Research Articles: Behavioral/Cognitive

Differentiation of human medial prefrontal cortex activity underlies long-term resistance to forgetting in memory

Youssef Ezzyat¹, Marika Inhoff² and Lila Davachi³

¹Department of Psychology, University of Pennsylvania, Philadelphia, Pennsylvania 19104
²Department of Psychology, University of California, Davis, California 95616
³Department of Psychology, Columbia University, New York, New York, 10027

DOI: 10.1523/JNEUROSCI.2290-17.2018

Received: 13 August 2017

Revised: 12 June 2018

Accepted: 12 June 2018

Published: 16 July 2018

Author contributions: Y.E., M.C.I., and L.D. designed research; Y.E. and M.C.I. performed research; Y.E. and M.C.I. analyzed data; Y.E. and L.D. wrote the paper; M.C.I. and L.D. edited the paper.

Conflict of Interest: The authors declare no competing financial interests.

This work was supported by DART Neuroscience and NIH R01 MH074692 to L.D.

Correspondence should be addressed to Contact information for the corresponding author: Lila Davachi, Ph.D., Department of Psychology, Columbia University, Schermerhorn Hall, 1190 Amsterdam Ave #406, New York, NY 10027, Email: ld24@columbia.edu, Phone: 212-854-1754

Cite as: J. Neurosci ; 10.1523/JNEUROSCI.2290-17.2018

Alerts: Sign up at www.jneurosci.org/cgi/alerts to receive customized email alerts when the fully formatted version of this article is published.
Research Article

Differentiation of human medial prefrontal cortex activity underlies long-term resistance to forgetting in memory

Youssef Ezzyat¹, Marika Inhoff² and Lila Davachi³

¹ Department of Psychology, University of Pennsylvania, Philadelphia, Pennsylvania 19104
² Department of Psychology, University of California, Davis, California 95616
³ Department of Psychology, Columbia University, New York, New York, 10027

Running title: MPFC pattern differentiation and memory retrieval

Contact information for the corresponding author:
Lila Davachi, Ph.D.
Department of Psychology
Columbia University
Schermerhorn Hall
1190 Amsterdam Ave #406
New York, NY 10027
Email: ld24@columbia.edu
Phone: 212-854-1754

This work was funded by DART Neuroscience and NIH R01 MH074692 to L.D.

Pages: 42
Tables: 0
Figures: 6

Keywords: medial prefrontal cortex, hippocampus, temporal context, pattern separation, episodic memory retrieval, fMRI, consolidation
Abstract

It is well known that distributing study events over time leads to better memory over long
timescales, compared to massing study events together. One explanation for such long-term re-
sistance to forgetting is that distributed study leads to neural differentiation in memory, which
supports retrieval of past experiences by disambiguating highly similar memory representations.

Neuroanatomical models of episodic memory retrieval propose that the hippocampus and medial
prefrontal cortex (MPFC) work together to enable retrieval of behaviorally appropriate memo-
ries. However, it is not known how representations in these regions jointly support resistance to
forgetting long after initial learning. Using fMRI, we measured differentiation in retrieved
memory representations following an extended delay in male and female human participants.

After one week, word-object associations were better remembered if studied across two days
(overnight), allowing associations to be learned in distinct temporal contexts, compared to learn-
ing within a single day (same day). MPFC retrieval patterns showed differentiation for overnight
relative to same day memories, while hippocampal patterns reflected associative retrieval suc-
cess. Overnight memory differentiation in MPFC was higher for associative than item memories
and higher than differentiation assessed over a brain-wide set of retrieval-active voxels. The
memory-related difference in MPFC pattern differentiation correlated with memory success for
overnight learning and with hippocampal—MPFC functional connectivity. These results show
that learning information across days leads to differentiated MPFC memory representations, re-
ducing forgetting after one week, and suggest this arises from persistent interactions between
MPFC and hippocampus.
Significance Statement

Neural activity in both the hippocampus and medial prefrontal cortex (MPFC) has been linked to memory-related representations, but prior work has not examined how these representations support episodic memory retrieval over extended timescales that are characteristic of everyday retrieval. We show that differentiation in MPFC activity one week after encoding is higher for retrieved information learned across two days compared to within a single day. In hippocampus, differentiation was greater for detailed memory retrieval but was not influenced by whether information had been learned over one or two days. Differentiation in MPFC predicted behavioral robustness to forgetting and was correlated with hippocampal—MPFC connectivity. The results suggest that context-based differentiation supports robust long-term memory via persistent MPFC—hippocampal interactions.
Introduction

Humans have the ability to retrieve episodic memories long after the initial experience, but not all memories maintain their fidelity over time. Behavioral and computational modeling work (Estes, 1955; Heusser et al., 2016; Howard & Kahana, 2002; Polyn et al., 2009a; Polyn et al., 2009b) suggests that context-based differentiation at encoding facilitates later memory retrieval. Memory in these studies is typically assessed following a relatively short delay, leaving open the question of how context-based differentiation at encoding might support retrieval over the longer timescales characteristic of everyday episodic retrieval.

Activity in both the hippocampus and medial prefrontal cortex (MPFC) during encoding has been shown to represent contextual information that supports memory, making each a candidate region for context representation following long delays (Kitamura et al., 2017). In rodents, ensemble activity in hippocampus becomes more differentiated over time in a way that predicts temporal memory for learned information (Mankin et al., 2012; Manns et al., 2007). Similarly, ensembles in MPFC show pattern differentiation with changes in context, although these representations appear to integrate a broader range of contextual changes (Hyman et al., 2012). In humans, blood-oxygen-level dependent (BOLD) fMRI encoding patterns in hippocampus (Du-Brow & Davachi, 2014; Ezzyat & Davachi, 2014; Hseih et al., 2014) and MPFC (Jenkins & Ranganath, 2016; Tompary and Davachi 2017) reflect the temporal context of learned information and predict temporal memory. Hippocampal activity also reflects contextual retrieval when memory is tested within one day of learning (Long et al., 2017; Ritchey et al., 2015).

Although activity in both the hippocampus and MPFC reflects memory-related context representations, less is known about how they support memory over delays that extend beyond one day. There is growing evidence that interactions between the hippocampus and MPFC are
critical to memory retrieval. These interactions occur during and following learning of new in-
formation (Guise & Shapiro, 2017; Schlichting & Preston 2016; Siapas et al., 2005; Tompary
and Davachi 2017), and are thought to reflect processes in which new hippocampal memory rep-
resentations are integrated into established MPFC cortical networks. This implies that the hippo-
campal and MPFC contextual representations that support memory should change over time,
with MPFC patterns assuming a more important role in supporting detailed memory retrieval
over time. While there is evidence that univariate MPFC fMRI activity is greater during retrieval
of temporally remote compared to recent memories (Gais et al., 2007; Nieuwenhuis & Ta-
kashima, 2011; Sterpenich et al., 2009; Takashima et al., 2006) and more differentiable for re-
mote compared to recent memories (Bonnici et al., 2012) there is little evidence that MPFC pat-
terns reflect contextual information for memories that are all equally remote.

