NORTHWESTERN UNIVERSITY

Odors as Reactivation Cues During Sleep: An Investigation of Memory Outcomes and Neural Mechanisms

A DISSERTATION

SUBMITTED TO THE GRADUATE SCHOOL

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

for the degree

DOCTOR OF PHILOSOPHY

Field of Neuroscience

By

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EVANSTON, ILLINOIS

March 2019

Abstract

Odors as Reactivation Cues During Sleep:

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For years, neuroscientists have strived to understand memory consolidation, where salient memories are sorted and organized into distributed cortical networks for long-term storage. A large body of sleep research suggests that slow-wave sleep is an optimal opportunity for memory consolidation, and that consolidation is driven, at least in part, by a memory replay mechanism, where the same neural activity that occurs during memory encoding comes back online during sleep. Recently, scientists have demonstrated that sleep-borne sensory cues can be used to direct memory consolidation. Specifically, when memory encoding occurs in the presence of odor or sound cues, delivery of the same cues during subsequent sleep (i.e., reactivation) reinforces the associated memories, enhancing retrieval for those memories upon waking. Recent studies suggest that reactivation cues might promote consolidation by inducing replay of the associated material, but direct evidence for such mechanisms is scant, and the relevant brain regions supporting these processes are poorly understood.

Here, we address these gaps by developing a novel olfactory reactivation paradigm, which included simultaneous EEG-fMRI recording in human subjects. We find that odor cues presented

during sleep improved performance on an object-location memory task. Using multivariate pattern analysis of fMRI data, we also find that these memory gains are supported by neural replay of category-level information in ventromedial prefrontal cortex and visual associative cortex. In identifying the potential mechanisms by which odor cues selectively modulate memory consolidation, our findings bring unique insights into how memories are stored and preserved in the sleeping human brain.

Acknowledgments

This work would not have been possible without support, encouragement, and inspiration from mentors, colleagues, family, friends, pets, and dozens of fiction novels. First and foremost, I want to thank my advisor, Dr. Jay Gottfried. Your scientific ambition and talent for creative writing are forces to be reckoned with. I truly value the opportunity to have grown as a scientist under your training and mentorship, and, thanks to your sincerity and sense of humor, it was a joy to be a part of your lab.

I would also like to thank my committee members, Drs. Ken Paller, Joel Voss, and Phyllis Zee. Thank you for your guidance, and for your thoughtful input on my research projects. Special thanks to Dr. Ken Paller, who has been an excellent collaborator and mentor. I also want to thank Dr. Thorsten Kahnt, who has contributed substantially to my research projects, as well as my scientific growth.

I am also grateful to all the members of the Gottfried Lab, past and present: Agnes Bao, Nicholas Bowman, Pei-Ching Chang, Sydni Cole, Mathieu d'Acremont, Eva Gjorgieva, Katherina Hauner, James Howard, Isabel Hutchison, Heidi Jiang, Olga Lacki, Shayna Levine, Daria Porter, Lisa Qu, Joanna Wu, and Christina Zelano. I would like to specially thank James and Agnes, who taught me a lot about fMRI data analysis, and to Eva, who helped me collect fMRI sleep data for my thesis project at all ungodly hours of the night.

I also want to thank the good friends I made in graduate school, because they got me through it: in particular, Nick Bush, Jessica Creery, James Ellis, Evan Fazio, Dominic Frank, Natalie Frederick, Josh Glaser, Jenna Harmon, Tom Harmon, Carmen Lin, Christina LoTempio, Marios Loukidis, Sonja Marziano, Dan Ohm, Nelly Papalambros, Brad Radvansky, Éanna Ryan, and Dan O'Young. Special thanks to the members of my fiction book club, who helped me escape from science sometimes, and to Carmen Lin, who has been my study partner, roommate, bridesmaid, and best friend in NUIN.

Finally, I want to thank my family. To my parents, Anne and Tim Shanahan, thank you for raising me, and for believing in me unconditionally. Thank you to my siblings, especially to Maggie Shanahan, my best friend and confidant. I would also like to thank my grandma, Barb Letcher, who lived in Chicago when I started graduate school, and made it feel more like home. Most of all, I want to thank my husband, Paul Dodd. Thank you for your love and support, for listening, and for not holding it over my head too much when you earned your PhD before me. Finally, I want to thank my puppy, Missoula, for helping to pace me while I wrote this thesis by resting her head on my keyboard periodically.

List of abbreviations

BCG .....Ballistocardiogram

ECG .....Electrocardiography

EEG .....Electroencephalography

EOG .....Electrooculography

EMG .....Electromyography

fMRI .....Functional magnetic resonance imaging

GLM .....General linear model

mPFC ..... Medial prefrontal cortex

MRI ..... Magnetic resonance imaging

MVPA .....Multivariate pattern analysis

PET .....Positron emission tomography

PPI ..... Psychophysiological interaction

PFC ..... Prefrontal cortex

nREM ..... Non-rapid eye movement sleep

RT .....Response time

ROI .....Region of interest

REM ..... Rapid eye movement sleep

SVM ..... Support vector machine

SWA ..... Slow-wave activity

SWR ..... Sharp-wave ripple

SWS ..... Slow-wave sleep

tDCS ..... Transcranial direct current stimulation

vmPFC ..... Ventromedial prefrontal cortex

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Chapter 1: Background

1.1 Memory consolidation

In his novel *Brain Jack*, Brian Falkner writes, "We are our memories. That's all we are. That's what makes us the person we are. The sum of all our memories from the day we were born. If you took a person and replaced his set of memories with another set, he'd be a different person. He'd think, act, and feel things differently[2]." Though *Brain Jack* is science fiction, this statement rings true. Memories are the very essence of identity, which is why investigating the brain's ability to form and retain a unique collection of memories is one of the most compelling avenues of neuroscience research.

