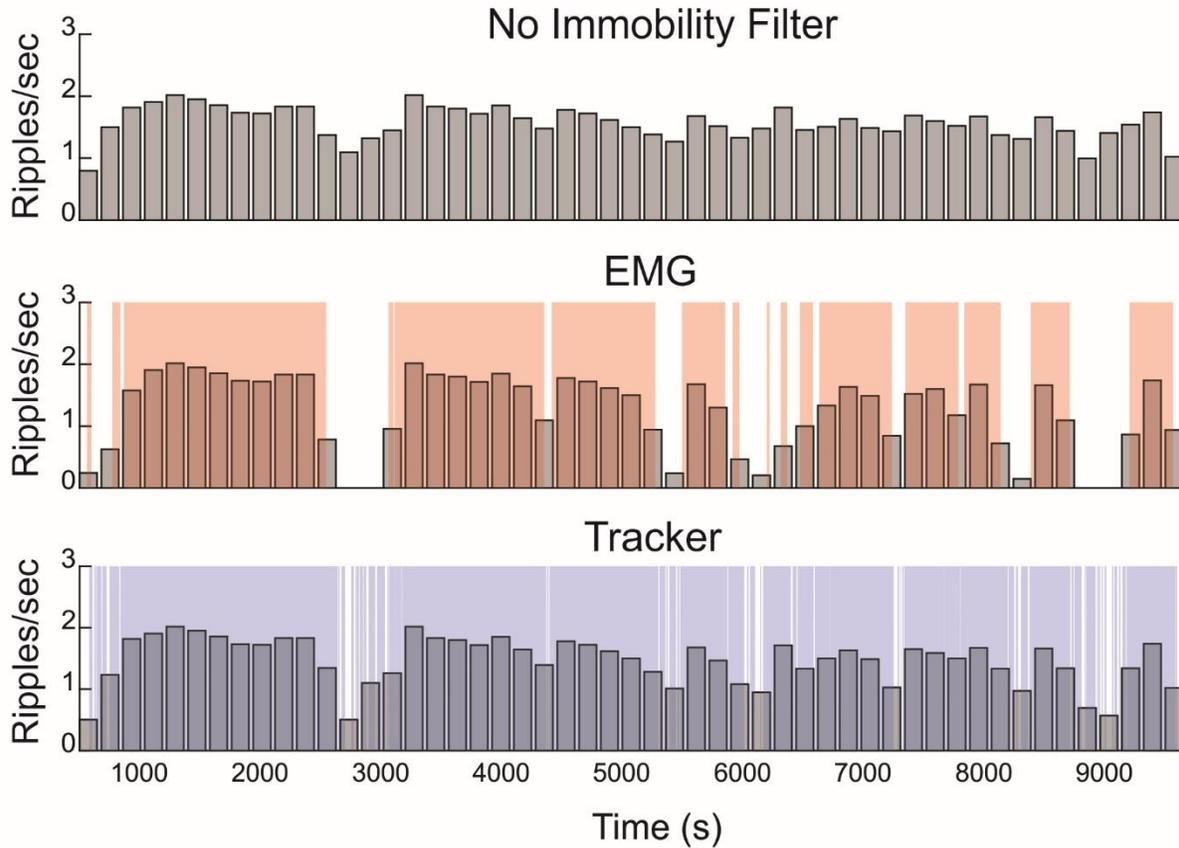
An important additional development from this data will be the study of single units in coordination with ripples and spindles. As described in the introduction, both prefrontal and hippocampal circuits have been shown to reactivate separately during sleep immediately following a task. It is still unknown how reactivation is coordinated within and across regions, and how spindle and ripple occurrences may guide it. Spike timing in the hippocampus and prefrontal cortex is known to relate to hippocampal ripples, but the role of spindles in this interaction remains unclear. A critical next step is to understand what correspondence spindles have with these phenomena, as well as to clarify the functional significance of the relationships that have been outlined in existing research.

This phenomenon is a likely candidate for influence over, or induction of, repeated reactivations during sleep. Though related to all theories of hippocampo-cortical coordination, the interactions reported here most closely support the multiple trace theory of systems consolidation: high baseline rates of oscillatory interaction may indicate the continuous integration of recent events with existing information, gradually expanding the network supporting a given memory trace.