Here, we ask how learning information across contexts leads to neural pattern differentia-
tion in MPFC and hippocampus that supports resistance to forgetting. Each participant was
trained to a high criterion (90%) on two separate lists of word-object associations. To manipulate
whether associations were learned in the same or distinct contexts, training sessions occurred ei-
ther across a 24-hour period (overnight) or within the same session (same day). One week later,
participants were scanned using fMRI during cued retrieval to compare the multivariate patterns
of retrieved memories that had been learned in the overnight and same day conditions. To fore-
shadow the core findings, we observed that learning across a 24-hour period led to more differ-
entiated long-term memory representations in MPFC, which correlated with the behavioral bene-
fit of overnight learning over same-day learning. Functional connectivity between MPFC and
hippocampus also correlated with the degree of MPFC differentiation, suggesting that context-
based differentiation supports memory through hippocampal-cortical interactions at least one week following learning.
Materials and Methods

Participants

Twenty-two healthy volunteers (15 female; mean age = 23.3, range = 19-29) from the New York University and New York City communities participated in this study for payment. All participants were native English speakers with normal or corrected-to-normal vision. Informed consent for this experiment was obtained in a manner approved by the University Committee on Activities Involving Human Subjects at New York University. Three participants were excluded from analysis due to mechanical issues with the fMRI scanner and all remaining 19 participants were included in behavioral analyses. For the fMRI analyses, two additional participants were excluded due to having fewer than 5 responses in at least one memory condition.

Stimuli

The words used in the experiment were taken from a set of 506 adjectives downloaded from the MRC psycholinguistics database (http://websites.psychology.uwa.edu.au/school/MRCDatabase/uwa_mrc.htm). For each participant, 72 words were randomly chosen from the pool to be used in the overnight, same day and single session study conditions; an additional 72 words were randomly chosen for each participant to be used as new lures for the Day 7 retrieval test. Within each of the overnight, same day and single session study lists, half of the words were paired with pictures of natural objects (e.g. animals, plants) and half were paired with pictures of manmade objects (e.g. tools, kitchen gadgets). All object stimuli were taken from an Internet image search. Our randomization procedure ensured that the assignment of words and images to each other and to the overnight/same day/single session conditions was equally likely for all stimuli.
Experimental Design and Statistical Analysis

General Procedure

On Day 1 participants came into the lab and were trained to criterion (90% correct) on a set of word-object pairings (overnight pairs). Twenty-four hours later (Day 2) participants returned to the lab and were trained to criterion on a novel set of word-object pairings (same day pairs). Following this training on the same day pairs, participants were re-trained to criterion (90% criterion, see Behavioral Procedures for details on training) on both sets from Day 1 and Day 2 (Figure 1A). Thus, participants were trained to criterion twice on both lists; for the overnight list this occurred across two days, for the same day list this occurred within the same day.

One week later (Day 7) participants came into the lab and were trained to criterion on a final novel set of word-object pairings (single session pairs). Immediately following this final training session, participants were brought to the fMRI scanner and were scanned while performing a retrieval test for all the word-object pairings learned across all three days (~15 minute delay between the final study session in the lab and the start of the scanned retrieval test). The results of this retrieval test were analyzed to look for behavioral evidence of differences in memory performance on the basis of study condition (overnight/same day/single session), as well as neural evidence for differentiation in memory representations on the basis of study condition when controlling for memory.

Behavioral Procedures

On Day 1 participants came into the lab and were trained to learn 72 word-object associations (overnight pairs). Training began with a study phase in which participants were presented
with all of the word-object pairs and asked to memorize the associations (Figure 1B, left panel; no behavioral response was required). Each study phase trial began with 500 ms of fixation, followed by presentation of the word-object pair for 4500 ms. After the study phase, participants began the training phase in which the words were presented alone and participants had to indicate whether the word had been paired with a ‘natural’ or ‘manmade’ object during study, or whether they were unsure (Figure 1B, middle panel). Each training trial began with 500 ms of fixation followed by the presentation of a word for a maximum of 12 s. Once a participant made a natural/manmade choice, feedback was given and the object stimulus was presented alongside the word for 8.5 s. Training was broken into three blocks of 24 trials based on pilot data suggesting faster learning for several smaller blocks compared to a single large block. Once all 24 trials were tested, training began again. If a participant correctly answered the natural/manmade decision on two consecutive training rounds, the pair was dropped from future training rounds. Participants continued with training until they reached 90% of the 24 pairs correct. The training procedure was then repeated for the second and third blocks of 24 pairs. After reaching the 90% criterion on the third and final block, participants performed a final test on all 72 pairs together and were required to reach 90% correct. This was done to ensure that participants still showed robust learning for pairs that had been learned in the first and second blocks. Three participants that performed slightly below the 90% criterion (minimum = 86%) on this final test were re-exposed to the full set of 72 pairs one final time. Participants were then dismissed until the following day.

Participants returned 24 hours later on Day 2 and were trained to 90% criterion on a novel set of 72 word-object pairs (same day pairs) using a training paradigm that was identically structured to the training on Day 1. After performing the final test on this new set of pairs, participants performed another training session that included all 72 pairs from Day 1 intermixed with
all 72 pairs from Day 2 (Figure 1A) in a single block. Thus, participants were re-trained to 90% criterion on both the overnight and same day lists; the critical difference is that overnight pairs were first trained on the previous day while the same day pairs were first trained earlier in that same session. All participants met or exceeded 90% correct for both overnight and same day lists, after which they were dismissed until the following week.

One week after the Day 1 session, participants returned to the lab for the Day 7 session. They began this session with another learning phase on a novel set of 72 word-object pairs (single session pairs). After reaching criterion on this set, participants were taken to the fMRI scanner for the final testing session. In the scanner, participants were presented with all of the studied words (72 each of overnight, same day, and single session) as well as 72 novel words (Figure 1B, right panel) in a randomly ordered sequence. On each trial, participants were presented with a word and were asked to indicate which type of object the word had been paired with during the learning phases (Old-Natural; Old-Manmade; Old-Unsure; New). Participants were given a maximum of 6 s to make their response. Following a response, participants performed an odd/even judgment on a series of numbers for the remainder of the trial, up to a total trial length of 24 s.