The process through which newly-made memories are eventually integrated into long-term storage in the brain is called memory consolidation. According to standard consolidation theory, memory traces are thought to depend on the hippocampus following initial encoding[3] (**Fig. 1.1a**). Over time, connections between neocortical sites are strengthened, eventually rendering memories hippocampus-independent. It has been suggested that the PFC may gradually overtake the role of the hippocampus in integrating memories across cortical modules, thereby serving as a remote memory hub (**Fig. 1.1b**). Note that the processes outlined here occur at a systems level, though memory consolidation can also be studied at the level of the synapse, where the variable ability of presynaptic neurons to trigger an action potential in their postsynaptic counterparts is dictated by a complex cascade of molecular changes. Though systems-level consolidation certainly requires synaptic plasticity, it is a fundamentally slower process that involves the reorganization of neural networks on a larger scale.

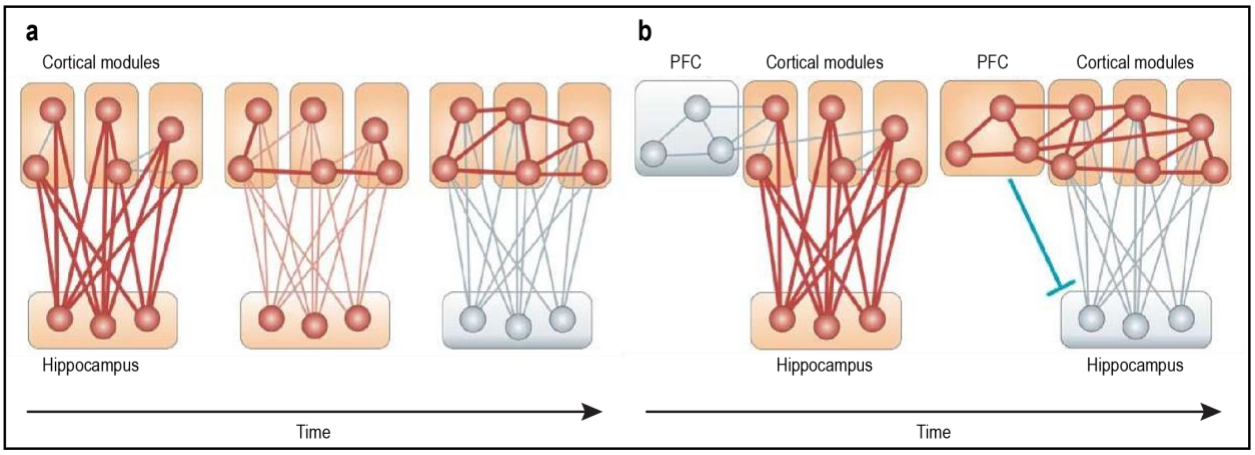

Figure 1.1. Memory consolidation schematic. (a) Model of standard consolidation theory. **(b)** Proposed role for PFC in remote memory consolidation. Adapted with permission from Frankland & Bontempi, 2005[3].

1.2 The role of sleep in memory consolidation

Although memories are likely consolidated during a variety of brain states, a convincing line of research suggests that sleep represents a particularly important time window for memory consolidation. As early as 1924, Jenkins and Dallenbach demonstrated the importance of sleep for memory retention[4]. In their experiment, human subjects learned a list of nonsense syllables, and then waited for an established length of time before taking a free recall test. Memory measurements were recorded for several time steps, during which the subjects slept or remained awake. The researchers found that subjects could recall more syllables after periods of sleep than after equivalent periods of wakefulness. Though their primitive study design was wrought with circadian confounds and limited by a tiny sample size of two, their core finding that sleep benefits memory has been replicated many times since. Moreover, sleep has been shown to improve performance across a slew of memory tasks, from visual texture discrimination[5,6] to motor sequence learning[7,8], to spatial navigation[9]. This line of research serves to demonstrate the broad influence of sleep on memory, the limits of which are not yet known.

Early studies like the one conducted by Jenkins and Dallenbach predated the practice of using EEG to stage sleep. In 1968, Rechtschaffen and Kales were the first to fastidiously describe the various stages of human sleep based on their unique EEG signatures. They developed a detailed manual to standardize the terminology and criteria used to score human sleep[10], and though the American Academy of Sleep Medicine released revised guidelines in 2007[11], much of Rechtschaffen and Kales' original scoring scheme informs modern practice.

In humans, overnight sleep is divided into approximately four to six 90-minute cycles (**Fig. 1.2a**). These cycles alternate regularly between nREM and REM. nREM is more prevalent in the first half of the night, while REM predominates the second half. nREM can be further divided into stages 1, 2, and 3. Stage 1 constitutes light sleep, while stage 2 can be identified by two distinctive waveforms – K-complexes and spindles. Stage 3 is also called SWS, because it is characterized by slow, high-amplitude brain oscillations (**Fig. 1.2b, top**). These oscillations reflect synchronized neural firing, and have been shown to promote communication between cortical regions[12]. In contrast, REM is characterized by fast, low-amplitude EEG activity resembling that observed during quiet wakefulness (**Fig. 1.2b, bottom**)[A].

[A] Note that there are still significant differences in EEG topography between REM and wake states[13].

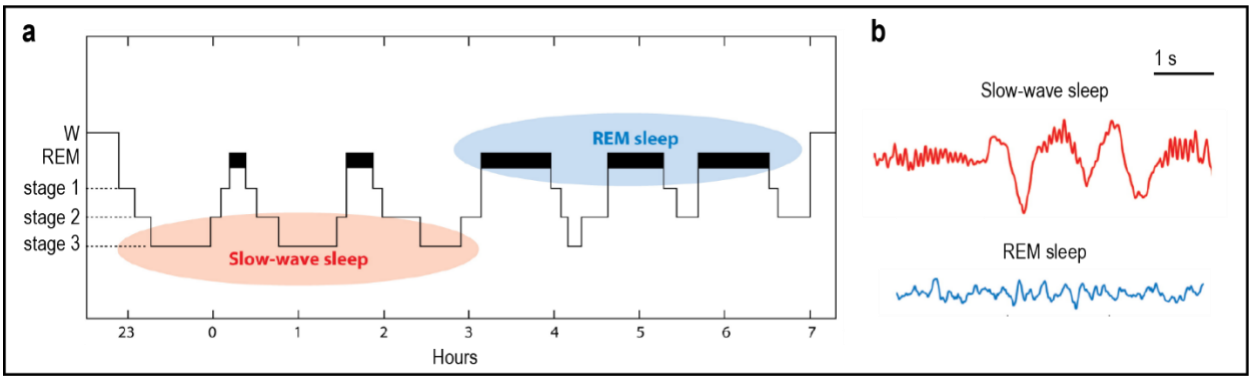

Figure 1.2. Sleep physiology. (a) Sleep stages progress in a cyclical fashion over nocturnal sleep. **(b)** Sample EEG trace to show characteristics of SWS (top) versus REM (bottom). Adapted with permission from Inostroza & Born, 2013[14].

