JNeurosci
THE JOURNAL OF NEUROSCIENCE

Research Articles: Behavioral/Cognitive

Sleep spindles promote the restructuring of memory representations in ventromedial prefrontal cortex through enhanced hippocampal-cortical functional connectivity

https://doi.org/10.1523/JNEUROSCI.1946-19.2020

Cite as: J. Neurosci 2020; 10.1523/JNEUROSCI.1946-19.2020

Received: 8 August 2019
Revised: 6 January 2020
Accepted: 8 January 2020

Sleep spindles promote the restructuring of memory representations in ventromedial prefrontal cortex through enhanced hippocampal-cortical functional connectivity

Abbreviated title: Sleep spindles promote memory trace reorganization

Emily Cowan[1], Anli Liu[2,3], Simon Henin[2,3], Sanjeev Kothare[2,3], Orrin Devinsky[2,3], Lila Davachi[4,5]

1. Center for Neural Science, New York University, 4 Washington Place, NY, NY 10003, USA
2. Comprehensive Epilepsy Center, New York University, 223 East 34th Street, NY, NY 10016, USA
3. Department of Neurology, New York University Langone Health, 222 East 41st St, 14th Floor, NY, NY 10017, USA
4. Psychology Department, Columbia University, Schermerhorn Hall, 1190 Amsterdam Ave, NY, NY 10027, USA
5. Nathan Kline Institute, 140 Old Orangeburg Rd, Orangeburg, NY, 10962, USA

Corresponding Author: Lila Davachi, Ph.D., ld24@columbia.edu

Number of pages: 38

6 Figures; 0 Tables

Number of words: Abstract: 250, Introduction: 648, Discussion: 1,489

Acknowledgements
This work was supported by the NIH Grant R01-MH076492 to LD, Dart Neuroscience grant to LD, a NYU Clinical Translational Science Initiative Grant to AL, and a NSF Graduate Research Fellowship to EC. We thank David Clewett and Kevin Ochsner for helpful comments on the manuscript.

Declaration of Interests
The authors declare no competing interests.

Abstract

Memory consolidation is hypothesized to involve the distribution and restructuring of memory representations across hippocampal and cortical regions. Theories suggest that, through extended hippocampal-cortical interactions, cortical ensembles come to represent more integrated, or overlapping, memory traces that prioritize commonalities across related memories. Sleep processes, particularly fast sleep spindles, are thought to support consolidation, but evidence for this relationship has been mostly limited to memory retention benefits. Whether fast spindles provide a mechanism for neural changes hypothesized to support consolidation, including the strengthening of hippocampal-cortical networks and integration across memory representations, remains unclear, as does the specificity of regions involved. Using functional connectivity analyses of human fMRI data (both sexes), we show that fast spindle density during overnight sleep is related to enhanced hippocampal-cortical functional connectivity the next day, when re-studying information learned before sleep. Spindle density modulated connectivity in distinct hippocampal-cortical networks depending on the category of the consolidated stimuli. Specifically, spindle density correlated with functional connectivity between anterior hippocampus and ventromedial prefrontal cortex (vmPFC) for object-word pairs, and posterior hippocampus and posteromedial cortex (PMC) for scene-word pairs. Using multivariate pattern analyses, we also show fast spindle density during post-learning sleep is associated with greater pattern similarity, or representational overlap, across individual object-word memories in vmPFC the next day. Further, the relationship between fast spindle density and representational overlap in vmPFC was mediated by the degree of anterior hippocampal-vmPFC functional connectivity. Together, these results suggest fast spindles support the network distribution of memory traces, potentially restructuring memory representations in vmPFC.

Significance statement

How new experiences are transformed into long-term memories remains a fundamental question for neuroscience research. Theories suggest that memories are stabilized as they are reorganized in the brain, a process thought to be supported by sleep oscillations, particularly sleep spindles. Although sleep spindles have been associated with benefits in memory retention, it is not well understood how spindles modify neural memory traces. This study found that spindles during overnight sleep correlate with changes in neural memory traces, including enhanced functional connectivity in distinct hippocampal-cortical networks and increased pattern similarity amongst memories in the cortex. The results provide critical evidence that spindles during overnight sleep may act as a physiological mechanism for the restructuring of neural memory traces.

Introduction

Accumulating evidence suggests sleep plays an important role in the retention of memories (Diekelmann and Born, 2010; Rasch and Born, 2013). However, precisely how sleep alters neural memory traces to support memory is still not well understood. Consolidation theories posit that new memories become stabilized as they are gradually reorganized and distributed across neocortical networks (Alvarez and Squire, 1994; McClelland et al., 1995). This memory transformation process is thought to be supported, at least partly, by neural oscillations occurring during non-rapid eye movement (non-REM) sleep (Sirota et al., 2003; Diekelmann and Born, 2010; Rasch and Born, 2013). For example, prior research has consistently linked sleep spindles (11-16 Hz) with benefits for later memory performance (Gais et al., 2002; Clemens et al., 2005, 2006; Mednick et al., 2013; Rasch and Born, 2013). Sleep spindles are proposed to help coordinate neural activity between the hippocampus and neocortex through interactions with sharp wave ripples (80-100 Hz), oscillations supporting hippocampal replay (Wilson and McNaughton, 1994; Siapas and Wilson, 1998; Clemens et al., 2011), and cortical slow oscillations (~0.5-1 Hz) (Mölle et al., 2002; Clemens et al., 2007; Staresina et al., 2015; Klinzing et al., 2016). Indeed, during sleep, fast spindle activity is coincident with increases in univariate activation in hippocampus and cortex (Schabus et al., 2007; Bergmann et al., 2012) and hippocampal-cortical functional connectivity (Andrade et al., 2011), as well as the reactivation of memories (Schönauer et al., 2017; Antony et al., 2018; Cairney et al., 2018).

While these data broadly support the notion that spindle activity promotes memory consolidation, gaps remain in our current understanding about this relationship. If spindles act as a mechanism for systems consolidation, they should be related to neural markers of consolidation that endure *after* sleep – yet, little work has examined how spindles affect neural memory

representations. Here, we address three critical questions concerning the way sleep spindles effect lasting changes on the neural organization of memories.

First, a central tenant of systems consolidation models is the enhanced communication between hippocampus and cortex. Prior work has shown hippocampal-cortical functional connectivity increases with time (Gais et al., 2007; Sterpenich et al., 2007; Vilberg and Davachi, 2013) (but see: Takashima et al., 2009; Baran et al., 2016), in a manner predicting memory retention (Vilberg and Davachi, 2013). However, whether spindles during sleep contribute to such enhancements in hippocampal-cortical functional connectivity remains unknown. We additionally queried the specificity of these effects. The anterior and posterior hippocampus have been shown to participate in distinct networks with differing functional specialization, processing object/item information or spatial relationships/context, respectively (Davachi, 2006; Libby et al., 2012; Ranganath and Ritchey, 2012; Nadel et al., 2013; Poppenk et al., 2013; Robin and Moscovitch, 2017). Using two stimulus categories, we tested if the relationship between spindles and hippocampal network connectivity is sensitive to the content of the pre-sleep information.

Second, models of consolidation suggest the cortex gradually extracts the central features ('gist') from new memories (Alvarez and Squire, 1994; McClelland et al., 1995; Robin and Moscovitch, 2017), resulting in more integrated traces. Prior work has shown that medial prefrontal cortex (mPFC) representations for similar experiences become more integrated, or overlapping, with time (Richards et al., 2014; Tompary and Davachi, 2017). Yet, it remains unclear if spindles during sleep contribute to this neural restructuring process.

Finally, connecting the preceding hypotheses, we tested whether the reorganization of cortical memory representations depends on the modulation of hippocampal-cortical networks. If sleep spindles promote the transfer of information *through* hippocampal-cortical interactions, we

hypothesized the magnitude of neural integration across cortical traces would be related to the strength of hippocampal-cortical functional connectivity.

We designed a three-day, within-participant experiment that included pre- and post-sleep encoding, measures of overnight polysomnography, a next-day fMRI scan, and behavioral memory tests (Figure 1). We examined how spindles measured during the intervening night relate to differences in functional connectivity and multivariate pattern similarity for memories learned before, versus after, sleep.

Materials and Methods

Participants

A total of 22 participants (7 female, 15 male) were recruited for this experiment (all protocols approved by the NYU School of Medicine's Institutional Review Board). Two participants were excluded due to problems with the MRI scanner, and one participant was excluded as an outlier for total sleep time (> 2.5 standard deviations below the group average). All participants were between 18-35 years of age (mean= 25.3), fluent in English, did not have any diagnoses of neurologic, psychiatric or sleep disorders, and were not using any psychoactive medications. Participants were included if they had not traveled across time zones or completed night-shift work in the month preceding the first study session, had a body-mass index (BMI) below 30, and did not have any contraindications for the MRI. For the 48-hours prior to the beginning of Session 1 (day 1), participants were asked to log their sleep patterns, refrain from any alcohol or drug use, and reduce caffeine intake to one cup per day. At the beginning of Session 1, participants were asked to complete questionnaires to assess eligibility for study participation and query sleep habits, including the MOCA, Insomnia Symptom Questionnaire, Morning-Eveningness Questionnaire, STOP-BANG Questionnaire and Epworth Sleepiness

Scale. All participants provided informed consent, and were compensated for their time. The sample size was based on similar studies examining sleep effects on univariate neural activation (Takashima et al., 2006; Gais et al., 2007; Sterpenich et al., 2007; Hennies et al., 2016).