**Behavioral Analysis**

Analysis of the behavioral data focused on the Day 7 retrieval test and was designed to identify memory differences relating to study list condition (overnight/same day/single session). Trials for which participants responded correctly to the Natural/Manmade decision (e.g. selecting Old-Natural when the word was old and had been paired with a ‘natural’ object) were coded as associative; trials for which participants responded incorrectly to the Natural/Manmade decision (e.g. selecting Old-Natural when the word was old but had been paired with a ‘manmade’
object) or responded Old-Unsure were coded as item-only. Trials for which participants responded New when the word was ‘old’ were coded as forgotten. Correctly identified new words were coded as correct rejections (CR), and new words that were given any Old response were coded as false alarms (FA). Overall memory performance was defined as the difference between the hit rate (any old response to an old trial) minus the false alarm rate. We defined the overnight learning benefit as (overnight associative) – (same day associative) proportion correct.

**FMRI Procedures**

As described in the Behavioral Procedures, on Day 7 participants first completed the learning phase for the single session stimuli in the lab. After completion, participants were brought to the scanner and performed a scanned retrieval test that included all of the learned words (overnight, same day, and single session) as well as novel words (response options: Old-Natural; Old-Manmade; Old-Unsure; New). Participants performed a total of 288 retrieval trials split evenly into 12 scanning runs.

**FMRI Data Acquisition**

Functional imaging was performed using a Siemens Allegra 3T head-only scanner with a custom head coil (NM-011; Nova Medical) located at the Center for Brain Imaging at New York University. Functional data were collected using an echo-planar (EPI) pulse sequence (34 contiguous slices; TR = 2000 ms; 3 mm isotropic voxels; TE = 15 ms; flip angle = 82°; field of view = 240 x 192 mm; slice gap = 0%) with slices oriented parallel to the AC-PC axis. Slices were positioned ventrally to provide full coverage of the anterior temporal lobes and prefrontal cortex; this resulted in omission of parts of the superior parietal cortex and, occasionally, parts of motor cortex.
A high-resolution T1-weighted anatomical scan (magnetization-prepared rapid-acquisition gradient echo sequence, 1 x 1 x 1 mm) was also obtained for each subject following the final block of the localizer task.

**Preprocessing of fMRI Data**

Images were preprocessed using SPM8 software (Wellcome Trust Centre for Neuroimaging, London UK). Functional images were realigned to the within-run mean to correct for head motion (one run from one participant was discarded due to head motion > 1 voxel). Realigned images were corrected for slice acquisition time and were then coregistered to the anatomical image to correct for between-run motion. For group-level analyses, the coregistered images were first spatially normalized to an EPI template in Montreal Neurological Institute space, resliced to 2 x 2 x 2 mm voxels and finally smoothed with a 6 mm FWHM isotropic Gaussian kernel. Low frequencies (< 1.5 cycles/run) were removed from the functional data in both the subject-specific and group analyses.

**ROI Definition**

Anatomical hippocampal ROIs were drawn manually on each participant’s T1-weighted anatomical scan using an in-house drawing tool written in Matlab (Mathworks, Sherborn MA) and according to standard anatomical convention (Insausti et al., 1998). For the analysis comparing pattern differentiation in anterior and posterior hippocampus, we split the hippocampus into thirds based on coronal slice position, taking the anterior and posterior thirds as the ROIs. We also defined functional ROIs based on fMRI activation during the retrieval task. We first created an across-participant random effects general linear model (GLM) that included eight conditions...
of interest (source correct/item-only for each of the overnight/same day/single session lists; and

correct rejections/false alarms) that modeled activation for each trial as a 2-TR boxcar convolved

with a canonical hemodynamic response function (HRF). Trials without a response and forgotten

trials were each separately modeled as confounds, as were estimates of across-run participant

motion. The resulting beta estimates were then used to define MPFC; specifically, a contrast of

single session associative and item-only trials was conducted against baseline at a voxel-wise

threshold of \( p < 0.001 \) to functionally identify the region [MNI coordinates: (0, 56, 18)]. Based

on a comparison of our region to available MNI coordinates of prior work, the center of our

MPFC region is more anterior and/or dorsal to some ROIs that have been reported previously

(e.g. Bonnici et al., 2012; Gais et al., 2007; Takashima et al., 2007), but is partially overlapping

with others (e.g. Tompary & Davachi, 2017). Importantly, we used only single session trials to

define the MPFC region in order to avoid biasing our main pattern similarity analyses, which

were limited to comparisons between the overnight and same day conditions.

We also defined, individually for each subject, a mask of the 5000 voxels showing the

strongest responses (positive or negative) in a contrast of Task > Baseline. We defined these

brain-wide task active masks as a proxy for voxels that might be expected to show reinstatement

of contextual information during retrieval (Howard et al., 2015).

Pattern Analysis of fMRI Data

Analysis of multivariate patterns evoked during memory retrieval was conducted on

functional data from the retrieval runs. In brief, estimates of activation on each trial were com-

puted at every voxel in the brain. These single-trial estimates were then extracted as spatially-

distributed patterns across relevant ROIs. To compute single-trial estimates of activation, each of
the 24 trials in a run was modeled as a separate condition in a general linear model (GLM). Trials were modeled as two-TR boxcars beginning at trial onset convolved with a canonical hemodynamic response function (HRF). Mean intensity across the run, linear drift and estimates of subject motion were modeled as nuisance regressors. The procedure was repeated within each of the 12 runs of the retrieval task, resulting in a GLM estimated for each run and a parameter estimate for each trial in each run in every voxel in the brain.

Patterns of parameter estimates were extracted from all voxels within an ROI and individual trial patterns were separated according to study condition (overnight/same day/single session) and memory (associative/item-only). We then measured pattern differentiation as follows: we computed the Pearson correlation between the pattern for each trial and all other trials in the same condition [i.e. a correlation value was computed between each overnight-associative trial and all other overnight-associative trials, (LaRocque et al., 2013)]. We then applied Fisher's r-to-z transformation to the between-trial similarities, converted the measures to differentiation by taking 1-z and averaged within participant to generate a global measure of differentiation across trials of the same condition. We then used ANOVAs and t-tests across participants to measure effects of learning condition and memory performance on neural differentiation.