Given the considerable electrophysiological differences between SWS and REM, it is not surprising that these stages also deviate in terms of their contributions to memory consolidation. To parse these differences, sleep parameters (e.g., time spent in each stage) are often correlated with pre- to post-sleep changes in memory performance. Alternatively, memory retention can be selectively tested after SWS- or REM-rich sleep using the night-half paradigm, which was originally conceived of by Ekstrand and colleagues in 1971[15]. In the night-half paradigm, one group encodes a memory task in the late evening, and then undergoes memory testing after an interval of early night (SWS-rich) sleep. A separate group encodes the same task in the middle of the night (after sleeping through the first half), and memory testing occurs after an interval of late night (REM-rich) sleep. In 1997, Plihal and Born implemented the night-half paradigm to demonstrate that SWS is essential for remembering associated word pairs, while REM is critical for enhancing mirror-tracing[16B]. This early study and a handful of others[18] are in line with the "Dual Process Hypothesis," which posits that SWS supports declarative (explicit) memory

[B] This is the same skill that patient H.M. famously acquired despite extensive hippocampal resection and anterograde amnesia[17].

consolidation, while REM supports non-declarative (implicit) memory consolidation. However, while more contemporary studies reinforce the notion that SWS is critical for declarative memory consolidation, the role of REM is less clear (see Ackermann & Rasch, 2014[19] for a review). Recent advances have allowed for direct manipulation of SWA through tDCS[20,21] and closed-loop auditory stimulation[22]. These technologies and others should provide a more nuanced understanding of how particular components of sleep physiology serve memory in its myriad forms.

Irrespective of memory class, the process through which memories are selected for within-sleep consolidation is not a stochastic one. Rather, there are several factors that influence whether a memory will be retained for long-term storage. For instance, memories that are linked to a future reward are more likely to endure. In a 2009 study by Fischer and Born[23], subjects learned two distinct motor patterns to criteria while performing the Finger Sequence Tapping Task (for task details, see Walker et al., 2002[8]). After learning, subjects were told that they would receive a monetary reward based on their ability to perform one of the two motor patterns following a 12-hour interval of overnight sleep or daytime wakefulness (as a control). Critically, immediately before testing, subjects were told that the reward would depend instead on their average ability to perform both motor patterns. In the sleep group, overnight improvements were significantly greater for the motor pattern that subjects initially believed would be rewarded, when compared to the other motor pattern. These results constitute convincing evidence that reward expectation spurs consolidation during sleep. In addition to reward, there are many other memory features that modulate sleep-based consolidation, including emotional tone[24,25], memory strength[26,27], and social praise [28].

1.3 Memory replay and sleep-dependent memory consolidation

But *how* are some memories strengthened, stabilized, and ultimately preserved in the brain, while others are not? One mechanism thought to promote consolidation is memory replay (**Fig. 1.3**), where the same neurons involved in encoding a new memory are active again later to facilitate the integration of that memory into established cortical networks. Thus, memories are essentially "replayed" so that they can be moved to long-term storage. This phenomenon was originally demonstrated in 1989 by Pavlides and Wilson[29], who recorded from hippocampal place cells[C] while rats explored an enclosure, and then again while they slept. They found that place cells that were active during exploration fired action potentials at an elevated rate during subsequent sleep, compared to those cells that were not previously active. They speculated that this pattern of activity might reflect information processing during sleep.

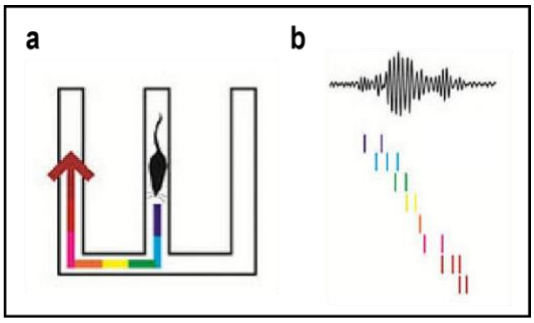

Figure 1.3 Memory replay schematic. **(a)** Place cells are active as the mouse traverses a maze. **(b)** Same place cells are active later on, during hippocampal SWRs. Adapted from NIH[1].

Since this rudimentary demonstration of within-sleep memory replay, there have been a plethora of other replay studies in animal models that have confirmed and extended these findings. For instance, replay events are synchronized with hippocampal SWRs[31,32], the sequence of neurons that fire during spatial navigation is conserved during replay events[33], and replay during nREM

[C] Place cells are active in particular spatial locations, and were first discovered by O'Keefe and Dostrovsky in 1971[30].

occurs on a compressed time scale[34,35D]. Though initial replay work focused on hippocampal place cells, more recent studies have provided evidence for replay in cortical regions, including parietal[37], visual[38], and prefrontal cortices[39]. Critically, in studies where experimenters recorded from hippocampus and cortex simultaneously, replay events in both regions were coordinated to reflect the same experience[37,38]. Moreover, replay is enhanced for salient information (e.g., novel environments[40]), which suggests that it may serve to promote consolidation of certain memories over others. Finally, blocking SWRs (and thereby memory replay) during sleep disrupts spatial memory performance[41-43]. This line of research heavily implicates memory replay as a central mechanism underpinning consolidation during sleep[E].