## Appendix A – Experimental Task and Histology



## Appendix B – Comparison of EMG and Tracker-Based Immobility



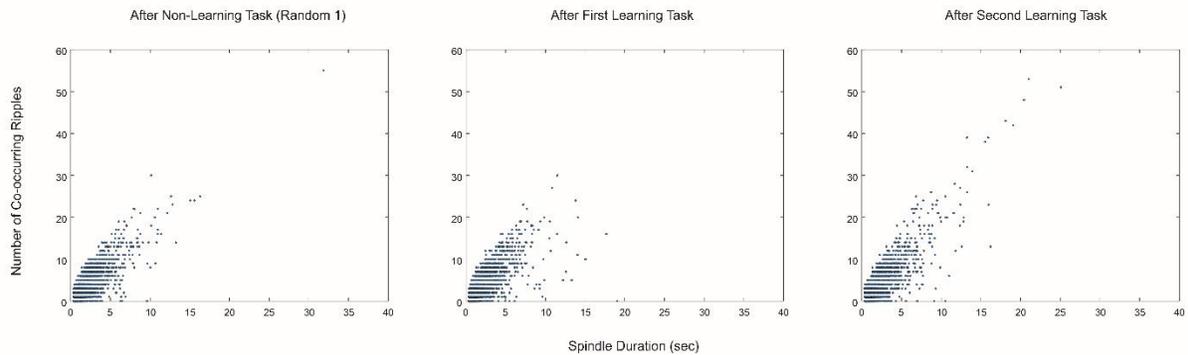
Representative Example of Differences in Immobility Thresholding Methods. Top, histogram of ripple extraction using no wake exclusion method (14419 detected ripples). Middle, histogram of ripple extraction using EMG-based stop times (red overlay, 11262 detected ripples). Bottom, histogram of ripple extraction using tracker-based stop times (blue overlay, 13453 detected ripples). Tracker extraction used a threshold of 2 cm/s velocity and minimum 5 seconds spent below threshold to include a time in stops. EMG-based wake exclusion is used in these analyses.

## Appendix C – Summary of Statistical Tests

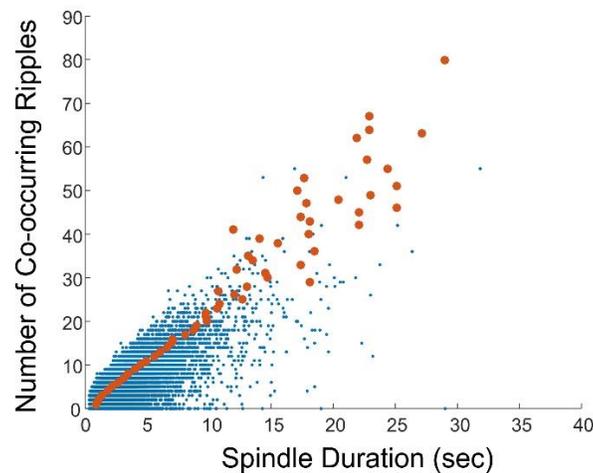
Test	Page	Results	Notes
Paired t-test	18	Pre-Learning 1, M = 9.91, SD = 3.21; Post-Learning 1, M = 11.46, SD = 3.53, $t(23) = -2.894$ , $p = 0.008$ . Pre-Learning 2, M = 8.46, SD = 3.85; Post-Learning 2, M = 10.73, SD = 2.90), $t(21) = -2.955$ , $p = 0.008$ .	Difference between pre- and post-task sleep mean spindle densities, considering full epoch.
Repeated Measures ANOVA	18	Learning 1: $F(1, 230) = 18.51$ , $p < 0.001$ . Learning 2: $F(1, 213) = 3.224$ , $p = 0.074$ .	Difference between pre- and post-task sleep mean ripple densities, in 2-minute bins.
Pearson correlation	20	$r = 0.097$ , $p < 0.001$	Correlation between spindle and ripple density, using 2-minute bins.
Spearman correlation	20	$r = 0.685$ , $p < 0.001$	Correlation between spindle duration and co-occurring ripple count.
Kruskal-Wallis test	20	$p > 0.05$	Difference between learning and non-learning tasks for spindle duration and co-occurring ripple count.
Spearman correlation	20	$r = 0.611$ , $p < 0.001$	Correlation between spindle duration and co-occurring ripple count (shuffled data).
Repeated Measures ANOVA	20	$p > 0.05$	Difference in % of tandem spindles across epochs (real data only).
Repeated Measures ANOVA	20	Mauchly's Test: $\chi^2(65) = 454.00$ , $p < 0.001$ . Within-Subjects effects: $p = 0.046$ (Greenhouse-Geisser correction). Between-Subjects effects: $F(1, 34) = 1774.13$ , $p < 0.001$ .	Difference in % of tandem spindles in epochs across real and shuffled data. W-S not reported due to inclusion of shuffled data with real.
Paired t-test	20	Sleep1: M = 72.18, SD = 11.85; M = 88.88, SD = 5.55; $t(20) = -8.99$ . Sleep2: M = 72.71, SD = 10.32; M = 88.39, SD = 5.57; $t(20) = -10.93$ . Sleep3: M = 72.25, SD = 10.43; M = 87.77, SD = 7.31; $t(20) = -10.94$ . Sleep4: M = 71.99, SD = 10.05; M = 87.54, SD = 5.89; $t(20) = -13.74$ . Sleep5: M = 71.91, SD = 11.78; M = 86.99, SD = 7.78; $t(18) = -8.84$ .	Differences in the above, for real vs. shuffle comparisons of individual epochs. All $p < 0.001$ .