Experiment Design

Participants completed a three-day experiment, as outlined in Figure 1. Each participant studied three separate Encoding Lists, consisting of unique scene-word and object-word pairs. The Sleep List was encoded before the overnight sleep period (day 1), during which polysomnography was recorded (lights out at approximately 10:30pm), while the Morning List was encoded after awakening the next morning (at approximately 7:30am, day 2). Participants were provided ~30 minutes to eat breakfast before beginning the Morning List encoding session. Approximately 2 hours later, both the Sleep and Morning Lists were studied for a second time in the fMRI scanner, intermixed with the third, novel list of word-image pairs (Single Study List). Participants completed two source memory tests, immediately after the scan (day 2) and after a 24-hour delay (day 3).

Encoding

For the encoding sessions, each trial consisted of the presentation of a central red fixation cross for 500ms, followed by a word and image (scene or object) pair for 4500ms, then a black fixation cross for 500ms. Participants were instructed to form a vivid mental association between the word and image on screen, and then rate how well the association could be formed, responding either ‘very well,’ ‘somewhat well,’ or ‘not well.’ Each of the three Encoding Lists consisted of 120 word-image pairs (60 object-word and 60 scene-word pairs). Encoding sessions were divided into two blocks of 60 trials, separated by a 30-second break. The order of word-

image pairs was randomized for each participant and intermixed between the categories. Word-image pairs were counterbalanced across Encoding Lists and across participants. Between trials, participants completed an active baseline task for 8.5 seconds (Stark and Squire, 2001). Numeric digits ranging between 1-9 were presented, and participants were instructed to respond 'even' or 'odd.' Digits were presented for 2 seconds each, or until a response was entered, and followed by a black fixation cross for 250ms. Outdoor scene stimuli were randomly selected from an online database (http://olivalab.mit.edu/MM/; Oliva and Torralba, 2001). Object stimuli were selected from the MIT Massive Memory set (Brady et al., 2008) and word stimuli were adjectives from the MRC psycholinguistics database (http://websites.psychology.uwa.edu.au/school/MRCDatabase/uwa_mrc.htm). Words were presented in size 36 black Helvetica font on a white background. All stimuli were presented on an Apple MacBook laptop, and responses were made using the computer's keyboard.

Overnight Polysomnography

Overnight polysomnography (PSG) was conducted at the NYU Langone Sleep Center using the Xltek data acquisition system (Natus Medical, San Carlos, California). PSG measurements included standard electroencephalography (International 10/20 Electrode Placement; Fp1, Fp2, F7, F3, F8, F4, Fz, Cz, T3, C3, C4, T4, T5, P3, Pz, P4, T6, O1, O2, and A1, A2 mastoid references), Electrooculogram (Left/Right EOG), Chin Electromyogram (EMG), as well as Chest and Leg movements, Respiratory monitoring, and Blood Oxygenation (SpO2). EEG data were digitized at a sampling rate of 256 Hz and filtered between 0.1-70 Hz.

fMRI session

213 Participants traveled to the Center for Brain Imaging at New York University for the
214 fMRI session. In the scanner, participants were re-presented with all previously studied word-
215 image pairs from both the Sleep and Morning Lists, as well as novel pairs ('Single Study List');
216 during the scanning session, the 120 word-image pairs from each list were randomly intermixed,
217 and divided into six separate runs, with 60 trials per run. Participants performed the same task as
218 in the previous two encoding sessions (forming an association between the word and image, rate
219 how well they could do so). Trials were presented with the same stimulus timing as described
220 above for the behavioral encoding sessions, and again included an odd/even active baseline task
221 between trials. All responses were made using an MRI-compatible button box. After the
222 encoding task, participants completed a localizer task in which they viewed novel objects, scenes
223 and scrambled objects, and was used in a separate line of inquiry. A high-resolution anatomical
224 image of each participant's brain was acquired at the end of the scan.
225
226 **fMRI parameters and preprocessing**
227 Scanning was completed on a 3T Siemens Allegra head-only scanner. Functional imaging
228 data was collected using an echo-planar (EPI) pulse sequence (TR= 2000ms, TE= 15ms, 34
229 interleaved slices oriented parallel to the AC-PC axis, flip angle= 82°**,** voxel size= 3x3x3 mm).
230 The first 6 volumes were discarded to allow for T1 stabilization. A high-resolution T1 weighted
231 anatomical scan (magnetization-prepared rapid-acquisition gradient echo sequence, voxel size=
232 1x1x1 mm) was acquired to aid in functional image co-registration.
233 Preprocessing on functional data was performed using the FSL (version 5.0.2.2) fMRI
234 Expert Analysis Tool version 6 (FSL: http://fsl.fmrib.ox.ac.uk/fsl/). Functional images were
235 brain-extracted, high-pass filtered (110s cutoff), realigned to correct for interleaved acquisition,
236 and MCFLIRT was applied for motion correction. For psychophysiological interaction analyses,

data was spatially smoothed with a 5mm FWHM kernel. FSL's motion outliers tool was used to identify outlier time-points, which were included as an additional regressor in subsequent general linear models to remove the outlier time-points. Functional data was registered to the high-resolution anatomical scans with FSL's FLIRT tool (12 DOF, non-linear registration 10mm warp resolution), then to standard Montreal Neurological Institute (MNI) space using FNIRT's nonlinear registration with a 10mm warp resolution.

Region of Interest (ROI) definition

Anatomical participant-specific hippocampal ROIs were defined using FSL's automatic segmentation tool, FIRST. For each participant, left and right hippocampal masks were generated, and in MATLAB, divided lengthwise into anterior and posterior halves.

Since the anatomical boundaries of the ventromedial prefrontal cortex (vmPFC) are not well defined, we adopted a vmPFC ROI previously identified in a paper examining changes in univariate activation over time, including a period of sleep (Takashima et al., 2006; coordinates: -2, 32, -10). In line with this prior study, these coordinates were used to create an 8mm-kernel sphere. Likewise to define an ROI for posterior medial cortex (PMC), we used a mask available from an online database (findlab.stanford.edu/functional_ROIs.html; Shirer et al., 2012). Additional cortical ROIs in perirhinal cortex and posterior parahippocampal cortex, which have both been implicated in memory and consolidation-related processes (Davachi, 2006; Ranganath and Ritchey, 2012; Vilberg and Davachi, 2013), were defined from a contrast of average brain activity for all word-image pairs versus baseline, a contrast agnostic to condition or category. These ROIs were generated using an 8mm-kernel sphere centered on the coordinate of peak activation identified within the known anatomical borders of these regions.

Immediate and Delayed Memory Tests

Immediately after participants were removed from the scanner, their memory for the word-image associations was probed by testing source memory, using the word as a cue. Participants also returned to the lab 24-hours later for a second, 'Delayed' memory test. Participants were asked to try to remember if they had seen the word before and if it had been paired with a scene or object image. The possible responses included: 'old - scene,' 'old - object,' 'word only,' or 'new.' The legend for these response options was presented under the cue-word on screen. Participants were instructed to use the 'scene' and 'object' responses only if they could remember the specific image with which the word had been paired, while the 'word only' key was to be used if they recognized the test word but could not remember the associated category of the image it had been paired with. The 'new' key was to be used if participants believed the word to be novel, or if they were unsure if it had been studied. Half of the words from each Encoding List (30 object-word, 30 scene-word) were included on each of the two tests (Immediate and Delayed) to avoid re-testing stimuli, along with 60 novel foil words. Each test trial consisted of a red fixation cross presented for 500ms, followed by a centrally presented word cue, which was on screen for a maximum of 12 seconds, or until a response was made, and was followed by a black fixation cross for 200ms.

Statistical Analyses

All reported statistical analyses are two-tailed. Repeated measures ANOVAs were performed and followed up with paired sample t-tests where applicable. Statistical tests were used to test the difference between correlations, including between two dependent correlations sharing a variable (Williams's test, e.g. functional connectivity correlations with fast spindle density) or between two dependent correlations with different variables (Steiger Test, e.g.

connectivity-similarity correlations for remembered vs forgotten pairs) with the r.test package. Tests of Bayes factor were computed on relevant analyses (referred to as BF in text) using the R package BayesFactor. Statistics were performed with R version 3.5.1, RStudio (RStudio, Inc version 0.99.903) and MATLAB (MathWorks) using both built-in and custom functions. $P < 0.05$ was considered significant for all statistical tests. Details for analyses are included in the subsections below.

Behavioral Analyses

Correct associative memory was defined as accurate source memory judgments ('remembered'), while misses, those previously studied pairs called 'new,' were counted as 'forgotten.' For all imaging analyses, memory was collapsed across the Immediate and Delayed tests to increase statistical power. A 3 x 2 x 2 repeated measures ANOVA was used to assess for differences amongst Encoding List (SL, ML, SS), Memory Test (Immediate, Delayed) and Category (Object, Scene).

Polysomnography

Each participant's night of sleep was scored by a sleep technician and checked over by a board-certified sleep physician, according to the American Academy of Sleep Medicine manual (Berry et al., 2012). The data was staged in 30-second epochs and categorized as Stage 1, 2, Slow Wave Sleep, or REM sleep. Together, the first three stages were defined as non-REM sleep.