**Beta-Series Connectivity**

To measure connectivity between hippocampus and MPFC we used the beta series method (Rissman, Gazzaley, & D’Esposito, 2004; Vilberg & Davachi, 2013). Within each subject, we used the same output of the single-trial GLM model that we used to compute pattern differentiation (see Pattern Analysis of fMRI Data above). These single-trial beta estimates were sorted by condition (overnight and same day) and averaged across voxels within the hippocampal and
MPFC ROIs. This yielded a single beta estimate for each region for each retrieval trial of the experiment. We then computed the Pearson correlation between the beta estimates across retrieval trials (the beta-series) from hippocampus and MPFC, and did so separately within the overnight and same day conditions. To assess the relationship between connectivity and pattern differentiation, we then correlated the difference in beta-series connectivity between the overnight and same day conditions with the difference in pattern similarity for the two conditions.
Results

Memory Performance

Participants performed well above chance on the Day 7 retrieval test. Collapsing across study condition (overnight/same day/single session) and memory status (associative/item only) status, the difference between the hit and false alarm rates for judging words as Old/New was significantly greater than zero \( [M = 0.60 \pm 0.04; t(18) = 14.8; p < 0.001] \). Participants also showed high levels of memory for the word-object associations. We compared the proportion of associative trials (collapsing across study list) to an expected mean of 0.5 (i.e. an equal number of associative and item-only trials), and found significantly better associative memory than expected by chance \([\text{associative } M = 0.74 \pm 0.03, \text{item-only } M = 0.22 \pm 0.03; t(18) = 9.0; p < 0.001; \text{Figure 2A}]\).

To determine whether associative memory differed by study list, we conducted a one-way ANOVA with study condition (overnight/same day/single session) as a within-participant factor, and observed a significant main effect \([F(2,36) = 55.0; p < 0.001]\). In addition to our primary conditions of interest (overnight/same day), participants were also trained to criterion on a novel set of word-object associations just prior to the Day 7 retrieval test (single session condition; see Experimental Procedures). Planned comparisons revealed that the proportion of associative memory in the single session condition \( (M = 0.93 \pm 0.02) \) was significantly greater than in both the overnight \( [M = 0.72 \pm 0.05; t(18) = 5.35, p < 0.001] \) and same day conditions \( [M = 0.57 \pm 0.03; t(18) = 10.9; p < 0.001] \). This effect was expected given that participants had just learned the single session pairs to criterion prior to the retrieval test, while they had last seen the overnight and same day pairs the week before. Importantly, however, associative memory was also significantly higher in the overnight condition than in the same day condition \( [t(18) = 4.9; p < \text{...}] \).
demonstrating that distributing learning across two days, relative to study within a single day, benefitted associative memory retrieval even after one week. Thus, we defined the **overnight learning benefit** as the difference in associative memory between the **overnight** and **same day** conditions, and use this measure in later analyses of the fMRI data. Finally, we also examined whether the different study conditions resulted in different levels of forgetting (i.e. **Old** trials given a **New** response). A one-way ANOVA with study list as a within-participant factor showed a significant main effect \[F(2,36) = 10.9; \ p < 0.001\] that was driven by significantly more forgetting of the **same day** list \[M = 0.07 \pm 0.02\] compared to the **overnight** \[M = 0.02 \pm 0.01; \ t(18) = 3.58, p = 0.002\] and **single session** \[M = 0.02 \pm 0.01; \ t(18) = 3.33, p = 0.004\] lists.

We also analyzed response times to determine whether they varied according to memory and study condition. A comparison of response times for **associative** and **item-only** memories, collapsed across study condition, showed that participants were significantly faster to make **associative** responses than **item-only** responses \[**associative** M = 2.60 \pm 0.08 \text{ s}, \ **item-only** M = 4.08 \pm 0.23 \text{ s}; \ t(18) = 7.17, p < 0.001, \text{Figure 2B}\]. Because six participants did not have any **single session** **item-only** trials, we focused our analysis on comparing **associative** response times across study conditions using a one-way ANOVA. The main effect of study condition was significant \[F(2,36) = 59.1, p < 0.001, \text{Figure 2B}\] and planned comparisons showed that **single session** **associative** response times \(M = 1.99 \text{ s} \pm 0.08 \text{ s}\) were significantly faster than both **overnight** \(M = 2.63 \text{ s} \pm 0.10 \text{ s}; \ t(18) = 7.09, p < 0.001\) and **same day** \(M = 3.18 \text{ s} \pm 0.12 \text{ s}; \ t(18) = 8.67, p < 0.001\) response times. Finally, **overnight** response times were also significantly faster than **same day** response times \(t(18) = 5.80, p < 0.001\). Taken together, object-word pairs learned across two days, relative to those learned on a single day, were better and more quickly recalled after
one week, consistent with the notion that distributing learning across contexts benefited the access to and accuracy of those memory representations.

Finally, although we observed the previously described differences between the overnight and same day conditions on Day 7, these were not due to differences between conditions during training. As described in the Methods, both conditions were trained to 90% criterion. In addition, the conditions did not differ in the number of training rounds required to reach criterion (mean overnight = 3.3, mean same day = 3.2, p = 0.60 by Wilcoxon signed rank test) and in the number of exposures to each item during the training phases on Days 1 and 2 (mean overnight = 7.6, mean same day = 7.5, p = 0.10 by rank sum test). We also found that the difference in number of presentations did not predict the difference in Day 7 hit rates [r(17) = -0.11, p = 0.66, Spearman rank correlation] nor source correct memory [r(17) = -0.07, p = 0.76, Spearman rank correlation].

**Pattern Differentiation in Hippocampus**

In order to measure whether memories are represented in a distinct manner that benefits retrieval, we computed the differentiation in hippocampal multivariate BOLD activity patterns evoked during retrieval of each memory with patterns evoked during retrieval of all the other memories from that learning session. We found that, in left hippocampus, pattern differentiation was significantly higher for associative compared to item-only memories [F(1,16) = 11.3, p = 0.004], but did not vary by overnight vs. same day learning [F(1,16) = 2.60, p = 0.12], with no interaction between factors (p = 0.58, Figure 3A). Planned comparisons confirmed more within-condition hippocampal pattern differentiation for associative trials compared to item-only trials in both the overnight [associative z = 0.90 ± 0.01, item-only z = 0.86 ± 0.03; t(16) = 2.17, p = 0.04] and same day [associative z = 0.88 ± 0.02, item-only z = 0.85 ± 0.02; t(16) = 2.36, p =
0.03] conditions. We also conducted our pattern differentiation analyses separately for voxels in the anterior and posterior thirds of the left hippocampus, defined by coronal slice position along the anterior/posterior axis. Using a 3-way ANOVA (anterior/posterior X associative/item-only X overnight/same day), we found that pattern differentiation in left hippocampus was greater for anterior compared to posterior ROIs [main effect: F(1,16) = 7.05, p = 0.02, Figure 3B], greater for associative compared to item-only trials [F(1,16) = 7.27, p = 0.02], and greater for overnight compared to same day trials [F(1,16) = 9.03, p = 0.008]. There was a trend for a 3-way interaction (p = 0.10), but all other interactions with anterior/posterior ROI were not significant (p > 0.41).