		<p>Sleep6: <math>t(20) = M = 71.21, SD = 12; M = 87.67, SD = 6.47; -11.11.</math></p> <p>Sleep7: <math>M = 65.94, SD = 20.57; M = 83.66, SD = 18.03; t(19) = -6.39.</math></p> <p>Sleep8: <math>M = 69.49, SD = 13.45; M = 85.83, SD = 7.42; t(20) = -8.38.</math></p> <p>Sleep9: <math>M = 68.34, SD = 17.12; M = 85.32, SD = 11.65; t(20) = -7.93.</math></p> <p>Sleep10: <math>M = 72.29, SD = 11.84; M = 88.05, SD = 5.80; t(20) = -9.53.</math></p>	
One-sample t-test	22	<p>Before: <math>M = 1.95, SD = 2.44; MD = 1.95 (95\% CI, 1.61 to 2.29); \mu = 0. t(204) = 11.44, p &lt; 0.001.</math></p> <p>During: <math>M = 4.77, SD = 2.43; MD = 4.77 (95\% CI, 4.44 to 5.11); \mu = 0. t(205) = 28.13, p &lt; 0.001.</math></p> <p>After: <math>M = 1.55, SD = 1.65; MD = 1.55 (95\% CI, 1.32 to 1.77); \mu = 0. t(205) = 13.51, p &lt; 0.001.</math></p>	Differences in distributions of ripples before/during/after spindles relative to shuffle mean (3 tests). $N = 58045$ spindles.
Paired t-test	22	<p>Before: <math>M = 3.08, SD = 1.19; After: M = 2.83, SD = 0.98, t(239) = 5.20, p &lt; 0.001</math></p>	Difference in raw mean proportions of ripples Before vs. After spindles. $N = 240$ epochs.
Pearson correlation	26	<p>Non-Learning: <math>M = 18.62, SD = 58.05.</math> Learning 1: <math>M = 18.72, SD = 55.47.</math> Learning 2: <math>M = 22.53, SD = 48.08.</math> NLxL1: <math>r = 0.960, p &lt; 0.001.</math> L1xL2: <math>r = 0.955, p &lt; 0.001.</math> NLxL2: <math>r = 0.944, p &lt; 0.001.</math></p>	Correlation between bin heights for non-learning and learning ripple-spindle cross-correlograms.
One-way ANOVA	39	<p>Same Context (<math>n = 16</math>): <math>M = 59.74, SD = 7.48.</math> Different Context (<math>n = 16</math>): <math>M = 62.11, SD = 8.33. F(1, 30) = 0.715, p = 0.404.</math></p>	Difference in mean recall rate in Same vs. Different Context conditions (n.s.).
One-way ANOVA	39	<p>Same Context (<math>n = 16</math>): <math>M = 8.78, SD = 5.12.</math> Different Context (<math>n = 16</math>): <math>M = 17.20, SD = 9.00. F(1, 30) = 10.59, p = 0.003.</math></p>	Difference in mean intrusion rate in Same vs. Different Context conditions.