To estimate spindle density across a night of sleep, a custom algorithm was implemented in MATLAB (The MathWorks, Inc). All data were first re-referenced to the left mastoid. Identification of spindles was computed on data from the Cz electrode from epochs identified as

either Stage 2 or Slow Wave Sleep. Evidence suggests there are two types of spindles, slow (<12 Hz) and fast (>12 Hz) spindles, differing both in their frequency range and cortical topography (Mölle et al., 2011; Rasch and Born, 2013). As fast spindles have more consistently been associated with hippocampal processing and memory (Schabus et al., 2007; Andrade et al., 2011; Mölle and Born, 2011; Bergmann et al., 2012; Rasch and Born, 2013; Staresina et al., 2015; Antony et al., 2018), analyses focused on fast spindles (12-16 Hz). To identify fast spindles, a morlet-wavelet filter centered on 14 Hz was applied to the data (a method similar to Wamsley et al., 2012). Spindles were identified if the wavelet power exceeded twice the averaged power over all S2/SWS epochs and its duration was between 0.4-3 seconds. For each participant, spindle density was calculated as the average number of spindles per 30-second epoch.

Functional connectivity analyses

We conducted psycho-physiological interaction (PPI) analyses, measuring how the correlation between activation in a 'seed' brain region and activation in other regions is modulated by a psychological parameter. In keeping with our interest in the dynamics of the long-axis of the hippocampus, both anatomically defined anterior and posterior hippocampal ROIs were used as "seed" regions.

Two different types of PPI analyses were performed: the first assessed functional connectivity for all trials on the Encoding Lists, and the second was broken down by subsequent memory status (remembered vs. forgotten). For the all-trial PPI GLMs, task regressors were created for each Encoding List and image category (object or scene), for a total of 6 regressors (SL- object, SL-scene, ML-object, ML-scene, etc.). The averaged hippocampal timecourse of activity was extracted from each participant's preprocessed functional data and modeled as a physiological regressor. Finally, four psychological regressors were modeled as the interaction

between the hippocampal timecourse of activity and the Sleep and Morning Lists with each category. Two additional contrasts were defined collapsing by category (object + scene analysis).

For the subsequent memory PPI GLMs, 24 regressors were included to model the four possible memory responses: Remembered (source correct), Source incorrect, Word only, or Forgotten (miss), broken down by Encoding List and the category of paired image, collapsed across memory test. As we were interested in comparing functional connectivity for trials with successful associative memory to those later forgotten trials, we specified our analysis to contrasts of remembered and forgotten pairs. Four psychological regressors were modeled as the interaction between the hippocampal timecourse of activity and the remembered or forgotten pairs from the Sleep and Morning Lists, respectively. Separate analyses were conducted for trials with each category of image (object vs. scene). Contrasts for each condition greater than baseline were then used in second-level analyses (across runs, within-participant), modeled using a fixed-effects analysis. From the relevant contrasts, average beta estimates were extracted from our ROIs to examine task-dependent changes in functional connectivity and correlations with overnight fast spindle density. Paired t-tests were used to assess the differences between the Sleep and Morning List functional connectivity values.

Inter-item pattern similarity analyses

For inter-item pattern similarity analyses, functional data was preprocessed as outlined above with a few exceptions: the data was smoothed using a 3mm FWHM kernel, and was kept in native space for each participant. After preprocessing, each run was aligned to the first run. For each run, trial-level general linear models (GLMs) were constructed by modeling one regressor per trial (60 regressors in total), with additional regressors for extreme head motion and temporal derivative (Mumford et al., 2014; Tompary and Davachi, 2017). Resulting t-stat maps

were imported into MATLAB, where t-stat activation maps were extracted for each ROI in native-space.

A linear vector containing the BOLD activation in each voxel in each ROI was extracted for each trial in the three Encoding Lists (Sleep List, Morning List, Single Study List), and the Pearson's correlation coefficient (*r*) was computed between each pairwise vector. This allowed assessment of the extent to which the activation patterns for each trial correlated with all other trials from the same list across scanning runs, thereby avoiding confounds from temporal autocorrelation (Mumford et al., 2014). The averaged trial-level correlations were then normalized using a Fisher r-to-z transformation and averaged, resulting in similarity estimates for each Encoding List (Figure 4a). Correlations were performed in two ways: first, with all other trials from the same Encoding List, irrespective of later memory outcome, and separately only amongst later remembered or forgotten trials from the same Encoding List ('subsequent memory'). Akin to earlier studies, this analysis allowed for the examination of representations amongst memories learned at the same time (LaRocque et al., 2013; Tompary and Davachi, 2017; Ezzyat et al., 2018). 3 x 2 repeated measures ANOVAs were used to assess for differences amongst Encoding List (SL, ML, SS) and Category (object, scene).

Mediation Analysis

To test the hypothesis that the extent of hippocampal-neocortical functional connectivity mediates the relationship between fast spindles and representational overlap in vmPFC, we employed a mediation analysis. Formally, this model tested if the influence of the measured fast spindle density during overnight sleep on the inter-item pattern similarity in vmPFC can be accounted for by the mediator variable, the functional connectivity between anterior hippocampus and vmPFC. For both brain measures we calculated the sleep-specific metric for

the object-word pairs that were later remembered. This effect was tested using the bootstrapping method (Shrout and Bolger, 2002), with 10000 iterations, using the mediation package in R.

Results

Behavioral results

We expected the twice-presented pairs from the Sleep and Morning Lists would be better remembered compared to the once-presented Single Study List pairs. On both the Immediate and Delayed memory tests, associative memory performance was significantly better for pairs from the Sleep and Morning Lists compared to the Single Study List, but memory did not significantly differ between the Sleep and Morning Lists (Encoding List main effect: $F(2,36)= 57.44$, $p< 0.0001$; Figure 2). For the Morning and Single Study Lists, scene-word pairs were better remembered than object-word pairs on both tests, but for the Sleep List, object- and scene-word source memory was equated on the immediate memory test (Figure 2).

Polysomnography: Sleep stage duration

On average, participants slept for a total of 463.13 minutes (SEM= 7.30), spending on average, 23.66 minutes in Stage 1 (SEM= 2.46), 233.5 minutes in Stage 2 (SEM= 7.78), 97.74 minutes (SEM= 5.97) in Slow Wave Sleep (SWS), and 108.24 minutes (SEM= 3.65) in REM sleep. Our main hypotheses focused on fast spindle density, as fast spindles (12-16 Hz) have more consistently been associated with hippocampal processing and memory (Mölle et al., 2011; Rasch and Born, 2013; Staresina et al., 2015; Antony et al., 2018). Fast spindle density was defined for each participant as the average number of spindles identified by a custom algorithm per 30-second epoch of Stage 2 and Slow Wave Sleep (see Methods). Average fast spindle density was 2.1 (SEM= 0.14).

406

407 **Functional Connectivity**

408 We hypothesized if spindles facilitate hippocampal-cortical interactions in the service of
409 memory consolidation, then spindle density during sleep would correlate with increased
410 functional connectivity measured the next day. We asked if fast sleep spindles modulate distinct
411 hippocampal-cortical networks based on the content of the restudied pairs (e.g. object-word or
412 scene-word). If fast spindles preferentially impact the networks in a manner that maintains
413 content specificity, we expect a dissociation to emerge when examining functional connectivity
414 in the anterior versus posterior hippocampal networks for the object- and scene-word pairs,
415 respectively (Davachi, 2006; Hoscheidt et al., 2010; Libby et al., 2012; Ranganath and Ritchey,
416 2012; Nadel et al., 2013; Robin and Moscovitch, 2017). In particular, anterior hippocampus has
417 greater connectivity with anterior medial temporal lobe regions including perirhinal cortex and
418 the ventromedial prefrontal cortex (vmPFC), while connectivity is greater between posterior
419 hippocampus and posterior parahippocampal cortex and posterior medial cortex (PMC) (Libby et
420 al., 2012; Ranganath and Ritchey, 2012; Eichenbaum, 2017). Thus, we examined functional
421 connectivity between anterior and posterior hippocampus and neocortical regions *separately* for
422 the object- and scene-word pairs (see Methods).

423

424 *Overnight fast spindle density and next day functional connectivity correlations: anterior*
425 *hippocampus*

426 First, we examined the relationship between overnight fast spindles and next-day anterior
427 hippocampal functional connectivity with targeted neocortical regions during the restudy of
428 paired associate trials from the Sleep and Morning Lists (see Methods). We examined right and
429 left hippocampal seed regions separately, since a 2(Encoding List) x 2(Hemisphere seed)

repeated measures ANOVA for anterior hippocampal-vmPFC functional connectivity resulted in a significant main effect of hemisphere ($F(1,18)= 6.51$, $p= 0.02$) such that functional connectivity was greater with right, compared to left, anterior hippocampus.