We also examined right hippocampus, which showed higher differentiation for associative compared to item memories [F(1,16) = 14.4, p = 0.002] and a trend for greater differentiation with overnight study [F(1,16) = 4.09, p = 0.06] with a significant difference observed in both the overnight [associative z = 0.89 ± 0.02, item-only z = 0.83 ± 0.03; t(16) = 2.48, p = 0.02] and same day conditions [associative z = 0.87 ± 0.02, item-only z = 0.82 ± 0.02; t(16) = 4.12, p < 0.001]. There was no interaction between study list and memory (p = 0.76). These results provide evidence for hippocampal pattern separation during a one-week memory test that is related to successful associative memory retrieval. When comparing anterior/posterior ROIs in right hippocampus, we also found a main effect such that pattern differentiation was greater in anterior compared to posterior right hippocampus [F(1,16) = 9.81, p = 0.006, Figure 3B] and greater for associative compared to item-only trials [F(1,16) = 13.0, p = 0.002]. There were no other main effects or interactions (all p > 0.12).

To determine whether the level of pattern differentiation in hippocampal memory representations was related to memory performance, we correlated overnight > same day associative
memory differentiation with the *overnight learning benefit*. We also conducted the same analysis between *overnight > same day item-only* memory differentiation and the *overnight > same day item-only* behavioral memory difference. In both left and right hippocampus, neither the *associative* correlation [left: $r(15) = 0.31, p = 0.23$, Figure 3A; right: $r(15) = 0.30, p = 0.24$] nor the *item-only* correlation [left: $r(15) = -0.28, p = 0.27$; right: $r(15) = 0.32, p = 0.21$] was significant.

In sum, pattern separation in hippocampus was higher for retrieved memories that contained associated source information compared to memories containing only item information, irrespective of whether information was learned the week before across 24 hours or within the same session. We observed greater differentiation in anterior compared to posterior hippocampus, which highlights a dissociation along the long-axis of the hippocampus (also see, Tompary & Davachi, 2017), but is not fully consistent with models that predict more separated representations in posterior compared to anterior subregions (Poppenck et al., 2013). In contrast we do think the current findings are consistent with a role for anterior hippocampus in a distributed network that represents specific item details in episodic memory (Ranganath & Ritchey, 2012).

### Pattern Differentiation in MPFC

As in left hippocampus, there was significantly more differentiation in MPFC for *associative* memories compared to *item-only* memories [$F(1,16) = 9.45, p = 0.007$]. Interestingly, however, unlike the results from hippocampus, there was a significant interaction between study list (*overnight/same day*) and memory [$F(1,16) = 7.39, p = 0.02$] that was driven by a difference in *associative/item-only* memory specific to the *overnight* list [$associative z = 0.92 \pm 0.02, item-only z = 0.84 \pm 0.03; t(16) = 3.18, p = 0.006$], with no difference in *associative/item-only* memory for the *same day* list ($p = 0.16$; Figure 4A). A direct comparison of *associative* trials in
the overnight and same day conditions showed that overnight differentiation was higher than
same day differentiation \[z = 0.88 \pm 0.02; t(16) = 3.70, p = 0.002\].

We next investigated whether MPFC differentiation was related to the behavioral en-
hancement seen for overnight associative memory relative to same day associative memory. We
found that overnight > same day pattern differentiation in MPFC for associative memory trials
was significantly correlated with the overnight learning benefit \[r(15) = 0.53, p = 0.03\], Figure 4A. Thus, greater differentiation in evoked MPFC neural patterns for the overnight condition
relative to the same day condition was associated with increases in overnight associative
memory, relative to same day associative memory (this correlation did not differ from the hippo-
campal correlation \( p = 0.18 \)). There was no correlation between overnight > same day differenti-
ation for item-only memories and overnight > same day item-only memory success \[r(15) = 0.38, \( p = 0.13 \)], but this did not differ from the associative correlation \( p = 0.30 \). These data show that,
relative to same day memories, greater pattern differentiation in MPFC patterns across overnight
associative memories predicts a larger overnight learning benefit. More broadly, these data are
consistent with the idea that differentiation in measured BOLD retrieval patterns in MPFC sup-
ports enhancements in associative memory derived from studying information across an over-
night delay.

One explanation for the differentiation of retrieval patterns in MPFC is simply that over-
night memories are tagged during encoding with information from two contexts, which is then
reinstated at retrieval. If this were the case, then one prediction that would follow is that over-
night memories should be more likely to cluster into two groups than same day memories. To
test this we applied \( k \)-means clustering to segment the overnight and same day patterns for each
subject into two clusters. We then assessed whether the between-cluster distance for the over-
night patterns was larger than for the same day patterns, as would be the case if there was greater evidence for two distinct clusters in the overnight condition. In MPFC we did not find greater evidence for two clusters in the overnight compared to same day conditions [mean within vs. between cluster correlation: overnight = 0.22, same day = 0.21, t(16) = 1.21, p = 0.24] (there was also no difference in the left hippocampus [overnight = 0.17, same day = 0.19, t(16) = -1.02, p = 0.32]).

To confirm that this null finding was not the result of lack of power to discriminate conditions of interest, we repeated the analysis and used it to cluster associative and item-only patterns into groups, as a way to classify the two trial types. We assessed classification for each subject using area under the curve (AUC) and then compared the AUC distribution across subjects to chance (0.50). We found that the mean of the group AUC distribution was significantly above chance (M = 0.55, p < 0.001), confirming that the analysis was sufficiently powered to separate associative and item-only MPFC patterns. Taken together, these results suggest that greater MPFC differentiation was not necessarily driven by the direct representation variable encoding of contextual information during learning.