## Appendix D – Spindle Duration vs. Co-occurring Ripple Count

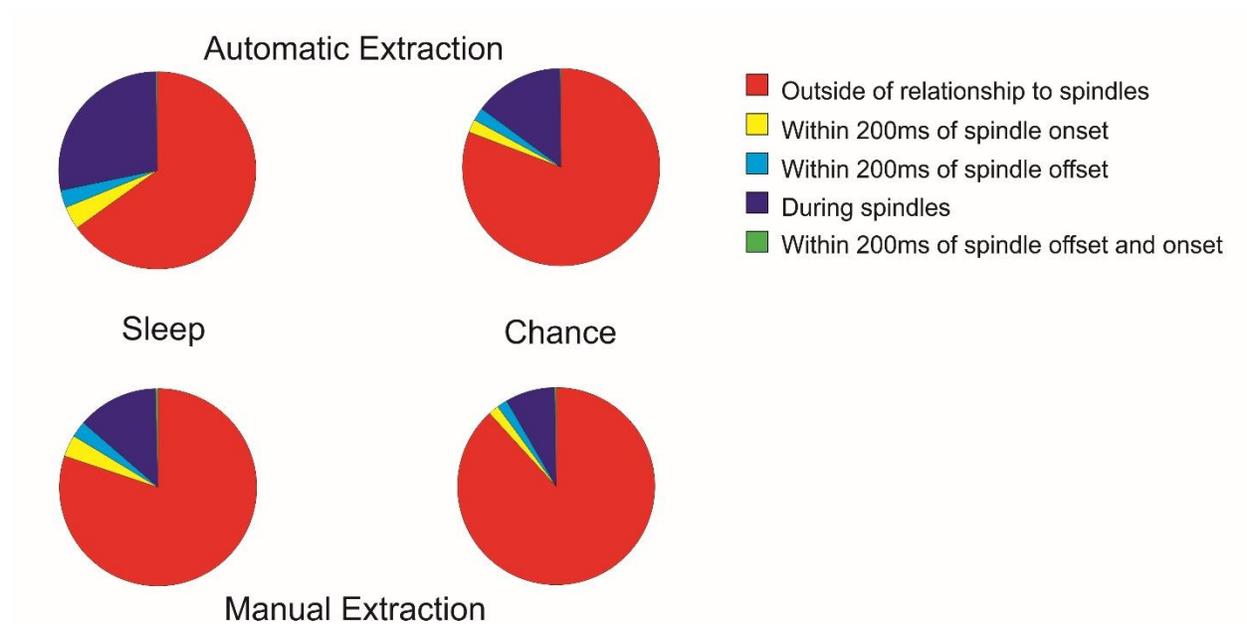


No Significant Effect of Learning on Number of Co-occurring Ripples. Left, a scatterplot of spindle durations with corresponding counts of co-occurring ripples during sleep after a non-learning task. Middle, the same for sleep after the first Learning session. Right, the same for sleep after the second Learning session. No significant differences are present in the relationship between spindle duration and co-occurring ripple count (Kruskal-Wallis test,  $p > 0.05$ ).