Overnight fast spindle density positively correlated with functional connectivity between right anterior hippocampus and vmPFC measured during the restudy of the Sleep List *object*-word pairs ($r= 0.68$, $p= 0.002$, $BF= 23.65$; Figure 3a). Thus, in line with our hypothesis, the greater the density of fast spindles during overnight sleep, the greater the functional connectivity between the anterior hippocampus and vmPFC when restudying the object-word pairs learned before sleep. Importantly, this relationship was *not* seen when restudying the more recently learned object-word pairs from the control Morning List ($r= -0.24$, $p= 0.32$, $BF= 0.71$; Figure 3a), which did not have an opportunity to undergo sleep-dependent consolidation. The spindle-connectivity correlations for the two Lists were significantly different from each other ($t= 2.94$, $p= 0.0097$), highlighting that the relationship between fast spindle density and Sleep List connectivity does not simply reflect trait-level differences across individuals. Without considering sleep measures, the Sleep and Morning List functional connectivity values did not significantly differ according to a repeated measures t-test ($t(18)= 0.07$, $p= 0.94$), suggesting that overnight spacing versus same day spacing did not strongly modulate overall connectivity between anterior hippocampus and vmPFC.

To further isolate the relationship between overnight fast spindle density and subsequent functional connectivity, and to normalize for individual differences, we calculated a "sleep-specific" functional connectivity measure by using the Morning List as a direct within-participant control, subtracting each participant's functional connectivity on the Morning List trials from the functional connectivity from the Sleep List trials. The Morning List provided a baseline that could better isolate sleep-specific effects evident for the Sleep List since all other

experimental variables (e.g. repeated study, overall performance, average functional connectivity, percept category) are matched for the Sleep and Morning Lists. Critically, we see a significant correlation between fast spindle density and this 'sleep-specific' right anterior hippocampal-vmPFC functional connectivity (r= 0.60, p= 0.007, Figure 3b). Therefore, even when taking into account the Morning List functional connectivity as a baseline, the density of overnight fast spindles is associated with enhanced network dynamics the next day, when restudying the pairs learned prior to sleep.

To address the content specificity, we ran the same analyses for the *scene*-word pairs. Fast spindle density did not significantly correlate with next day functional connectivity between right anterior hippocampus and vmPFC for either the Sleep or Morning List (SL: r= 0.19, p= 0.45, BF= 0.61; ML: r= 0.26, p= 0.28, BF= 0.63). However, within the Sleep List, the spindle-connectivity correlations for the object- and scene-word pairs were only marginally different (t= 1.79, p= 0.09). There were no significant correlations with fast spindle density when considering left anterior hippocampal functional connectivity with vmPFC for either object- or scene-word pairs (object-word pairs: SL: r= 0.09, p= 0.72, ML: r= -0.13, p= 0.59; scene-word pairs: SL: r= 0.04, p= 0.89, ML: r= 0.03, p= 0.9), perhaps consistent with the hemispheric difference reported above. Likewise, fast spindle density did not significantly correlate with functional connectivity between right anterior hippocampus and perirhinal cortex or PMC for either the Sleep or Morning List pairs of either category, though did correlate with anterior hippocampal-right parahippocampal functional connectivity (scene-word pairs SL: r= 0.46, p= 0.05, ML: r= -0.5, p= 0.03).

Overnight fast spindle density and next day functional connectivity correlations: posterior hippocampus

By contrast, when analyzing the network involving *posterior* hippocampus, a different pattern emerged, with the fast spindle-connectivity correlation significant only for the *scene*-word pairs, and not the object-word pairs. Specifically, right posterior hippocampal-PMC functional connectivity during restudy of the scene-word pairs from the Sleep List positively correlated with fast spindle density (r= 0.51, p= 0.02, BF= 4.83; Figure 3c). This relationship was not significant for the more recently learned Morning List pairs (r= 0.12, p= 0.62, BF= 0.45), and these correlations again were significantly different from each other (t= 2.52, p= 0.02; Figure 3c). Additionally, there was a significant positive correlation between spindle density and the sleep-specific right posterior hippocampal-PMC functional connectivity measure (r= 0.48, p= 0.04, Figure 3d). There was no overall difference between the Sleep and Morning List connectivity ($t(18)$= 0.33, p= 0.75).

Right posterior hippocampal-PMC functional connectivity during the object-word pair restudy did not significantly correlate with fast spindles for either List (SL: r= 0.1, p= 0.69, BF= 0.52; ML: r= -0.06, p= 0.82, BF= 0.49). A direct comparison of the Sleep List scene- and object-word pair spindle-connectivity correlations was marginally different (t= 1.85, p= 0.08). There was also not a significant correlation between left posterior hippocampal functional connectivity with PMC and fast spindle density for the Sleep List scene-word pairs (r= 0.36, p= 0.13), though the correlation was significant for the Morning List scene-word pairs (r= 0.51, p= 0.03) - however, these correlations were not significantly different (t= 0.89, p= 0.39), and the correlation with the sleep-specific measure was not significant (r= -0.26, p= 0.28). Functional connectivity between right posterior hippocampus and right parahippocampal cortex marginally correlated with fast spindle density for the Sleep List scene-word pairs (r= 0.45, p= 0.053), and not the Morning List (r= -0.04, p= 0.89), but connectivity between posterior hippocampus and vmPFC or perirhinal cortex did not significantly correlate with fast spindle density.

Together, these results demonstrate that functional connectivity in *both* the anterior and posterior hippocampal-neocortical networks is related to fast spindle activity during the prior night's sleep. Critically, the hippocampal networks sensitive to fast spindles seemed to differ based on the content of the memories: fast spindles correlated with increased right anterior hippocampal-vmPFC connectivity when restudying the Sleep List object-word pairs, but with the right posterior hippocampal-PMC functional network when restudying the Sleep List scene-word pairs. Importantly, these effects cannot be driven by the on-screen category presentation during the scanning session because the same effects were not evident for the Morning List object- or scene-word pairs. This suggests that the networks supporting sleep-consolidated information is at least somewhat sensitive to the visual category of the stimulus. As such, we proceeded to focus on the category relevant to each network of interest in subsequent analyses.

Inter-item Pattern Similarity

Thus far we have shown that fast spindle density during overnight sleep is associated with increased hippocampal-cortical network connectivity measured during the restudy of information learned prior to, but not after, the sleep period. We next wanted to test the hypothesis that sleep spindles play a role in the integrative, gist-extraction process, resulting in more overlapping neural representations for memories learned prior to sleep. We hypothesized that this consolidation-related gist extraction might be supported by the ventromedial prefrontal cortex (vmPFC), which has been implicated in the emergence and maintenance of schemas (Tse et al., 2007; van Kesteren et al., 2010, 2013b; Preston and Eichenbaum, 2013; Schlichting and Preston, 2015; Spalding et al., 2018), as well as the retrieval of remote, compared to recent, memories more broadly (Takashima et al., 2006, 2007, 2009; Gais et al., 2007; Sterpenich et al., 2007, 2009).

We employed a multivariate pattern analysis to measure neural similarity between representations of individual memories from the same Encoding List (e.g. Sleep List), by extracting the BOLD activity pattern for each word-image pair (each trial during the scan), and computing its similarity with all other trials encoded as part of the same List and within the same category (Figure 4a; see Methods). We refer to this as an *inter-item pattern similarity score*.

We first examined whether there were differences in inter-item pattern similarity between the Encoding Lists, separately for the object- and scene-word pairs. In vmPFC, there was no main effect of Encoding List ($F(2,36)= 0.996$, $p= 0.38$), or List x Category interaction effect ($F(2,36)= 0.41$, p= 0.67). However, we found significantly lower inter-item pattern similarity amongst object-word pairs compared to scene-word pairs (Category main effect: $F(1,18)= 5.67$, $p= 0.03$).

As with the functional connectivity analyses, we investigated whether fast spindle density during overnight sleep was related to the inter-item pattern similarity measured during the Sleep versus Morning List restudy trials. We found that, for object-word pairs, the sleep-specific inter-item pattern similarity in vmPFC significantly correlated with overnight fast spindle density ($r= 0.54$, $p= 0.02$, $BF= 4.44$; Figure 4b). In other words, greater fast spindle density was associated with greater pattern similarity, or representational overlap, amongst the Sleep List object-word pairs, relative to those studied in the morning. For scene-word pairs, this sleep-specific similarity-spindle relationship was not significant ($r= 0.11$, $p= 0.67$, $BF= 0.52$), though these two correlations were not significantly different from each other ($t= 1.32$, $p= 0.21$).

Next we examined inter-item pattern similarity in PMC. We again found that similarity was greater across scene-word compared to object-word pairs (Category main effect: $F(1,18)= 30.79$, $p< 0.0001$), as in vmPFC. There was not a significant main effect of List ($F(2,36)= 1.92$, $p= 0.16$), or interaction effect ($F(2,36)= 0.4$, $p= 0.67$). Based on the above functional

connectivity results involving PMC, we first examined the spindle-similarity relationship specifically for the scene-word pairs. However, this correlation was not significant (r= -0.05, p= 0.84, BF= 0.49; Figure 4b). Fast spindle density also did not correlate with sleep-specific inter-item pattern similarity in PMC for object-word pairs (r= 0.29, p= 0.23, BF= 0.84). A direct test showed a marginal difference between the spindle-similarity correlations for the vmPFC (object-word pairs) and PMC (scene-word pairs) (t= 1.95, p= 0.07). Thus, it seems that fast spindles may particularly affect representational similarity in vmPFC.