Though memory replay has been most thoroughly investigated in animal models, and especially in rodents, there is also limited evidence from human studies that within-sleep replay drives memory consolidation[F]. In 2004, Peigneux and colleagues used PET to measure brain activity while human subjects navigated a virtual town, and then again during the following sleep period[48]. In line with previous studies[49-51], they found that hippocampus and parahippocampal gyrus were active during spatial navigation. During the next bout of nREM, the same regions

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[D] See Louie & Wilson, 2001[36] for an account of replay during REM, which may unfold in real-time.

[E] Memory replay has probably been so heavily studied during sleep because it was initially discovered during the sleep state. However, replay has since been demonstrated during the wake state as well[44], suggesting that memory processing may begin immediately after encoding (see Carr et al., 2011[45] for a review of wake replay). Still, given the strong behavioral evidence that memories benefit preferentially from sleep, there may be something special about within-sleep replay and its neural context that promotes consolidation.

[F] The term "memory replay" is usually reserved to describe the sequential firing of individual neurons, whereas corresponding systems-level changes in activity are more often deemed "reactivation". In this thesis, the term replay will be used to describe both phenomena, as has been done previously[46,47].

demonstrated elevated neural activity, when compared to a control condition where sleep was not preceded by spatial navigation. Critically, the level of activity in these brain areas during nREM was correlated with the subjects' ability to successfully navigate the virtual town upon waking. In a more recent study that employed MVPA of fMRI data (see Section 1.6), the Axmacher group found that specific neural representations of learned visual objects (e.g., pumpkin, hamster) are replayed during quiet wakefulness and nREM, and that replay strength predicts later memory for object locations[46]. These studies and a handful of others[52,53] provide nascent systems-level evidence that memory replay promotes consolidation during human sleep, but so far there is no parallel proof at the cellular level. This may change soon, given advances in invasive electrophysiological monitoring of epilepsy patients, where local field potentials and single-unit recordings can be collected via microelectrodes.

1.4 Presenting sensory cues in sleep modulates memory consolidation

The experiments and evidence outlined thus far regarding consolidation mechanisms pertain to spontaneous replay, where memory replay occurs naturally in the brain. That is not to say that replay events are indiscriminate (as they are likely biased toward experiences that are important to remember), but rather that they are not externally prompted. In a related line of work, researchers have developed a method that can be used to direct memory consolidation toward specific content during sleep. This method, often called "targeted memory reactivation", or simply "reactivation", involves establishing an association between learned material and external sensory cues, and then re-presenting the same cues in the subsequent sleep period. In the first reactivation study from 2007, human subjects learned card pair locations (like in the memory game "Concentration") in the presence of a background rose odor[54] (**Fig. 1.4**). After subjects

learned the task to criteria, experimenters delivered the same odor to their noses while they slept in the lab. They found that, although subjects were naïve to the experimental conditions (they did not consciously smell the rose odor during the sleep period), they were better at recalling card locations upon waking if they had received the odor during both learning and SWS. They did not observe this memory boost if the rose odor was presented during learning and again during wake or REM, or if the odor was presented during SWS but not during prior learning. Thus, when subjects learned a declarative memory task in the presence of an odor cue, that odor gained the ability to reactivate the task, specifically in SWS, to facilitate consolidation of card pair locations.

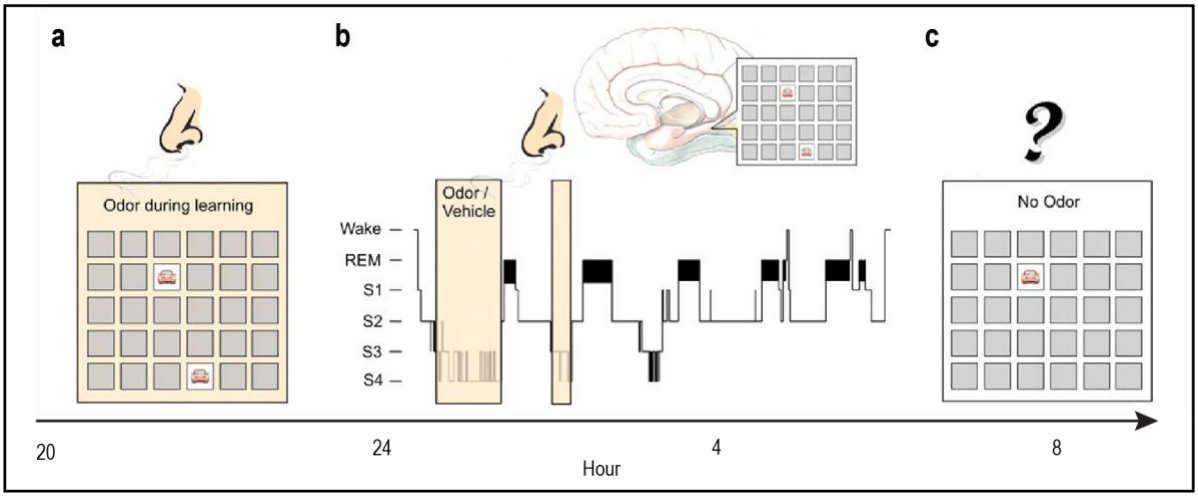


Figure 1.4. Memory reactivation paradigm. (a) Subjects learned card pair locations in the presence of a rose odor, and then **(b)** received the same rose odor during SWS. **(c)** Upon waking, subjects were tested on their knowledge of card paid locations. Adapted with permission from Rasch et al., 2013[54].

Since this first demonstration that sleep-born cues can enhance associated memories, there have been several others. In one key study, Rudoy and colleagues used sound cues to reactivate picture locations in a similar visuospatial task[55]. This time, they paired a unique sound cue with each individual picture throughout learning, and cues matched picture content (e.g., cat picture +

meow sound, kettle picture + whistle sound). They then presented half of the sound cues during nREM, and found that cues boosted spatial recall upon waking, specifically for the subset of pictures that was reactivated. Though most reactivation studies have involved declarative memory tasks, reactivation has also been shown to encourage creative problem solving[56,57], improve procedural skills[58,59], promote explicit knowledge of a motor sequence[60], bolster multisensory recalibration[61], and reinforce counter-stereotype training to combat implicit social biases[62]. A study from our lab further demonstrated that odor cues facilitate extinction of associated fear memories following fear conditioning[63], and another group found a similar effect[64G]. Other investigations of the effects of reactivation on emotional (non-fear) memories are rather inconclusive[67-69].