Brain-Wide Pattern Differentiation

In our final set of analyses, we were interested in whether large-scale activity distributed across the brain exhibited similar patterns as observed in hippocampus and MPFC. For each subject we defined a mask of the 5000 most active voxels in a contrast of Task > Baseline and computed pattern differentiation across this distributed set of voxels (single-subject example, Figure 4B). Unlike the hippocampus and MPFC, brain-wide pattern differentiation did not differ based on memory retrieval success [associative vs. item-only main effect F(1,16) = 0.11, p = 0.74] but
was higher overall for pairs learned overnight compared to same day \(F(1,16) = 7.89, p = 0.01, \text{ Figure 4B}\). When comparing pattern differentiation across the three ROIs (MPFC/hippocampus/brain-wide) we found that the interaction of region \(\times\) study list (overnight/same day) \(\times\) memory \(F(2,32) = 2.82, p = 0.07\] was driven by greater study list and memory-dependent pattern differentiation in MPFC than over the distributed task active brain mask \(p = 0.03\). In left hippocampus, study list and memory-dependent pattern differentiation did not differ from that observed in the task active brain mask \(p = 0.29\).

As in MPFC and hippocampus, we next examined whether the difference in pattern differentiation for overnight compared to same day pairs predicted the overnight-same day behavioral enhancement in associative memory performance. Brain-wide pattern differentiation was not significantly correlated with the behavioral enhancement \[r(15) = 0.03, p = 0.88, \text{ Figure 4B}\] and showed a trend for being lower than observed in MPFC \(p = 0.08\).

**MPFC—Hippocampal Connectivity Predicts Differentiation**

To shed light on the mechanism by which MPFC representations of overnight memories were more differentiated than same day memories, our final analysis measured beta-series connectivity between hippocampus and MPFC during correct associative retrieval. We hypothesized that if context-based MPFC differentiation arises from reorganization in mnemonic networks (Siapas & Wilson, 1998; Takashima et al., 2009; van Kesteren, Fernández, Norris, & Hermans, 2010), then connectivity with hippocampus may be related to this mnemonic differentiation in MPFC. Indeed, we found that, across participants, the difference in hippocampal-MPFC connectivity for overnight memories relative to same day memories correlated with the increase in rep-
resentational differentiation for *overnight* compared to *same day* memories in MPFC \[r(15) = 0.60, p = 0.01; \text{Figure 5}\]. Thus, those participants who exhibited greater hippocampal-MPFC connectivity during retrieval of *overnight* memories compared to *same day* memories also showed more pattern separation for *overnight* compared to *same day* memories in MPFC. This suggests that study across an overnight delay leads to enhanced hippocampal-MPFC interactions that may support the development of differentiated memory representations over time.

-Univariate Effects in Left Hippocampus and MPFC-

Although we were focused on assessing pattern differentiation, we also examined univariate activity in our primary regions of interest. Specifically, we tested whether parameter estimates in left hippocampus and MPFC significantly differed for the *overnight/same day* and *associative/item-only* conditions. A 2x2 ANOVA applied to the data from the left hippocampus (Figure 6A) showed no significant main effects or interaction (all \(p > 0.12\)). Figure 6B shows that MPFC was more active for the overnight compared to the same day condition \[F(1,16) = 8.27, p = 0.01\] and also showed a study condition x memory interaction \[F(1,16) = 6.73, p = 0.02\]. A 3x2 ANOVA comparing the two ROIs showed a significant main effect of region \[F(1,16) = 138, p < 0.001\] but no interactions of region with study condition or memory.
Discussion

Understanding the mechanisms that promote the longevity of learning is a critical focus of cognitive neuroscience. We find that learning word-object pairs across the distinct temporal contexts of two days benefited long-term associative memory retrieval compared to word-object pairs learned on one day, even though both overnight and same day associations were trained to the same high learning criterion (90%) and were matched in the number of training sessions. At one-week retrieval, multivariate activity patterns in MPFC were more differentiated for associations learned across temporal contexts compared to associations learned within a single temporal context. Furthermore, the increase in pattern separation for overnight compared to same day memories in MPFC correlated with the behavioral memory benefit for the overnight memories, suggesting that differentiation of MPFC retrieval patterns contributes to the memory benefit for information studied across contexts. We also found a correlation between context-driven pattern differentiation of MPFC activity for individual memories and functional connectivity between hippocampus and MPFC, suggesting that persistent hippocampal-cortical connectivity one week after learning supports the observed MPFC differentiation. These data show that robust long-term memory is supported by differentiation in the neural activity patterns that represent memories and suggest that this differentiation arises as a consequence of context change during learning that promotes sustained MPFC—hippocampal interactions.

Given that participants encoded overnight pairs on two days and same day pairs on a single day, one explanation for greater MPFC pattern differentiation for the overnight condition is that participants encoded, and subsequently retrieved, associative details from two temporal contexts (Estes, 1955). One prediction that might follow from such a model is that overnight patterns should be more likely to cluster into two groups than same day memories, perhaps reflecting dif-
ferential levels of contextual associations across memories. However, we did not find evidence that overnight memories were more likely to cluster into two groups than same day memories, suggesting that MPFC pattern differentiation may arise as a consequence of repeated learning across days, but does not directly reflect variability in contextual associations across conditions. Repeated learning across contexts may facilitate the long-term retention of unique memory traces through mechanisms that enhance the fidelity of individual memory representations (Karlsson Wirebring et al., 2015; Thios & D’Agostino, 1976).

Future work could investigate the degree to which the night of sleep itself (in the overnight condition) is important for promoting differentiation. We observed differentiation for overnight compared to same-day memories in spite of the fact that both types of memories were matched in several ways. First, both types were trained to the same high behavioral criterion. Second, both overnight and same-day memories had the opportunity for significant consolidation to occur during the one week between study and retrieval. These unique features of our design highlight the important role that reactivation following a 24-hour period played in the overnight condition. One possibility is that the overnight-delayed reactivation tagged overnight memories with enhanced behavioral relevance, leading these memories to undergo enhanced consolidation the following week (Lesburguères et al., 2011; Oudiette, Antony, Creery, & Paller, 2013; Singer & Frank, 2009; Wilhelm et al., 2011). Another possibility is that one night of sleep prior to res-study establishes a schema (in the sense of a pre-existing neural network), which then promotes additional learning following the night of sleep (Tse et al., 2007; Tse et al., 2011; van Kesteren et al., 2012). Each of these studies includes some period of rest before reactivation, which appears to be critical in enhancing memory retention (Litman & Davachi, 2008). Thus, future work that
can compare representations at encoding, rest and retrieval over extended timescales will be needed to adjudicate between these and other possibilities.