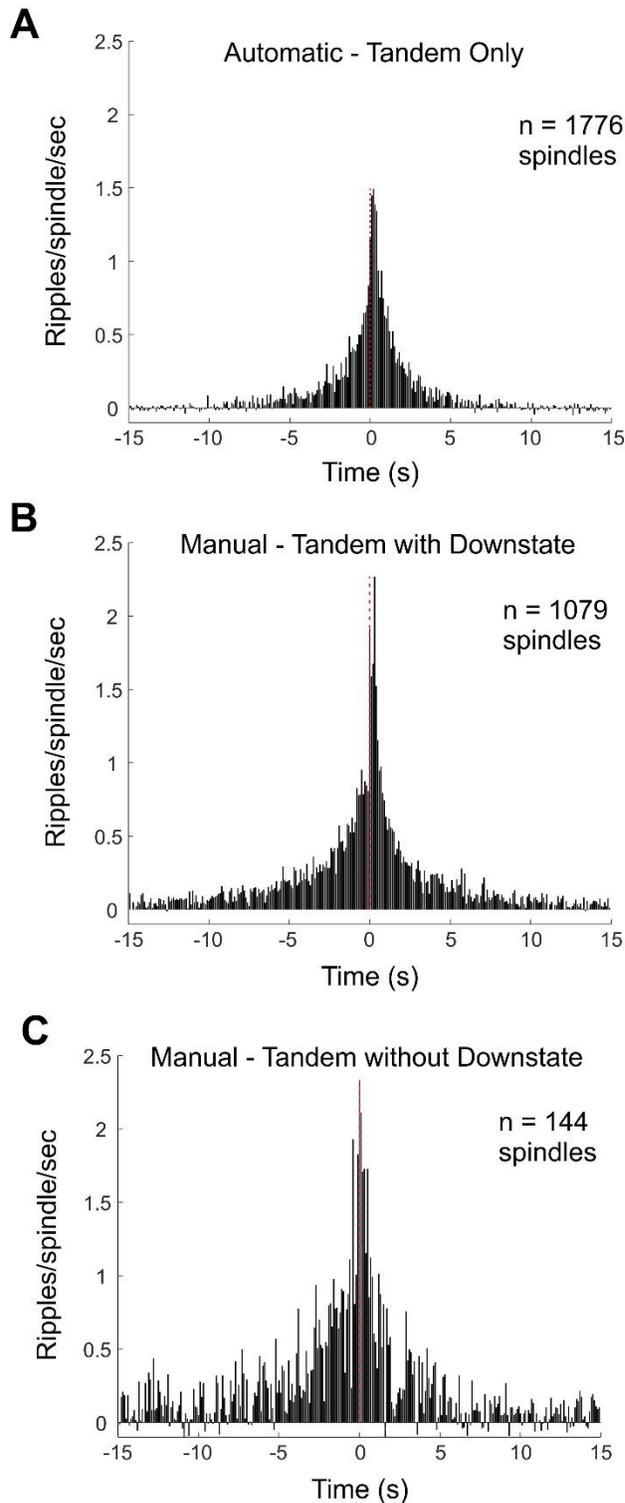
Spindle Duration Correlates with Number of Co-occurring Ripples after Shuffling. Shuffle maintains a significant correlation between spindle duration and co-occurring ripple count (Spearman  $r = 0.611$ ,  $p < 0.001$ ). Average spindle duration for each ripple count is shown in red.

## Appendix E – Technical Considerations



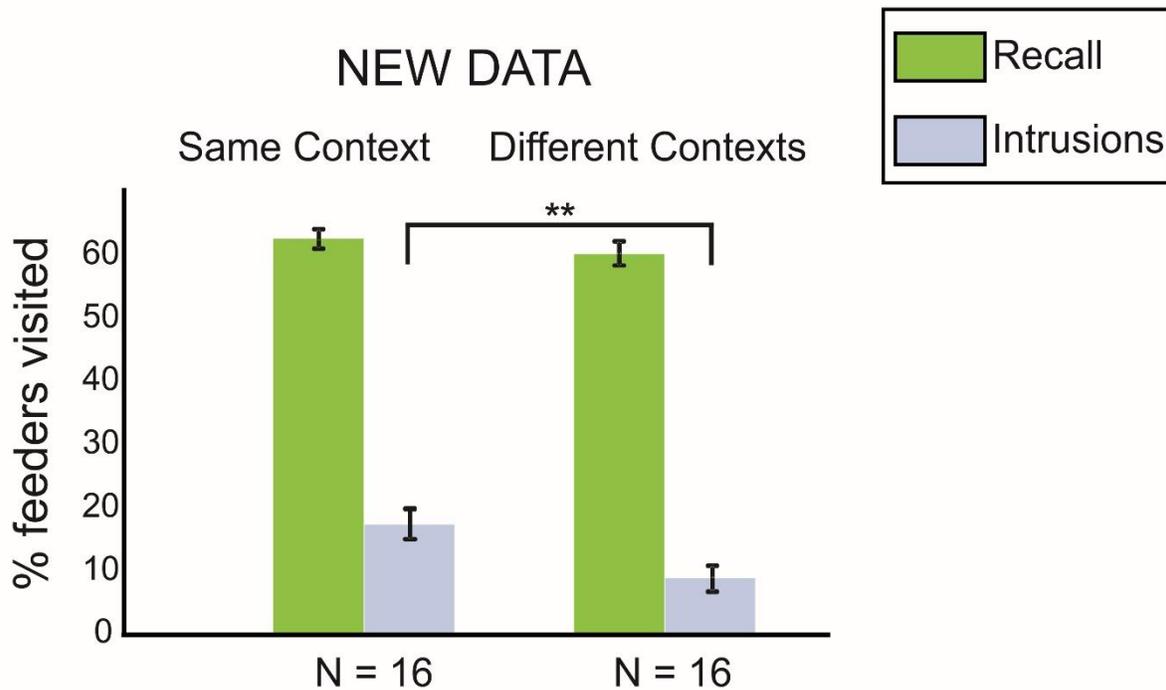
Automatic and Manual Spindle Extraction Differ, but Preserve Effects. To continue evaluating and improving the automatic extraction method used in this study, manual spindle scoring is underway to establish a ground truth for comparison. The above figure shows ripple categorization results relative to automatically-detected spindles (top row) and manually-detected spindles (bottom row) from a single experiment day. From this example there seem to be differences in the results; however, the relationship to chance appears to remain. Statistics will follow with a larger basis for comparison.