For the majority of reactivation studies, olfactory or auditory cues are presented during nREM, and particularly during SWS. As in the seminal Rasch study[54], more recent attempts have failed to boost memory performance when reactivation cues are delivered during REM[70,71]. In a recent meta-analysis, the Paller lab demonstrated that auditory cues are only successful in driving consolidation when they are presented during the "up" phase of slow waves[72], suggesting that the unique physiology of SWS may be essential for reactivation. Still, given the challenges associated with cue delivery during REM (i.e., cues are more likely to provoke arousal, and overnight studies are often required to observe sufficient REM), and the relative dearth of literature on the subject, the idea that REM reactivation could improve memory under

[G] A similar study in rodents found the opposite effect – namely, within-sleep odor cues strengthened associated fear memories[65]. For a review that seeks to reconcile these disparate findings, see Ouidette et al., 2013[66].

unexplored conditions is still viable. Even considering the growing number of SWS cueing studies, the optimal conditions and practical boundaries for reactivation have yet to be clearly defined.

1.5 Neural correlates of memory reactivation

Given these exciting behavioral outcomes, researchers are keen to investigate the neural mechanisms underpinning reactivation. That is, *how* do reactivation cues strengthen memories in the sleeping brain? The predominant theory is that these cues promote memory replay. Specifically, external cues could serve to manipulate the content of replay events, biasing them toward associated memories. The most definitive evidence in support of this theory comes from a rodent study conducted by the Wilson lab in 2012[73]. In their study, rats were trained to run toward the left or right end of a linear track in response to two distinct sound cues (i.e., an ascending sound cue prompted rightward running, and a descending sound cue prompted leftward running). They recorded from hippocampal place cells to determine which cells were active during leftward and rightward running, and then they exposed the rats to both sound cues several times in a random order during nREM[H]. They found that cues biased replay events toward the associated spatial trajectory. For example, presenting the ascending sound cue would preferentially evoke replay in hippocampal place cells with place fields on the right side of the track. A couple years later, another rodent study from a different Wilson lab demonstrated that artificially imposing "odor" replay in the olfactory bulb during nREM via ensemble electrical stimulation strengthened associative memory in a fear conditioning paradigm[74].

[H] In rodents, sleep stages are classified simply as REM or nREM, without further dividing nREM into multiple stages, as is done in humans.

In human work, evidence that reactivation cues elicit memory replay is less direct. For instance, as part of the same study that was previously described, the Rasch group also collected fMRI data from a small number of subjects during the sleep reactivation period[54]. They found that odor cues activated left hippocampus during sleep, a finding which was replicated in another fMRI study four years later[75]. Moreover, a study of chronic temporal lobe epileptic patients showed that auditory reactivation only improved memory for subjects that were healthy or had unilateral hippocampal damage, but not for those that had bilateral hippocampal damage[76]. Interestingly, the structural integrity of the hippocampus was predictive of the magnitude of the cueing effect. Together, these studies suggest that the hippocampus is an important structure for reactivation, which is thoroughly unsurprising given its established role in memory function.

Since memory consolidation is thought to involve a dialogue between hippocampus and neocortex to strengthen neocortical networks, hippocampal replay is only one side of the story. In 2014, Cox and colleagues used EEG to show that reactivation modulates sleep physiology locally[77]. In their experiment, two olfactory cues were associated with words presented at different screen locations, such that each cue was paired with words appearing exclusively in the right or left visual hemifield. They found that delivering one of the two odor cues during nREM increased the number and amplitude of sleep spindles in contralateral posterior brain regions (i.e., contralateral to the cued hemifield). Given that visual information from each hemifield is initially processed by the opposite side of the brain, they speculated that their results might

reflect local reprocessing of memory traces[I]. More recently, the Lewis lab used MVPA to demonstrate that auditory cues evoke content-specific replay of a motor sequence learning task during sleep[79]. Around the same time, another group used a similar approach to show that auditory cues trigger replay of visuospatial memory content (objects versus scenes), and that decoding fidelity was predictive of later memory performance[80]. These recent experiments suggest that reactivation cues promote cortical replay of associated memory traces, and tentatively show that such replay might affect memory outcomes.

1.6 fMRI multivariate pattern analysis

As referenced in previous sections, MVPA is a type of statistical analysis that can be applied to neural data[J]. Briefly, to apply MVPA to fMRI data, researchers consider the pattern of activity across voxels in response to a stimulus, rather than treating each voxel as an independent entity (as in a traditional univariate analysis). Haxby and colleagues were the first to use an MVPA approach to analyze fMRI data in 2001[81]. In their experiment, subjects studied pictures from several categories (e.g., faces, cats, houses, chairs, scissors) during fMRI scanning while performing a one-back repetition detection task[K]. They found that images from each category evoked a unique signature of fMRI activity across voxels in ventral temporal cortex. As MVPA

[I] In a study that came out earlier this year, Antony and colleagues further demonstrated the important role of spindles in memory reactivation[78]. They found that spindles manifest rhythmically, and that auditory cues are only effective in enhancing associated declarative memories when presented outside of the "spindle refractory period".

[J] The abbreviation MVPA generally stands for multivariate pattern analysis. However, in the fMRI literature, MVPA often stands more specifically for multi*voxel* pattern analysis.

[K] In an N-back repetition detection task, subjects indicate when the perceived stimulus matches the one presented N steps previously.

has generally proven to be more sensitive than traditional univariate analyses, the approach quickly gained traction in the field.