Additionally, we examined the inter-item pattern similarity in parahippocampal and perirhinal cortices, however we found no significant relationships between fast spindle density and sleep-specific pattern similarity in these regions. Overall, for both right and left perirhinal cortex, inter-item pattern similarity differed by category, with object-word pairs more similar than scene-word pairs (main effect of Category: right PrC $F(1,18)$= 8.19, p= 0.01); left PrC $F(1,18)$= 18.93, p= 0.0004), but was not modulated by Encoding List (right: $F(2,36)$= 1.85, p= 0.17, left: $F(2,36)$= 1.54, p= 0.23). In contrast, for right parahippocampal cortex inter-item pattern similarity was greater for scene-word pairs compared to object-word pairs (Category main effect: $F(1,18)$= 5.02, p= 0.04), and similarity differed by Encoding List for both right and left parahippocampal cortex (List main effect: right $F(2,36)$= 6.06, p= 0.005; left $F(2,36)$= 4.1, p= 0.025).

Relationship between functional connectivity and inter-item pattern similarity

Thus far, our results indicate that, when reactivating information originally learned before sleep, fast spindles during overnight sleep are correlated with (1) greater anterior hippocampal-vmPFC functional connectivity for object-word pairs, (2) greater posterior hippocampal-PMC functional connectivity for scene-word pairs, and (3) greater pattern similarity in vmPFC for

575 object pairs. As theories suggest that memory representations are reorganized via extended
576 hippocampal-cortical communication, we expected that our two neural measures showing effects
577 for the Sleep List, hippocampal-cortical functional connectivity and the degree of cortical
578 representational overlap, would be related. Since we were particularly interested in if this
579 relationship was dependent on later memory success, we conducted this analysis separately for
580 later remembered and forgotten pairs (see Methods).

581 For later remembered object-word pairs, the sleep-specific right anterior hippocampal-
582 vmPFC functional connectivity did indeed positively correlate with the inter-item pattern
583 similarity in vmPFC for those pairs (r= 0.62, p= 0.005, BF= 10.61; Figure 5). In other words, for
584 Sleep List memories, greater functional connectivity was associated with more overlapping
585 representations in vmPFC. This relationship was specific to trials that were later remembered;
586 there was not a significant correlation between the measures for forgotten pairs (r= -0.09, p=
587 0.70, BF= 0.51; Figure 5). The sleep-specific connectivity-similarity correlations for
588 remembered and forgotten pairs were significantly different from each other (z= 2.17, p= 0.03).

589 There was no such relationship, however, for the scene-word pairs (r= -0.35, p= 0.14),
590 consistent with the emerging pattern of results. Together, these results suggest that for object-
591 based memories given the opportunity for sleep-dependent consolidation, the extent of functional
592 connectivity between the anterior hippocampus and vmPFC is related to the overlap in the
593 representations in vmPFC for those memories.

594 We next examined if posterior hippocampal-PMC functional connectivity was related to
595 the representational structure in PMC. We focused the analysis on scene-word pairs, as fast
596 spindle density was related to the functional connectivity for this category. Unlike the results
597 reported above in vmPFC, there was no significant correlation between the sleep-specific
598 functional connectivity and inter-item pattern similarity in PMC (r= 0.09, p= 0.73). According to

599 a direct statistical test, the correlations for vmPFC and PMC were significantly different (z=
600 1.93, p= 0.05), suggesting a dissociation between these two midline neocortical brain regions.
601
602 *Mediation analysis: fast spindles, functional connectivity and representational overlap*
603 Together, the results presented so far suggest that the co-activation between the anterior
604 hippocampus and vmPFC may be related to the extent of local transformation of the Sleep List
605 object-word memory traces in vmPFC. Spindles are theorized to help facilitate the information
606 transfer through the hippocampal-cortical dialogue (Siapas and Wilson, 1998; Sirota et al., 2003;
607 Steriade, 2006; Rasch and Born, 2013; Staresina et al., 2015), and consolidation theories posit
608 that, through the cross-regional communication, the cortex builds up an integrated memory trace
609 (McClelland et al., 1995), which suggests that spindles' mechanism for affecting the
610 representational structure of cortical traces may be indirect, depending on the strength of the
611 functional connectivity.
612 Using a mediation analysis, we formally tested if the relationship between fast spindle
613 density and sleep-specific inter-item pattern similarity for remembered object-word pairs in
614 vmPFC is mediated by the sleep-specific anterior hippocampal-vmPFC functional connectivity
615 for those same trials (Figure 6, see Methods). Indeed, we found that the direct relationship
616 between overnight fast spindles and next-day vmPFC representational overlap was attenuated by
617 the addition of the anterior hippocampal-vmPFC functional connectivity in the model, resulting
618 in a significant mediation effect (ACME= 0.02, p= 0.039), as assessed using the bootstrapping
619 method (see Methods). This analysis suggests that fast spindles exert their effects on memory
620 representations in vmPFC *through* their direct modulation of anterior hippocampal-vmPFC
621 functional connectivity.
622

Discussion

The current study combined overnight polysomnography, next-day fMRI, and behavioral measures of associative memory, to probe if, and how, fast sleep spindles promote lasting changes in neural memory traces. We tested three specific questions, asking if fast spindles during overnight sleep after learning were associated with: (1) strengthening of hippocampal-cortical functional networks measured the next day, (2) changes in the representational structure of pre-sleep memories in cortical regions, and, critically, (3) the mechanistic relationship between these measures. Fast spindles during overnight sleep were related to the next-day neural representation of information learned prior to, and importantly, not after, the sleep period. In particular, fast spindle density positively correlated with functional connectivity in both anterior and posterior hippocampal-neocortical networks. Fast spindle density also correlated with greater overlap amongst the multivariate pattern representations in vmPFC for the object-word pairs learned before sleep. Further, the representational overlap in vmPFC positively correlated with the extent of anterior hippocampal-vmPFC functional connectivity for the successfully remembered object-word pairs, and a significant mediation analysis demonstrated that fast spindles promote this representational overlap vmPFC *through* the enhancement of anterior hippocampal-vmPFC functional connectivity. Taken together, these results provide new evidence suggesting that fast spindles during overnight sleep may alter the neural representation of memory traces, distributing memories between hippocampal-cortical networks, and through this cross-regional communication, promoting the restructuring of memory traces within the vmPFC.

Sleep spindles are proposed to play an important role in systems memory consolidation. Considerable evidence has linked spindles during post-learning sleep to post-sleep memory retention benefits (Gais et al., 2002; Schabus et al., 2004; Clemens et al., 2006; Mednick et al.,

2013; Rasch and Born, 2013; Hennies et al., 2016). Current theories suggest that spindles, through the temporal coupling with sharp wave ripples and slow oscillations, facilitate the coordination of reactivated memories from hippocampus to cortex (Siapas and Wilson, 1998; Sirota et al., 2003; Steriade, 2006; Clemens et al., 2007; Mölle and Born, 2011; Rasch and Born, 2013; Staresina et al., 2015). Evidence for this theory has generally come from studying brain dynamics during sleep; fast spindle activity has been linked with overall activation in hippocampus and cortex (Schabus et al., 2007; Bergmann et al., 2012), functional connectivity measures (Andrade et al., 2011), and with memory reactivation during sleep (Schönauer et al., 2017; Antony et al., 2018; Cairney et al., 2018). The current study adds to this growing body of work by showing that spindles not only have transitory effects on the brain *during* sleep, but also contribute to lasting neural changes associated with the distribution and representation of those memories. Our results show that post-learning fast spindle density is associated with next-day enhancements in hippocampal-cortical functional connectivity specifically for the memories learned prior to sleep, and through this functional pathway, spindles also promoted the local reorganization of multivariate representations in vmPFC.

As reported here, the representational organization in vmPFC, but not PMC, was sensitive to fast spindle density, despite significant spindle-connectivity relationships for these regions with anterior and posterior hippocampus, respectively. One possible explanation is that vmPFC representations are particularly reliant on sleep-dependent consolidation. Prior work suggests consolidation modulates retrieval-related activity in vmPFC (Takashima et al., 2006, 2007, 2009; Gais et al., 2007; Sterpenich et al., 2007, 2009; Sekeres et al., 2018b). Further, processes within vmPFC are thought to promote generalization across memories, including the emergence of schemas and integration of memories with overlapping content (van Kesteren et al., 2010, 2013a; Preston and Eichenbaum, 2013; Richards et al., 2014; Schlichting and Preston,

671 2015; Tompary and Davachi, 2017; Spalding et al., 2018), which aligns with theories suggesting
672 neocortical regions come to represent the gist, or central tendencies, of new memories with
673 consolidation (McClelland et al., 1995; Lewis and Durrant, 2011; Winocur and Moscovitch,
674 2011; Sekeres et al., 2018a). Here, fast spindle density during overnight sleep correlated with
675 greater multivariate pattern similarity in representations for object-word pairs learned prior to
676 sleep, which may be indicative of generalization across these memories. Thus, spindles may act
677 as a physiological mechanism, via functional networks, for the neural integration of vmPFC
678 traces. This may be adaptive for memory retention, as only later remembered pairs showed a
679 significant correlation between anterior hippocampal-vmPFC functional connectivity and
680 representational overlap. However, since our memory measure was a fairly general source
681 memory test, future work will be needed to directly test the behavioral consequences of such
682 neural representational changes.