## Appendix F – Ripple-Spindle Cross-correlations for Spindles with and without Downstates



Spindles do not always occur with a visible downstate, so manual scoring includes this category (details in Methods). In the manual scoring, 85.7% of spindles (2059 out of 2402 detected spindles; 1 experiment) were associated with a downstate. The distributions of spindles with and without downstates are similar. Decrease in cross-correlation appears to occur immediately after spindle onset at time 0 for spindles without downstates, but not those with downstates.

## Appendix G – Behavioral Results



Memory Interference is Reduced by Differential Context Binding. Recall rate is constant across learning conditions. Intrusion rate, however, is reduced when learning occurs in different contexts rather than the same context (one-way ANOVA,  $F(1, 30) = 10.59$ ,  $p = 0.003$ ). This finding replicates previously published data using this spatial memory paradigm (Jones et al., 2012). Further analysis of single unit and oscillation interactions will pursue a mechanistic explanation for this effect.

## References

- Andrillon T, Nir Y, Staba RJ, Ferrarelli F, Cirelli C, Tononi G, Fried I (2011) Sleep Spindles in Humans: Insights from Intracranial EEG and Unit Recordings. *The Journal of Neuroscience* 31:17821-17834.
- Benchenane K, Peyrache A, Khamassi M, Tierney PL, Gioanni Y, Battaglia FP, Wiener SI (2010) Coherent theta oscillations and reorganization of spike timing in the hippocampal-prefrontal network upon learning. *Neuron* 66:921-936.
- Bi GQ, Poo MM (1998) Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 18:10464-10472.
- Blaskovich B, Szollosi A, Gombos F, Racsmany M, Simor P (2017) The Benefit of Directed Forgetting Persists After a Daytime Nap: The Role of Spindles and Rapid Eye Movement Sleep in the Consolidation of Relevant Memories. *Sleep* 40.
- Bower MR, Euston DR, McNaughton BL (2005) Sequential-context-dependent hippocampal activity is not necessary to learn sequences with repeated elements. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 25:1313-1323.
- Buzsaki G (2015) Hippocampal sharp wave-ripple: A cognitive biomarker for episodic memory and planning. *Hippocampus* 25:1073-1188.
- Buzsaki G, Horvath Z, Urioste R, Hetke J, Wise K (1992) High-frequency network oscillation in the hippocampus. *Science* 256:1025-1027.
- Cellini N, Torre J, Stegagno L, Sarlo M (2016) Sleep before and after learning promotes the consolidation of both neutral and emotional information regardless of REM presence. *Neurobiology of learning and memory* 133:136-144.
- Clemens Z, Molle M, Eross L, Barsi P, Halasz P, Born J (2007) Temporal coupling of parahippocampal ripples, sleep spindles and slow oscillations in humans. *Brain : a journal of neurology* 130:2868-2878.
- Clemens Z, Molle M, Eross L, Jakus R, Rasonyi G, Halasz P, Born J (2011) Fine-tuned coupling between human parahippocampal ripples and sleep spindles. *The European journal of neuroscience* 33:511-520.
- Cohen NJ, Eichenbaum H (1993) *Memory, amnesia, and the hippocampal system*. Cambridge, Mass.: MIT Press.
- Cohen NJ, Poldrack RA, Eichenbaum H (1997) Memory for items and memory for relations in the procedural/declarative memory framework. *Memory (Hove, England)* 5:131-178.
- Contreras D, Destexhe A, Sejnowski TJ, Steriade M (1997) Spatiotemporal patterns of spindle oscillations in cortex and thalamus. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 17:1179-1196.
- Debiec J, LeDoux JE, Nader K (2002) Cellular and systems reconsolidation in the hippocampus. *Neuron* 36:527-538.
- Ego-Stengel V, Wilson MA (2010) Disruption of ripple-associated hippocampal activity during rest impairs spatial learning in the rat. *Hippocampus* 20:1-10.
- Eichenbaum H (2000) A cortical-hippocampal system for declarative memory. *Nature reviews Neuroscience* 1:41-50.
- Eschenko O, Molle M, Born J, Sara SJ (2006) Elevated sleep spindle density after learning or after retrieval in rats. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 26:12914-12920.