For instance, since the seminal Haxby study, visual neuroscientists have used MVPA of fMRI data to demonstrate pattern specificity for visual edge detection[82], images belonging to subcategories (e.g., rural buildings versus skyscrapers)[83], and even within-category exemplars[84,85]. Interestingly, MVPA has also revealed that visual perception evokes patterns of fMRI activity that are similar to those elicited by recollection[86] and mental imagery[87] of the same stimuli, implying that experience and imagination recruit similar neural resources. Of course, MVPA has also been used widely outside of the realm of visual category processing. For instance, MVPA of fMRI data has been implemented in studies of odor perception[88-90], emotion [91,92], and reward [93-96]. These examples represent a tiny fraction of the hundreds of fMRI-MVPA studies that have been published over the past two decades.

In terms of analysis, the MVPA approach employed by Haxby and colleagues involves comparing correlations for within-category activity patterns (e.g., face pattern versus face pattern) to those for between-category activity patterns (e.g., face pattern versus cat pattern), where significantly higher within-category correlations indicate pattern specificity. This relatively straightforward method is still used today. More recently, neuroscientists have also begun to implement MVPA machine learning techniques, such as SVM, to decode fMRI signals (see Mur et al., 2009[97] for a review of MVPA methods, and see Haxby, 2012[98] for a historical perspective). The advent of MVPA methodology in neuroscience research has enabled detection

of finer-grained brain states. Thus, MVPA is an ideal tool for investigating neural replay of specific memory traces in the human brain.

Chapter 2: Introduction

2.1 Problem statement

The ability of sensory cues to guide memory consolidation during sleep was discovered only recently[L], and so there are many basic questions – both applied and mechanistic – that have yet to be investigated. For instance, how many reactivation cues are needed to influence behavior? Is it more beneficial to present cues during SWS compared to stage 2? Or during overnight sleep compared to a daytime nap? Are odors or sounds more effective as reactivation cues, and are there other sorts of cues (e.g., somatosensory) that could influence memory consolidation? Could memory benefits be amplified over multiple nights of cueing? Do cues truly evoke replay of specific memory content in the brain? If so, how does cued replay compare to spontaneous replay in terms of fidelity? And is cued replay similarly compressed during nREM? These are just a few examples of countless unanswered questions in the field, many of which are likely to be addressed in the next decade. The goal of this thesis work was to gain a better understanding memory reactivation and its neural correlates.

[L] The Rasch study afforded the first persuasive scientific evidence for reactivation in 2007[54], but earlier attempts to impart wisdom during sleep have been made. For instance, in 1942 Leshan tried to cure a group of summer campers of their nail-biting habit by playing the message "my fingernails taste terribly bitter" via phonograph while the children slept[99]. Though this endeavor proved moderately successful (12 of 20 children from the experimental group stopped biting their fingernails), there was no technology available at the time to ensure that message delivery was restricted to the sleep period.

2.2 Specific aims

Another unresolved question is whether different odors can reactivate multiple task components. Although olfactory stimuli have served as reactivation cues across several studies (e.g., Rasch et al., 2007[54]; Diekelmann et al., 2011[75]; Diekelmann et al., 2012[100]), none of those studies have involved presenting more than one odor cue during sleep. Rather, a single odor usually serves as a contextual cue to reactivate an entire task (as in Rasch et al., 2007[54]), or (less often) as a more precise cue to reactivate a single task component (as in Hauner et al, 2013[63] or Cox et al., 2014[77]). In 2014, Rihm and colleagues demonstrated that reactivation effects are odor-specific, which means that the odor presented in sleep must be the same as the odor paired with previous learning to improve memory[101]. These findings imply a certain level of specificity, but the study still only implemented a single reactivation odor during sleep, which was compared to a control odor that was not previously paired with learning. In contrast, several auditory cues are routinely used to reactivate highly specific memories (as in Rudoy et al., 2009[55]), and the cues are almost always semantically related to the linked content. Further research is required to determine whether odor cues could be employed in a similar fashion. Thus, the first aim of this thesis work was to discover whether multiple odor cues can facilitate consolidation of separate task components during sleep.

While studying the application of various reactivation strategies is certainly important, understanding how cues manage to strengthen and stabilize memories in the brain is arguably the more intriguing mystery. A small collection of recent EEG experiments suggests that reactivation cues evoke replay of specific memory traces during sleep[79,80], but this line of research is still in early stages. More studies are needed to reaffirm these findings, and to

establish a connection between neural replay and behavior. Moreover, the poor spatial resolution of EEG is not well-suited to pinpoint the specific brain regions that participate in memory replay, so alternative methods are needed to properly address this important gap. Therefore, the second aim of this thesis work was to determine whether odor cues evoke content-specific replay in the brain during sleep, and to investigate the relationship between replay and memory consolidation.

2.3 Research approach

To address these two specific aims, we implemented a novel olfactory reactivation paradigm that involved simultaneous EEG-fMRI acquisition and MVPA of fMRI data. Briefly, human subjects encoded a memory task during fMRI scanning, and then learned to associate each of four discrete task components with a familiar odor. Subjects then took a nap during EEG-fMRI acquisition, and two of the odors were presented during sleep to reactivate the associated memories. Moreover, subjects underwent extensive memory testing before and after the nap. By presenting two reactivation odors during sleep, we tested whether olfactory cues could selectively enhance two separate task components (Aim 1). By collecting fMRI data during learning and again during sleep, we studied the reemergence, or replay, of specific mnemonic information in response to odor cues, and the links between odor-evoked replay and memory performance (Aim 2).

Chapter 3: Olfactory reactivation paradigm

3.1 Overview

To address the specific aims outlined in the previous chapter, we designed a novel experimental paradigm where odors served as reactivation cues (**Fig. 3.1**). Human subjects first performed a visuospatial memory task, in which they learned the locations of objects from four categories (animals, buildings, faces, tools) during fMRI scanning. The purpose of this initial learning phase was to define fMRI ensemble representations (effectively, pattern templates) of each object category (**Fig. 3.1a**). Next, subjects learned to associate each of the four object categories with one of four distinct odors (**Fig. 3.1b**). Then, subjects were fitted with MRI-compatible EEG caps and took a nap during fMRI scanning, during which time two of the odors were re-presented in sleep, to selectively reactivate object representations from the associated categories (**Fig. 3.1c**). Memory for object locations was tested both before and after the sleep phase. The following sections contain a detailed explanation of each study phase, as well as some additional notes regarding paradigm development and rationale.