The observation that distributing study episodes across time is beneficial for memory relative to massing study within a shorter time span is one of the most robust findings in the study of memory (Cepeda et al., 2006; Dempster, 1989; Ebbinghaus, 1964; Litman & Davachi, 2008; McGeoch & Irion, 1952; Ruch, 1928). Our behavioral results are consistent with this literature, showing that object-word associations were better remembered one week later if initially learned in two sessions distributed across two days than associations learned in two sessions within the same day. Our design equated the number of learning sessions across the overnight and same-day lists, as well as the level of immediate memory for the two lists. Thus, our data suggest that the memory benefit for the overnight condition one week later is due to learning across two days.

Furthermore, the fMRI results highlight a potential mechanism for the efficacy of distributed learning – namely, that it promotes efficient, differentiated neural representations of memories that facilitates successful retrieval.

Prior fMRI work has suggested that the extent to which patterns overlap (pattern similarity) across repeated learning trials of the same stimulus is related to successful recognition memory. This work has shown that repeated individual stimuli that are later remembered were associated with greater similarity in the evoked fMRI activity patterns across encoding repetitions (Gordon et al., 2013; Ward et al., 2013; Watanabe et al., 2011; Xue et al., 2010, 2013). Similarity across trial-unique same-category stimuli during encoding is predictive of memory success (Kuhl, Rissman, & Wagner, 2012), as is similarity between patterns present during encoding and retrieval (Ritchey, Wing, Labar, & Cabeza, 2012; Zeithamova & Preston, 2010; Tompary et al, 2016; Danker et al, 2016), again suggesting the important role that similarity
plays in processing the same events in memory. This general approach is distinct from the one we adopt here in which we measure how memories are represented compared to one-another: an inter-item similarity analysis. Prior work using a similar approach suggests that both similarity and differentiation during encoding support later memory, but are instantiated in distinct brain areas, the MTL cortex and hippocampus, that support slow extraction of commonalities across memories vs. rapid differentiation of overlapping experiences (LaRocque et al., 2013, Norman & O'Reilly, 2003, Tompary & Davachi 2017). It is possible that enhanced similarity across learning repetitions of the same item could promote differentiation across items at retrieval (i.e. our primary neural measure) by sharpening the representations of individual memories relative to one another, following repeated study/test attempts during learning. Testing-related neural differentiation is known to predict memory performance after a one-week delay (Karlsson Wirebring et al., 2015); therefore, one critical question for future research is to examine the extent to which repeated study supports later memory through engagement of similarity-based mechanisms, while repeated testing does so through differentiation-based mechanisms.

The present work extends our knowledge of how MPFC represents contextual information over time. Previous work showed that differentiation in MPFC BOLD activity patterns at encoding predicts temporal order memory (Jenkins & Ranganath, 2016). MPFC activity is also enhanced during retrieval of temporally remote memories compared to recent memories (Gais et al., 2007; Nieuwenhuis & Takashima, 2011; Sterpenich et al., 2009; Takashima et al., 2006).

Furthermore, multivariate classification of MPFC BOLD patterns is better for remote than recent autobiographical memories, suggesting remote memory representations are more differentiated (Bonnici et al., 2012). Our work connects these data on MPFC encoding and retrieval activity by showing that MPFC pattern differentiation is related to the fidelity of individual associative
memories after one week, when those associations were learned across temporal contexts. Furthermore, our univariate analysis showing greater MPFC activity in the overnight compared to same day condition is consistent with a role for MPFC in retrieval of consolidated memories and extends prior work by contrasting conditions that are matched in terms of remoteness. Given that we scanned only at retrieval, an open question concerns the timecourse by which differentiation across memory representations emerges following study (Bonnici & Maguire, 2018). Understanding the timeline will help distinguish between the influence of processes engaged during encoding, retrieval or in between (Hulbert & Norman, 2015). For example, future work in which the encoding phase is scanned could determine whether differences in neural representation emerge during study. This work will be important to determine the relation between the context-based differentiation we observed and other mechanisms such as study-phase retrieval (Braun & Rubin, 1998; Thios & D’Agostino, 1976). Neural markers of memory stabilization in cortical networks have also been identified shortly after encoding of a to-be-remembered experience (Ben-Yakov & Dudai, 2011; Tambini et al., 2010; 2014; Tompary et al., 2015). Hippocampal-cortical connectivity following a one-day delay predicts subsequent resistance to forgetting (Vilberg & Davachi, 2013) and persistent connectivity between hippocampus and MPFC during the one-week delay is a potential mechanism that could lead to later pattern differentiation as representations become established in cortical networks (Peyrache et al., 2009; Richards et al., 2014). Our data showing hippocampal-MPFC connectivity predicting neural differentiation in MPFC are consistent with this hypothesis, but future work will be needed to address how such connectivity emerges and evolves over time. Prominent theories propose that MPFC contributes to long-term memory by supporting the integration of new information into established memory networks (Tse et al., 2007; van
Kesteren, Ruiter, Fernández, & Henson, 2012), reducing forgetting of new information by providing a set of stable associations that can be used to access specific memories during later retrieval. A computational challenge that arises when integrating new information into established networks is avoiding interference between similar representations (McClelland, McNaughton, & O’Reilly, 1995; O’Reilly & Rudy, 2001). Our findings suggest that one way to avoid such interference is to use contextual information to differentiate similar input representations, a notion that has figured prominently in theoretical and experimental work on the role of hippocampus in episodic memory encoding (Bakker et al., 2008; Hulbert & Norman, 2015; LaRocque et al., 2013; Leutgeb et al., 2007; Leutgeb et al., 2004; Marr, 1971; Norman & O’Reilly, 2003). Our data show that distributing study context across two days leads to differentiation in activity patterns in MPFC. The extent of this differentiation predicts the increase in memory performance that results from distributed study and is related to functional connectivity between MPFC and hippocampus. These data implicate a role for MPFC in representing contextual information in long-term memory over long timescales and provide novel evidence that distributed study leads to greater memory retention via persistent hippocampal-MPFC interactions.

Author Contributions


Acknowledgements
This work was supported by DART Neuroscience and NIH R01 MH074692 to L.D.
References


DuBrow, S., & Davachi, L. (2014). Temporal memory is shaped by encoding stability and inter-


Thios S. J., & D'Agostino, P. R. (1976). Effects of repetition as a function of study-phase


Vilberg, K., & Davachi, L. (2013). Perirhinal-hippocampal connectivity during reactivation is a


Figure 1. Study design.

(A) General Procedures for the experiment. Day 1: Participants were trained to criterion on a set of word-object associations on Day 1 (overnight condition). Day 2: Twenty-four hours later participants returned to the lab and were trained on a new set of word-object associations (same day condition) before being re-trained to criterion on both the overnight and same day lists intermixed. Day 7: One week after the Day 1 session, participants were trained to criterion on a novel set of word-object associations (single session) before being placed in the fMRI scanner and performing a retrieval test that included all lists (overnight, same day and single session) and novel lures.