683 In contrast to vmPFC, mnemonic representations in PMC may be more important in
684 maintaining episodic detail rather than representing similarities across memories. Prior work has
685 implicated PMC in episodic detail reinstatement during short- and long-term retrieval (Bird et
686 al., 2015; Chen et al., 2017), with little evidence for integration across memories sharing
687 overlapping content (Tompary and Davachi, 2017). It is thus possible fast spindles could
688 promote the stabilization of *individual* episodic memories in PMC, but future work will be
689 needed to address this specificity.

690 The results presented here suggest sleep-dependent consolidation effects may also be
691 localized to specific networks depending on the content of the pre-sleep learning experience. A
692 prior study measuring simultaneous EEG-fMRI during sleep reported that fast spindle amplitude
693 modulated univariate activation selectively in brain regions activated during the preceding
694 encoding task (Bergmann et al., 2012). Likewise, reports have shown experience-dependent

changes in post-encoding hippocampal functional connectivity with cortical regions previously engaged during encoding (Tambini et al., 2010; van Kesteren et al., 2010; Vilberg and Davachi, 2013; Schlichting and Preston, 2014; Murty et al., 2017; Collins and Dickerson, 2018). Here, in line with the proposed dichotomy in anterior and posterior hippocampal networks and functional specialization (Davachi, 2006; Libby et al., 2012; Ranganath and Ritchey, 2012; Poppenk et al., 2013; Robin and Moscovitch, 2017), we found that fast spindle density correlated with next-day anterior hippocampal-vmPFC and posterior hippocampal-PMC functional connectivity for the object-word and scene-word pairs, respectively. Importantly, these effects cannot be explained by perceptual differences between the categories alone; if the fast spindle-connectivity correlations only reflected processing of the stimuli on screen, the pairs learned in the morning should show the same results, yet no spindle-connectivity correlations were significant for the Morning List. This is particularly important as neural responses were measured during re-study, a design adopted to allow equal opportunity to reactivate all previously studied pairs without engaging in an explicit memory search, thus avoiding potential recency confounds when retrieving the Morning over the Sleep List. Further, differences in memory retention cannot explain the specificity of these effects on pre-sleep information, as associative memory performance for the Sleep and Morning Lists was matched on both memory tests.

There are some limitations to the current experiment that warrant further consideration. While our data suggest that sleep may promote more *similar* representational patterns in vmPFC, other research has shown that years-old autobiographical memories become more distinctive and amenable to decoding in vmPFC (Bonnici et al., 2012; Bonnici and Maguire, 2018). These results are not in direct conflict with our findings, as the extent of representational overlap or distinctiveness may depend on many factors, including the similarity in the content of the memories (Tompary and Davachi, 2017) and the learning schedule (Ezzyat et al., 2018). In

719 autobiographical memory studies the procedure of choosing personal memories that are
720 remembered years later could also result in the selection of memories that are distinctive, and
721 hence represented in different vmPFC networks. Future work should examine how the content
722 and context of memories alter their representational overlap in vmPFC, providing a deeper
723 understanding of how experiences are integrated or kept distinctive in this, and other, cortical
724 regions.

725 Additionally, the design of this experiment necessitated unequal encoding-restudy delay
726 periods for the Sleep and Morning Lists; to examine how spindles contribute to next-day
727 organization of memories in the scanner, we compared memories learned prior to the sleep
728 period with those that did not have an opportunity for sleep-dependent consolidation. While our
729 results suggest that fast spindles selectively influence representations for the content encountered
730 *prior* to sleep, more work is needed to directly compare the effects of an equal wake delay. For
731 example, the overall magnitude of functional connectivity did not generally differ between the
732 Sleep and Morning Lists, potentially due to consolidation-related enhancements that also
733 occurred during the wake delay. Thus, more work is necessary to tease apart the contributions
734 and mechanisms of sleep versus wake in consolidation.

735 For decades, research has linked memory improvements with sleep, with recent research
736 focused on understanding the physiological events during sleep, such as spindles, that contribute
737 to these effects. We conducted a multi-modal, multi-day study to ascertain if, and how, fast
738 spindles are related to neural markers of consolidation that endure *after* sleep. In demonstrating
739 that fast spindle-related reorganization involves lasting enhancements in hippocampal-cortical
740 functional network connectivity and the restructuring of vmPFC memory representations, this
741 report provides critical evidence that sleep does more than just stabilize memories, and indeed

supports enduring changes in the organization of memories both within, and across, regions of the brain that may support the retention of memories over time.

References

Alvarez P, Squire LR (1994) Memory consolidation and the medial temporal lobe: a simple network model. Proc Natl Acad Sci U S A 91:7041–7045 Available at: http://www.pnas.org/cgi/doi/10.1073/pnas.91.15.7041.

Andrade KC, Spoormaker VI, Dresler M, Wehrle R, Holsboer F, Samann PG, Czisch M (2011) Sleep Spindles and Hippocampal Functional Connectivity in Human NREM Sleep. J Neurosci 31:10331–10339 Available at: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.5660-10.2011.

Antony JW, Piloto L, Wang M, Pacheco P, Norman KA, Paller KA (2018) Sleep Spindle Refractoriness Segregates Periods of Memory Reactivation. Curr Biol 28:1736-1743.e4.

Baran B, Mantua J, Spencer RMC (2016) Age-related Changes in the Sleep-dependent Reorganization of Declarative Memories. J Cogn Neurosci 28:792–802 Available at: http://www.mitpressjournals.org/doi/10.1162/jocn_a_00938.

Bergmann TO, Mölle M, Diedrichs J, Born J, Siebner HR (2012) Sleep spindle-related reactivation of category-specific cortical regions after learning face-scene associations. Neuroimage 59:2733–2742 Available at: http://linkinghub.elsevier.com/retrieve/pii/S1053811911012006.

Berry RB, Budhiraja R, Gottlieb DJ, Gozal D, Iber C, Kapur VK, Marcus CL, Mehra R, Parthasarathy S, Quan SF, Redline S, Strohl KP, Ward SLD, Tangredi MM (2012) Rules for scoring respiratory events in sleep: Update of the 2007 AASM manual for the scoring of sleep and associated events. J Clin Sleep Med 8:597–619.

Bird CM, Keidel JL, Ing LP, Horner AJ, Burgess N (2015) Consolidation of Complex Events via Reinstatement in Posterior Cingulate Cortex. J Neurosci 35:14426–14434 Available at: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.1774-15.2015.

Bonnici HM, Chadwick MJ, Lutti A, Hassabis D, Weiskopf N, Maguire EA (2012) Detecting Representations of Recent and Remote Autobiographical Memories in vmPFC and Hippocampus. J Neurosci 32:16982–16991 Available at: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.2475-12.2012.

Bonnici HM, Maguire EA (2018) Two years later – Revisiting autobiographical memory representations in vmPFC and hippocampus. Neuropsychologia 110:159–169 Available at: http://linkinghub.elsevier.com/retrieve/pii/S002839321730180X.

Brady TF, Konkle T, Alvarez GA, Oliva A (2008) Visual long-term memory has a massive storage capacity for object details. Proc Natl Acad Sci 105:14325–14329 Available at: www.pnas.orgcgidoi10.1073pnas.0803390105.

Cairney SA, Guttesen A á V, El Marj N, Staresina BP (2018) Memory Consolidation Is Linked to Spindle-Mediated Information Processing during Sleep. Curr Biol 28:948-954.e4 Available at: https://linkinghub.elsevier.com/retrieve/pii/S0960982218301532.

Chen J, Leong YC, Honey CJ, Yong CH, Norman KA, Hasson U (2017) Shared memories reveal shared structure in neural activity across individuals. Nat Neurosci 20:115–125 Available at: http://www.nature.com/articles/nn.4450.

Clemens Z, Fabó D, Halász P (2005) Overnight verbal memory retention correlates with the number of sleep spindles. Neuroscience 132:529–535 Available at: http://linkinghub.elsevier.com/retrieve/pii/S0306452205000941.

Clemens Z, Fabó D, Halász P (2006) Twenty-four hours retention of visuospatial memory correlates with the number of parietal sleep spindles. Neurosci Lett 403:52–56.

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 130:2868–2878 Available at: https://academic.oup.com/brain/article-lookup/doi/10.1093/brain/awm146.

Clemens Z, Mölle M, Eross L, Jakus R, Rásonyi G, Halász P, Born J (2011) Fine-tuned coupling between human parahippocampal ripples and sleep spindles. Eur J Neurosci 33:511–520.

Collins JA, Dickerson BC (2018) Functional connectivity in category-selective brain networks after encoding predicts subsequent memory. Hippocampus:1–11 Available at: http://doi.wiley.com/10.1002/hipo.23003.

Davachi L (2006) Item, context and relational episodic encoding in humans. Curr Opin Neurobiol 16:693–700 Available at: http://linkinghub.elsevier.com/retrieve/pii/S0959438806001528.

Diekelmann S, Born J (2010) The memory function of sleep. Nat Rev Neurosci 11:114–126.

Eichenbaum H (2017) Prefrontal–hippocampal interactions in episodic memory. Nat Rev Neurosci 18:547–558 Available at: http://www.nature.com/doifinder/10.1038/nrn.2017.74.

Ezzyat Y, Inhoff M, Davachi L (2018) Differentiation of human medial prefrontal cortex activity underlies long-term resistance to forgetting in memory. J Neurosci 38:2290–17 Available at: http://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.2290-17.2018.