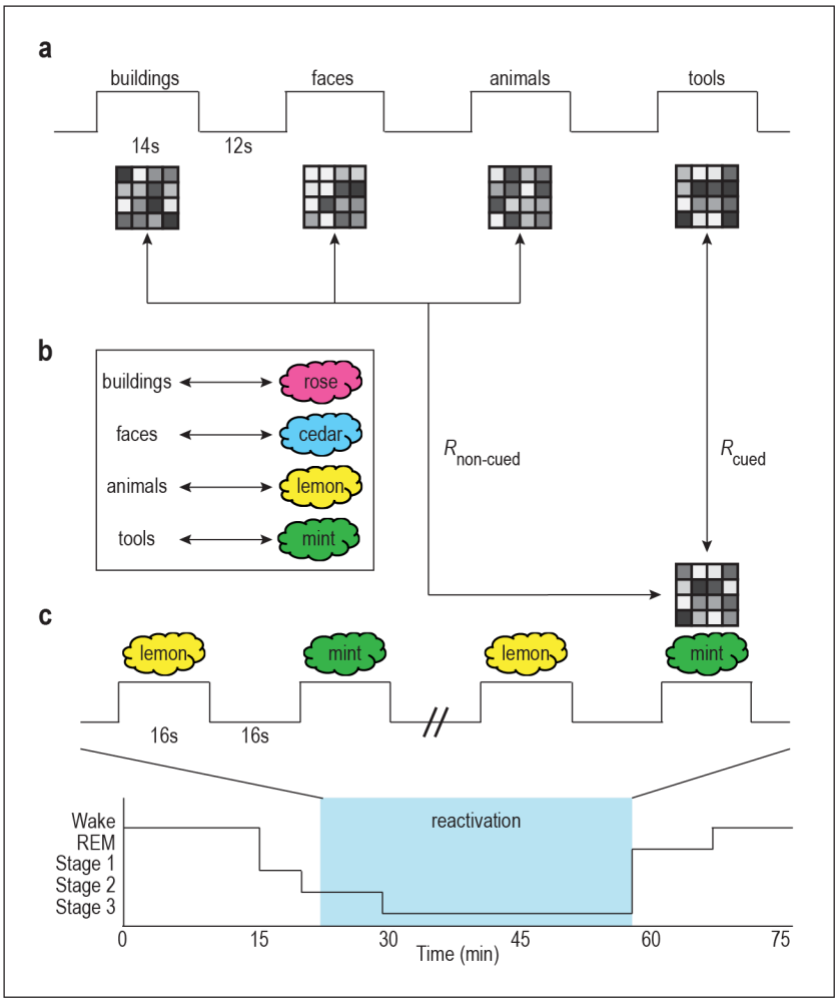


Figure 3.1. Olfactory reactivation paradigm. (**a)** During initial learning, category templates (depicted as 4-x-4 greyscale grids of voxels) were defined for each subject. **(b)** Subjects learned to associate each object category with a unique odor (e.g., mint odor + tool images). **(c)** Subjects napped during simultaneous EEG-fMRI acquisition. When subjects entered nREM sleep stages 2 and 3, two of the four odor cues were presented in 16s-on/16-s off blocks (i.e., during reactivation, blue box) to cue content from the associated object categories.

3.2 Experimental timeline

The study consisted of three days of experimental testing: (1) odor selection, (2) the main experiment, and (3) a follow-up visit. Odor selection took place the day before the main experiment, and the follow-up visit took place one week after the main experiment. Sleep-wake activity was monitored via actigraphy (Spectrum, Phillips) the night before the main experiment,

and subjects completed an online sleep diary (adapted from the National Heart, Lung, and Blood Institute) for one week prior to the main experiment.

3.3 Subjects

Thirty-two healthy human subjects (21 female, mean age = 25.25 years, age range, 19-37 years) gave written informed consent to take part in the study, which was approved by the Institutional Review Board at Northwestern University. All subjects were right-handed non-smokers under 40 years of age. Exclusionary criteria included history of significant medical or psychiatric illness, history of sleep disorder, use of psychotropic medications, and nasal congestion (to ensure that subjects could smell olfactory stimuli). Moreover, those that self-identified as frequent snorers were excluded, to improve the chances that subjects would breath through their noses during sleep (to receive olfactory reactivation cues).

To increase the likelihood that subjects would fall asleep in the MRI scanner during the sleep phase, subjects were required to go to bed at their habitual bedtime the night before the main experiment, and to wake up three hours earlier than their habitual wake time the following morning. Subjects were also asked to refrain from napping, and from consuming caffeine or alcohol on the day of the main experiment. Finally, in prior work with a collaborator, we found that first-time fMRI scanner subjects were often too anxious to fall asleep during scanning. Thus, subjects were only eligible to participate if they had undergone fMRI scanning at least once prior to the study, and reported that they thought they could feel relaxed and fall asleep during fMRI scanning.

Ultimately, fourteen subjects were excluded from the analysis. Eleven subjects were excluded because they had insufficient or fragmented SWS during the sleep phase, so we were not able to present each of the two odor cues a minimum of 14 times. An additional three subjects were excluded due to arousal and subsequent odor perception during the sleep phase. Eighteen subjects, equal to 56% of all subjects, were retained for analysis (11 female, mean age = 25.11 years ± .96 SEM). This success rate was in line with prior studies reporting subjects' ability to fall asleep and stay asleep in the fMRI scanner environment[75,102].

3.4 Stimuli

Visual stimuli consisted of 128 high-resolution portable network graphic images obtained from the internet. Images were cropped and displayed on a grey background with identifying labels, and included 32 well-known objects from each of four categories: animals, buildings, faces, and tools (**Fig. 3.2a-d**). To ensure that objects were familiar to subjects, an independent group of 12 subjects provided labels for each image of a larger stimulus set, and the most easily-identifiable images were retained for use in the study[M]. Thirty-two additional scrambled images were generated from a subset of category images, and accompanying labels consisted of arbitrary combinations of letters (**Fig. 3.2e**). Scrambled images were included as a localizer, so that we could identify functionally-defined voxels during analysis. Ultimately, this was not necessary.