- Eschenko O, Ramadan W, Molle M, Born J, Sara SJ (2008) Sustained increase in hippocampal sharp-wave ripple activity during slow-wave sleep after learning. *Learning & memory* 15:222-228.
- Euston DR, Tatsuno M, McNaughton BL (2007) Fast-forward playback of recent memory sequences in prefrontal cortex during sleep. *Science* 318:1147-1150.
- Girardeau G, Benchenane K, Wiener SI, Buzsaki G, Zugaro MB (2009) Selective suppression of hippocampal ripples impairs spatial memory. *Nature neuroscience* 12:1222-1223.
- Groch S, Wilhelm I, Diekelmann S, Born J (2013) The role of REM sleep in the processing of emotional memories: evidence from behavior and event-related potentials. *Neurobiology of learning and memory* 99:1-9.
- Harper B, Sampson A, Sejnowski TJ, Fellous J-M (2016) Sleep spindles and single-cell reactivation in the rodent medial prefrontal cortex during context-dependent memory reconsolidation Poster presentation, Annual Meeting of the Society for Neuroscience.
- Hennies N, Lambon Ralph MA, Kempkes M, Cousins JN, Lewis PA (2016) Sleep Spindle Density Predicts the Effect of Prior Knowledge on Memory Consolidation. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 36:3799-3810.
- Johnson LA, Euston DR, Tatsuno M, McNaughton BL (2010) Stored-trace reactivation in rat prefrontal cortex is correlated with down-to-up state fluctuation density. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 30:2650-2661.
- Jones B, Bukoski E, Nadel L, Fellous JM (2012) Remaking memories: reconsolidation updates positively motivated spatial memory in rats. *Learning & memory* 19:91-98.
- Kandel A, Buzsaki G (1997) Cellular-synaptic generation of sleep spindles, spike-and-wave discharges, and evoked thalamocortical responses in the neocortex of the rat. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 17:6783-6797.
- Kouvaros S, Kotzadimitriou D, Papatheodoropoulos C (2015) Hippocampal sharp waves and ripples: Effects of aging and modulation by NMDA receptors and L-type Ca<sup>2+</sup> channels. *Neuroscience* 298:26-41.
- Lacroix MM, Bagur S, Benchenane K (2015) Characterizing sub-stages of Non REM sleep: Importance for memory consolidation In: Poster presentation, Annual Meeting of the Society for Neuroscience.
- Luthi A (2014) Sleep Spindles: Where They Come From, What They Do. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry* 20:243-256.
- Maingret N, Girardeau G, Todorova R, Goutierre M, Zugaro M (2016) Hippocampo-cortical coupling mediates memory consolidation during sleep. *Nature neuroscience* 19:959-964.
- McClelland JL, McNaughton BL, O'Reilly RC (1995) Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. *Psychological review* 102:419-457.
- Miyamoto D, Hirai D, Fung CC, Inutsuka A, Odagawa M, Suzuki T, Boehringer R, Adaikkan C, Matsubara C, Matsuki N, Fukai T, McHugh TJ, Yamanaka A, Murayama M (2016) Top-down cortical input during NREM sleep consolidates perceptual memory. *Science* 352:1315-1318.
- Molle M, Eschenko O, Gais S, Sara SJ, Born J (2009) The influence of learning on sleep slow oscillations and associated spindles and ripples in humans and rats. *The European journal of neuroscience* 29:1071-1081.