Gais S, Albouy G, Boly M, Dang-Vu TT, Darsaud A, Desseilles M, Rauchs G, Schabus M, Sterpenich V, Vandewalle G, Maquet P, Peigneux P (2007) Sleep transforms the cerebral trace of declarative memories. Proc Natl Acad Sci 104:18778–18783 Available at: http://www.pnas.org/cgi/doi/10.1073/pnas.0705454104.

Gais S, Mölle M, Helms K, Born J (2002) Learning-dependent increases in sleep spindle density. J Neurosci 22:6830–6834 Available at: http://www.ncbi.nlm.nih.gov/pubmed/12151563.

Hennies N, Lambon Ralph MA, Kempkes M, Cousins JN, Lewis PA (2016) Sleep Spindle Density Predicts the Effect of Prior Knowledge on Memory Consolidation. J Neurosci 36:3799–3810 Available at: http://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.3162-15.2016.

Hoscheidt SM, Nadel L, Payne J, Ryan L (2010) Hippocampal activation during retrieval of spatial context from episodic and semantic memory. Behav Brain Res 212:121–132 Available at: http://dx.doi.org/10.1016/j.bbr.2010.04.010.

Klinzing JG, Mölle M, Weber F, Supp G, Hipp JF, Engel AK, Born J (2016) Spindle activity phase-locked to sleep slow oscillations. Neuroimage 134:607–616.

LaRocque KF, Smith ME, Carr VA, Witthoft N, Grill-Spector K, Wagner AD (2013) Global Similarity and Pattern Separation in the Human Medial Temporal Lobe Predict Subsequent Memory. J Neurosci 33:5466–5474 Available at: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.4293-12.2013.

Lewis PA, Durrant SJ (2011) Overlapping memory replay during sleep builds cognitive schemata. Trends Cogn Sci 15:343–351 Available at: http://linkinghub.elsevier.com/retrieve/pii/S1364661311001094.

Libby LA, Ekstrom AD, Ragland JD, Ranganath C (2012) Differential connectivity of perirhinal and parahippocampal cortices within human hippocampal subregions revealed by high-resolution functional imaging. J Neurosci 32:6550–6560 Available at: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.3711-11.2012.

McClelland JL, McNaughton BL, O'Reilly RC (1995) Why There Are Complementary Learning Systems in the Hippocampus and Neo-cortex: Insights from the Successes and Failures of Connectionists Models of Learning and Memory. Psychol Rev 102:419–457.

Mednick SC, McDevitt EA, Walsh JK, Wamsley EJ, Paulus M, Kanady JC, Drummond SPA

(2013) The Critical Role of Sleep Spindles in Hippocampal-Dependent Memory: A Pharmacology Study. J Neurosci 33:4494–4504 Available at: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.3127-12.2013.

Mölle M, Bergmann TO, Marshall L, Born J (2011) Fast and Slow Spindles during the Sleep Slow Oscillation: Disparate Coalescence and Engagement in Memory Processing. Sleep 34:1411–1421 Available at: https://academic.oup.com/sleep/article-lookup/doi/10.5665/SLEEP.1290.

Mölle M, Born J (2011) Slow oscillations orchestrating fast oscillations and memory consolidation. In: Progress in Brain Research, pp 93–110 Available at: https://linkinghub.elsevier.com/retrieve/pii/B9780444538390000077.

Mölle M, Marshall L, Gais S, Born J (2002) Grouping of spindle activity during slow oscillations in human non-rapid eye movement sleep. J Neurosci 22:10941–10947.

Mumford JA, Davis T, Poldrack RA (2014) The impact of study design on pattern estimation for single-trial multivariate pattern analysis. Neuroimage 103:130–138 Available at: http://linkinghub.elsevier.com/retrieve/pii/S105381191400768X.

Murty VP, Tompary A, Adcock RA, Davachi L (2017) Selectivity in Postencoding Connectivity with High-Level Visual Cortex Is Associated with Reward-Motivated Memory. J Neurosci 37:537–545 Available at: http://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.4032-15.2016.

Nadel L, Hoscheidt S, Ryan LR (2013) Spatial Cognition and the Hippocampus: The Anterior–Posterior Axis. J Cogn Neurosci 25:22–28 Available at: http://www.mitpressjournals.org/doi/10.1162/jocn_a_00313.

Oliva A, Torralba A (2001) Modeling the Shape of the Scene: A Holistic Representation of the Spatial Envelope. Int J Comput Vis 42:145–175 Available at: http://link.springer.com/10.1023/A:1011139631724.

Poppenk J, Evensmoen HR, Moscovitch M, Nadel L (2013) Long-axis specialization of the human hippocampus. Trends Cogn Sci 17:230–240 Available at: http://www.ncbi.nlm.nih.gov/pubmed/23597720.

Preston AR, Eichenbaum H (2013) Interplay of Hippocampus and Prefrontal Cortex in Memory. Curr Biol 23:R764–R773 Available at: http://linkinghub.elsevier.com/retrieve/pii/S0960982213006362.

Ranganath C, Ritchey M (2012) Two cortical systems for memory-guided behaviour. Nat Rev Neurosci 13:713–726 Available at: http://www.nature.com/articles/nrn3338.

Rasch B, Born J (2013) About Sleep's Role in Memory. Physiol Rev 93:681–766 Available at: http://www.physiology.org/doi/10.1152/physrev.00032.2012.

Richards BA, Xia F, Santoro A, Husse J, Woodin MA, Josselyn SA, Frankland PW (2014) Patterns across multiple memories are identified over time. Nat Neurosci 17:981–986 Available at: http://www.nature.com/articles/nn.3736.

Robin J, Moscovitch M (2017) Details, gist and schema: hippocampal–neocortical interactions underlying recent and remote episodic and spatial memory. Curr Opin Behav Sci 17:114–123 Available at: http://linkinghub.elsevier.com/retrieve/pii/S2352154616302777.

Schabus M, Dang-Vu TT, Albouy G, Balteau E, Boly M, Carrier J, Darsaud A, Degueldre C, Desseilles M, Gais S, Phillips C, Rauchs G, Schnakers C, Sterpenich V, Vandewalle G, Luxen A, Maquet P (2007) Hemodynamic cerebral correlates of sleep spindles during human non-rapid eye movement sleep. Proc Natl Acad Sci 104:13164–13169 Available at: http://www.pnas.org/cgi/doi/10.1073/pnas.0703084104.

Schabus M, Gruber G, Parapatics S, Sauter C, Klösch G, Anderer P, Klimesch W, Saletu B,

907 Zeitlhofer J (2004) Sleep spindles and their significance for declarative memory
908 consolidation. Sleep 27:1479–1485 Available at:
909 http://www.ncbi.nlm.nih.gov/pubmed/15683137.
910 Schlichting ML, Preston AR (2014) Memory reactivation during rest supports upcoming learning
911 of related content. Proc Natl Acad Sci 111:15845–15850 Available at:
912 http://www.pnas.org/cgi/doi/10.1073/pnas.1404396111.
913 Schlichting ML, Preston AR (2015) Memory integration: neural mechanisms and implications
914 for behavior. Curr Opin Behav Sci 1:1–8 Available at:
915 https://linkinghub.elsevier.com/retrieve/pii/S2352154614000072.
916 Schönauer M, Alizadeh S, Jamalabadi H, Abraham A, Pawlizki A, Gais S (2017) Decoding
917 material-specific memory reprocessing during sleep in humans. Nat Commun 8:15404
918 Available at: http://www.nature.com/articles/ncomms15404.
919 Sekeres MJ, Winocur G, Moscovitch M (2018a) The hippocampus and related neocortical
920 structures in memory transformation. Neurosci Lett 680:39–53 Available at:
921 https://linkinghub.elsevier.com/retrieve/pii/S0304394018303331.
922 Sekeres MJ, Winocur G, Moscovitch M, Anderson JAE, Pishdadian S, Martin Wojtowicz J, St-
923 Laurent M, McAndrews MP, Grady CL (2018b) Changes in patterns of neural activity
924 underlie a time-dependent transformation of memory in rats and humans. Hippocampus
925 28:745–764.
926 Shirer WR, Ryali S, Rykhlevskaia E, Menon V, Greicius MD (2012) Decoding subject-driven
927 cognitive states with whole-brain connectivity patterns. Cereb Cortex 22:158–165.
928 Shrout PE, Bolger N (2002) Mediation in experimental and nonexperimental studies: new
929 procedures and recommendations. Psychol Methods 7:422–445 Available at:
930 http://www.ncbi.nlm.nih.gov/pubmed/12530702.
931 Siapas AG, Wilson MA (1998) Coordinated interactions between hippocampal ripples and
932 cortical spindles during slow-wave sleep. Neuron 21:1123–1128.
933 Sirota A, Csicsvari J, Buhl D, Buzsaki G (2003) Communication between neocortex and
934 hippocampus during sleep in rodents. Proc Natl Acad Sci 100:2065–2069 Available at:
935 http://www.pnas.org/cgi/doi/10.1073/pnas.0437938100.
936 Spalding KN, Schlichting ML, Zeithamova D, Preston AR, Tranel D, Duff MC, Warren DE
937 (2018) Ventromedial prefrontal cortex is necessary for normal associative inference and
938 memory integration. J Neurosci 38:2501–2517 Available at:
939 http://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.2501-17.2018.
940 Staresina BP, Bergmann TO, Bonnefond M, van der Meij R, Jensen O, Deuker L, Elger CE,
941 Axmacher N, Fell J (2015) Hierarchical nesting of slow oscillations, spindles and ripples in
942 the human hippocampus during sleep. Nat Neurosci 18:1679–1686 Available at:
943 http://www.nature.com/articles/nn.4119.
944 Stark CEL, Squire LR (2001) When zero is not zero: The problem of ambiguous baseline
945 conditions in fMRI. Proc Natl Acad Sci 98:12760–12766 Available at:
946 http://www.pnas.org/cgi/doi/10.1073/pnas.221462998.
947 Steriade M (2006) Grouping of brain rhythms in corticothalamic systems. Neuroscience
948 137:1087–1106 Available at:
949 http://linkinghub.elsevier.com/retrieve/pii/S030645220501153X.
950 Sterpenich V, Albouy G, Boly M, Vandewalle G, Darsaud A, Balteau E, Dang-Vu TT,
951 Desseilles M, D'Argembeau A, Gais S, Rauchs G, Schabus M, Degueldre C, Luxen A,
952 Collette F, Maquet P (2007) Sleep-related hippocampo-cortical interplay during emotional
953 memory recollection. PLoS Biol 5:2709–2722.