(B) Left, Each training session began with a Study Phase in which participants were exposed to the word-object associations. Middle, Training then began with a test in which word-object association memory was tested by having participants respond ‘Natural’ or ‘Manmade’ for the object that had been associated with each word (Nat, Natural; Man, Manmade; Uns, Unsure). After each response, feedback (‘Correct!/Incorrect!’) was given along with presentation of the associated object. A correct response was required in two consecutive training rounds in order for the word-object pair to be dropped from future training. This training protocol was performed for three blocks of 24 stimuli until all 72 pairs from the list were learned. Following the final block of 24, all 72 words were then re-presented for a final test in order to ensure that participants were above 90% across the entire list. Participants who did not meet the 90% criterion on this final test were re-exposed to all 72 pairs in a final Study Phase. Right: In the scanner participants performed the final Retrieval Test for all of the words from the overnight, same day and single session lists intermixed with novel lures.

Figure 2. Behavioral data for day 7 retrieval test.

(A) The proportions of associative, item-only and forgotten pairs are plotted for the overnight, same day, and single session lists. Associative memory was higher for overnight pairs compared to same day pairs ($p < 10^{-4}$), evidence of a distributed learning effect. Associative memory for single session pairs was higher than both overnight and same day pairs (both pairwise comparisons $p < 10^{-4}$).

(B) Response times for associative and item-only conditions. In addition to showing higher source memory, participants were also faster to respond for overnight associative memories compared to same day associative memories ($p < 10^{-5}$). Participants were overall fastest for single session associative memories (both pairwise comparisons with overnight and same day trials, $p < 10^{-6}$). Error bars denote SEM.

Figure 3. Hippocampal pattern differentiation.

(A) Left, example subject left hippocampus anatomical region of interest. Middle, in left hippocampus, differentiation did not vary between overnight and same day memory ($p = 0.12$) but was significantly higher for associative compared to item-only memory (main effect: $p = 0.004$), a difference that was significant within the overnight and same day conditions individually. Right, the difference in overnight/same day pattern differentiation in hippocampus did not correlate with the overnight memory benefit ($p = 0.23$).

(B) Left, in left hippocampus pattern differentiation was significantly greater in anterior compared to posterior regions ($p = 0.02$). Right, in right hippocampus, pattern differentiation was al-
so significantly greater for anterior compared to posterior regions ($p = 0.006$).

**Figure 4. MPFC and brain-wide pattern differentiation**

(A) *Left*, MPFC region of interest rendered on the group-average brain. *Middle*, in MPFC, there was significantly more differentiation for *associative* versus *item-only* memories only within the overnight condition ($p = 0.006$) but not in the *same day* condition ($p > 0.16$; interaction $p = 0.02$). Differentiation for *overnight associative* trials was also significantly greater than for *same day associative* memories ($p = 0.002$). Error bars denote SEM. ⊗ indicates significant interaction ($p < 0.05$). *Right*, the difference in *overnight/same day* pattern differentiation on Day 7 correlated with the overnight memory benefit [$r(15) = 0.53$, $p = 0.03$].

(B) *Left*, example subject brain-wide task active mask. *Middle*, pattern differentiation was greater for overnight than same day retrieval ($p < 0.02$), but did not differ as a function of memory success. Error bars denote SEM. *Right*, the difference in *overnight/same day* pattern differentiation on Day 7 was not correlated with the overnight memory benefit [$r(15) = 0.03$, $p = 0.88$].

**Figure 5. Hippocampal-MPFC connectivity predicts MPFC pattern differentiation.**

The difference in hippocampal-MPFC connectivity between the *overnight/same day* conditions correlates with the level of pattern differentiation in MPFC [$r(15) = 0.60$, $p < 0.02$].

**Figure 6. Univariate activity in hippocampus and MPFC.**

*A*, we did not observe significant modulation of univariate activity in left hippocampus across conditions. (all $p > 0.12$). *B*, in MPFC, there was a main effect of overnight/same day condition ($p = 0.01$), as well as an interaction ($p = 0.02$). Errorbars denote SEM. ⊗ indicates significant interaction ($p < 0.05$).
A Day 1 Day 2 Day 7
Overnight Same Day Both Restudy
24 hrs ~ 15 min 6 days

ARCTIC LOOSE DRY JOVIAL LOOSE
DRY

Day 1
Both Restudy
Final Retrieval (fMRI scanner)

B Study Phase Training Phase Scanned Retreival
+ + +
500 ms 500 ms 500 ms
4500 ms +
12000 ms max
500 ms +
8500 ms Correct!
500 ms +
6000 ms variable ITI

- DRY
- Nat / Man / Uns
- DRY
- Nat / Man / Uns / New
- DRY
- natural / manmade unsure / new
-
A

B

proportion of trials

response times (s)

Overnight Same Day Single Session

Overnight Same Day Single Session

p < .0001

p < 10^-5

p < 10^-6
**A**

**Left Hippocampus**

- **Associative**
- **Item Only**

Pattern Differentiation

- Overnight: p < 0.05
- Same Day: p < 0.04

**Overnight-Same Day Pattern Differentiation**

- r(15) = 0.31
- p = 0.23

**B**

**Left Hippocampus**

- **Anterior**
- **Posterior**

Overnight: p < 0.02

**Right Hippocampus**

- **Anterior**
- **Posterior**

Overnight: p < 0.007
**Medial PFC**

- **A**
  - **Associative Item Only**
    - Pattern Differentiation: 
      - Overnight: 0.90
      - Same Day: 0.85
    - p < 0.002
    - p < 0.006
    - r(15) = .53
    - p < 0.03

- **B**
  - **Task Active Voxels**
    - Pattern Differentiation: 
      - Overnight: 0.92
      - Same Day: 0.88
    - p < 0.02

**Overnight-Same Day Memory Benefit**

- Overnight-Same Day Pattern Differentiation:
  - r(15) = .53
  - p < 0.03
  - p = 0.88
mPFC Pattern Differentiation
(Overnight-Same Day Difference)

Left Hippocampus-mPFC Beta Series
(Overnight-Same Day Difference)

Associative Memory

$r(15) = .60$
$p < 0.02$
A Left Hippocampus

B Medial PFC

Parameter Estimate (a.u.)

Overnight Same Day

Left Hippocampus Medial PFC Parameter Estimate (a.u.)
p = 0.01

Overnight Same Day

×