- Muller L, Piantoni G, Koller D, Cash SS, Halgren E, Sejnowski TJ (2016) Rotating waves during human sleep spindles organize global patterns of activity that repeat precisely through the night. *eLife* 5.
- Nadel L, Moscovitch M (1997) Memory consolidation, retrograde amnesia and the hippocampal complex. *Current opinion in neurobiology* 7:217-227.
- Nadel L, Hupbach A, Gomez R, Newman-Smith K (2012) Memory formation, consolidation and transformation. *Neuroscience and biobehavioral reviews* 36:1640-1645.
- Nir Y, Staba RJ, Andrillon T, Vyazovskiy VV, Cirelli C, Fried I, Tononi G (2011) Regional slow waves and spindles in human sleep. *Neuron* 70:153-169.
- O'Reilly RC, Bhattacharyya R, Howard MD, Ketz N (2014) Complementary learning systems. *Cognitive science* 38:1229-1248.
- Pereira SI, Bejamini F, Weber FD, Vincenzi RA, da Silva FA, Louzada FM (2017) Tactile stimulation during sleep alters slow oscillation and spindle densities but not motor skill. *Physiology & behavior* 169:59-68.
- Peyrache A, Khamassi M, Benchenane K, Wiener SI, Battaglia FP (2009) Replay of rule-learning related neural patterns in the prefrontal cortex during sleep. *Nature neuroscience* 12:919-926.
- Piantoni G, Halgren E, Cash SS (2016) The Contribution of Thalamocortical Core and Matrix Pathways to Sleep Spindles. *Neural plasticity* 2016:3024342.
- Plihal W, Born J (1997) Effects of early and late nocturnal sleep on declarative and procedural memory. *Journal of cognitive neuroscience* 9:534-547.
- Rothschild G, Eban E, Frank LM (2017) A cortical-hippocampal-cortical loop of information processing during memory consolidation. *Nature neuroscience* 20:251-259.
- Schlichting ML, Preston AR (2015) Hippocampal-medial prefrontal circuit supports memory updating during learning and post-encoding rest. *Neurobiology of learning and memory*.
- Siapas AG, Wilson MA (1998) Coordinated interactions between hippocampal ripples and cortical spindles during slow-wave sleep. *Neuron* 21:1123-1128.
- Sirota A, Buzsaki G (2005) Interaction between neocortical and hippocampal networks via slow oscillations. *Thalamus & related systems* 3:245-259.
- Sirota A, Csicsvari J, Buhl D, Buzsaki G (2003) Communication between neocortex and hippocampus during sleep in rodents. *Proceedings of the National Academy of Sciences of the United States of America* 100:2065-2069.
- Staresina BP, Bergmann TO, Bonnefond M, van der Meij R, Jensen O, Deuker L, Elger CE, Axmacher N, Fell J (2015) Hierarchical nesting of slow oscillations, spindles and ripples in the human hippocampus during sleep. *Nature neuroscience* 18:1679-1686.
- Studte S, Bridger E, Mecklinger A (2016) Sleep spindles during a nap correlate with post sleep memory performance for highly rewarded word-pairs. *Brain and language*.
- Tamminen J, Lambon Ralph MA, Lewis PA (2017) Targeted memory reactivation of newly learned words during sleep triggers REM-mediated integration of new memories and existing knowledge. *Neurobiology of learning and memory* 137:77-82.
- Wagner U, Gais S, Born J (2001) Emotional memory formation is enhanced across sleep intervals with high amounts of rapid eye movement sleep. *Learning & memory* 8:112-119.
- Wang DV, Ikemoto S (2016) Coordinated Interaction between Hippocampal Sharp-Wave Ripples and Anterior Cingulate Unit Activity. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 36:10663-10672.

- Wang SH, Morris RG (2010) Hippocampal-neocortical interactions in memory formation, consolidation, and reconsolidation. *Annual review of psychology* 61:49-79, C41-44.
- Wiegand JP, Gray DT, Schimanski LA, Lipa P, Barnes CA, Cowen SL (2016) Age Is Associated with Reduced Sharp-Wave Ripple Frequency and Altered Patterns of Neuronal Variability. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 36:5650-5660.
- Wierzynski CM, Lubenov EV, Gu M, Siapas AG (2009) State-Dependent Spike-Timing Relationships between Hippocampal and Prefrontal Circuits during Sleep. *Neuron* 61:587-596.
- Ylinen A, Bragin A, Nadasdy Z, Jando G, Szabo I, Sik A, Buzsaki G (1995) Sharp wave-associated high-frequency oscillation (200 Hz) in the intact hippocampus: network and intracellular mechanisms. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 15:30-46.