954 Sterpenich V, Albouy G, Darsaud A, Schmidt C, Vandewalle G, Dang-Vu TT, Desseilles M,
955 Phillips C, Degueldre C, Balteau E, Collette F, Luxen A, Maquet P (2009) Sleep Promotes
956 the Neural Reorganization of Remote Emotional Memory. J Neurosci 29:5143–5152
957 Available at: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.0561-09.2009.
958 Takashima A, Nieuwenhuis ILC, Jensen O, Talamini LM, Rijpkema M, Fernandez G (2009)
959 Shift from Hippocampal to Neocortical Centered Retrieval Network with Consolidation. J
960 Neurosci 29:10087–10093 Available at:
961 http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.0799-09.2009.
962 Takashima A, Nieuwenhuis ILC, Rijpkema M, Petersson KM, Jensen O, Fernández G (2007)
963 Memory trace stabilization leads to large-scale changes in the retrieval network: a
964 functional MRI study on associative memory. Learn Mem 14:472–479 Available at:
965 http://www.learnmem.org/cgi/doi/10.1101/lm.605607.
966 Takashima A, Petersson KM, Rutters F, Tendolkar I, Jensen O, Zwarts MJ, McNaughton BL,
967 Fernández G (2006) Declarative memory consolidation in humans: a prospective functional
968 magnetic resonance imaging study. Proc Natl Acad Sci U S A 103:756–761 Available at:
969 http://www.pnas.org/cgi/doi/10.1073/pnas.0507774103.
970 Tambini A, Ketz N, Davachi L (2010) Enhanced Brain Correlations during Rest Are Related to
971 Memory for Recent Experiences. Neuron 65:280–290.
972 Tompary A, Davachi L (2017) Consolidation Promotes the Emergence of Representational
973 Overlap in the Hippocampus and Medial Prefrontal Cortex. Neuron 96:228-241.e5
974 Available at: http://linkinghub.elsevier.com/retrieve/pii/S0896627317308401.
975 Tse D, Langston RF, Kakeyama M, Bethus I, Spooner P a, Wood ER, Witter MP, Morris RGM
976 (2007) Schemas and Memory Consolidation. Science (80-) 316:76–82 Available at:
977 http://www.ncbi.nlm.nih.gov/pubmed/17412951.
978 van Kesteren MTR, Beul SF, Takashima A, Henson RN, Ruiter DJ, Fernández G (2013a)
979 Differential roles for medial prefrontal and medial temporal cortices in schema-dependent
980 encoding: from congruent to incongruent. Neuropsychologia 51:2352–2359 Available at:
981 http://www.ncbi.nlm.nih.gov/pubmed/23770537.
982 van Kesteren MTR, Fernandez G, Norris DG, Hermans EJ (2010) Persistent schema-dependent
983 hippocampal-neocortical connectivity during memory encoding and postencoding rest in
984 humans. Proc Natl Acad Sci 107:7550–7555 Available at:
985 http://www.pnas.org/cgi/doi/10.1073/pnas.0914892107.
986 van Kesteren MTR, Rijpkema M, Ruiter DJ, Fernández G (2013b) Consolidation Differentially
987 Modulates Schema Effects on Memory for Items and Associations Martinez LM, ed. PLoS
988 One 8:e56155 Available at: http://dx.plos.org/10.1371/journal.pone.0056155.
989 Vilberg KL, Davachi L (2013) Perirhinal-Hippocampal Connectivity during Reactivation Is a
990 Marker for Object-Based Memory Consolidation. Neuron 79:1232–1242 Available at:
991 http://linkinghub.elsevier.com/retrieve/pii/S0896627313006132.
992 Wamsley EJ, Tucker MA, Shinn AK, Ono KE, McKinley SK, Ely A V., Goff DC, Stickgold R,
993 Manoach DS (2012) Reduced Sleep Spindles and Spindle Coherence in Schizophrenia:
994 Mechanisms of Impaired Memory Consolidation? Biol Psychiatry 71:154–161 Available at:
995 https://linkinghub.elsevier.com/retrieve/pii/S0006322311008146.
996 Wilson MA, McNaughton BL (1994) Reactivation of hippocampal ensemble memories during
997 sleep. Science (80-).
998 Winocur G, Moscovitch M (2011) Memory transformation and systems consolidation. J Int
999 Neuropsychol Soc 17:766–780 Available at:
1000 http://www.journals.cambridge.org/abstract_S1355617711000683.

Figure Legends

Figure 1. Study design
For all encoding and restudy sessions, participants were asked to form an association between a word and image. Participants first encoded the Sleep List (blue) before sleeping overnight while polysomnography was recorded. The next morning (Day 2), participants encoded a second set of word-image pairs (Morning List). After a short delay (~2 hrs), participants restudied these two sets of pairs, intermixed with novel pairs (Single Study List) in the fMRI scanner. Source memory was tested immediately after the scan and after a 24-hour delay (Day 3).

Figure 2. Correct associative memory on Immediate and Delayed Memory tests
Correct source memory was greater for the twice-studied Sleep and Morning List pairs than the once presented Single Study List pairs (Main effect of List $F(2,36)=57.44$, $P < 0.0001$, SL < SS $P < 0.0001$, ML < SS $P < 0.0001$), and better for the Immediate test than Delayed test (Main effect Test $F(1,18)= 136$, $P < 0.001$), consistent with forgetting over time. There was additionally a main effect of Category ($F(1,18)= 13.03$, $p= 0.002$), with scene-word pairs generally better remembered than object-word pairs. A significant List x Category x Test interaction ($F(2,36)= 4.42$, $p= 0.019$) seemed driven by a significant difference between accuracy on the object and scene pairs for all comparisons except the Sleep List during the Immediate Test ($p= 0.21$), including the Delayed test ($p= 0.036$, object< scene).

Figure 3. Hippocampal-neocortical functional connectivity correlates with fast spindle density for the Sleep List
a) Greater fast spindle density during overnight sleep positively correlated with increased right anterior hippocampal-vmPFC functional connectivity only during restudy of the Sleep List (blue), but not Morning List (red), object-word pairs.
b) In a control analysis, the sleep-specific functional connectivity (SL-ML) for object-word pairs still positively correlated with fast spindle density during overnight sleep.
c) Greater fast spindle density positively correlated with right posterior hippocampal-PMC functional connectivity only for the Sleep List (blue), and not Morning List (red), scene-word pairs.
d) Fast spindle density also positively correlated with the control, sleep-specific posterior hippocampal-PMC functional connectivity (SL-ML) for scene-word pairs.

Figure 4. Inter-item pattern similarity
a) To compute the inter-item pattern analysis, patterns of activation across voxels within ROIs were extracted for each trial and sorted by the Encoding List it was first learned during. All trials within a list were correlated, across runs, and averaged, resulting in a similarity metric within each list.
b) Fast spindle density correlated with inter-item pattern similarity in vmPFC for object pairs (left, $r= 0.54$, $p= 0.017$) but not for scene pairs in PMC (right, $r= -0.05$). For all plots, black dots represent individual participants and gray ribbon indicates 95% confidence intervals.

Figure 5. Relationship between right anterior hippocampal-vmPFC functional connectivity and inter-item pattern similarity in vmPFC
For remembered object-word pairs (left), sleep-specific functional connectivity positively

correlates with sleep-specific inter-item pattern similarity (r= 0.62, p= 0.005), such that greater connectivity for the Sleep List, relative to the Morning List, was related to greater similarity across these pairs in vmPFC. For object-word pairs that were subsequently forgotten (right), anterior hippocampal-vmPFC functional connectivity did not correlate with the representational similarity (r= -0.09). These correlations are significantly different from one another (z= 2.17, p= 0.03). For all plots, black dots represent individual participants and gray ribbon indicates 95% confidence intervals.

Figure 6. Schematic illustration of mediation analysis
A significant mediation effect demonstrated that fast spindle density during overnight sleep is related to greater right anterior hippocampal-vmPFC functional connectivity for the sleep-specific remembered object-word pairs, and through this relationship, is indirectly associated with greater representational overlap in vmPFC amongst these pairs.