[M] Labmate James Howard completed this task, and demonstrated an exceptional ability to identify international landmarks that dwarfed that of other subjects.

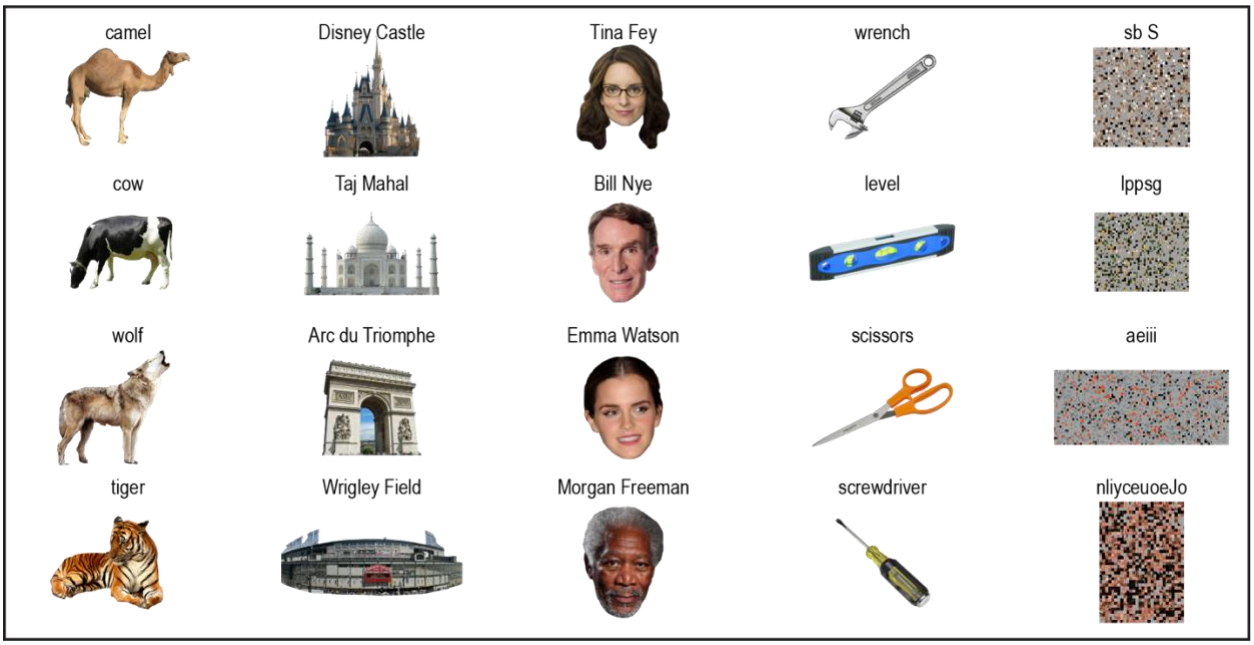


Figure 3.2. Examples of visual stimuli. Subjects learned the locations of visual object from five categories: animals **(a)**, buildings **(b)**, faces **(c)**, tools **(d)**, and scrambled **(e)**.

Olfactory stimuli comprised four easily distinguishable, familiar odorants. To ensure that odor cues could be easily discriminated from each other, four odorants were selected from a larger set of eight well-known odorants (banana, cedar, cinnamon, garlic, lemon, mint, rose, vanilla) on an individual subject basis (see Section 3.5). Odors were obtained from a variety of sources (e.g., Sigma Aldrich, Aroma Workshop in Lincoln Park), and were replenished on a regular schedule to prevent weakening. Odorants were delivered by a custom 12-channel computer-controlled olfactometer ("Horton") at a flow rate of 6.72 L/min, via a Teflon tube secured beneath the nose.

Notes on rationale and development. We opted to use familiar, nameable odors, because we felt it would be easier for subjects to build meaningful associations between those odors and object categories. Along these lines, after the main experiment, subjects would often comment that they devised mnemonic strategies to internalize associations (e.g., "tools are used to cut cedar", "faces

chew mint gum"). Moreover, we chose to implement a faster flow rate than is typical for studies in our lab, because this meant we could pause odor delivery more quickly if subjects were to wake up suddenly during reactivation. We ultimately regretted this decision, since the higher flow occasionally dried out subjects' nasal passages after prolonged exposure.

3.5 Odor selection

The day before the main experiment, subjects took part in an odor selection task so that we could determine which four odors (from the larger set of eight) were most discriminable for that subject. Immediately prior to the task, we familiarized subjects with all eight odors by instructing them to smell the odorant contained in each of eight amber bottles, and providing the respective labels. Subjects then completed a computer task, where they made pairwise similarity ratings between all possible odor pairs (8 choose 2 = 28 unique pairs). Each odor pair was presented two times, and odor order was counterbalanced across the two trials. There were 56 trials in total. During each trial, subjects were cued to sniff two consecutive odors presented 4.5-s apart, and then they made a pairwise similarity rating on a visual analog scale from 'extremely different' to 'extremely similar'.

After subjects completed the rating task, we asked them whether they were highly averse to any of the odor stimuli. This was very unusual, as the odors were generally perceived as pleasant (see Section 3.6.1). However, if the subject reported that they were highly averse to an odor, we removed it from the stimulus set prior to selecting four odors for the main experiment.

A “pairwise similarity score” was then calculated for each odor pair by taking the average similarity rating across two trials. Based on pairwise similarity scores, four odors were selected to minimize perceptual overlap (i.e., low pairwise similarity scores for odor pairs included in the final set of four). Thus, a “total similarity score” was computed for each possible combination of four odors (8 choose 4 = 70 total combinations). For instance, for a set of four odors (A, B, C, and D), with all possible pairwise combinations among these odors, the total similarity score would be the sum of similarity ratings for A versus B, A versus C, A versus D, B versus C, B versus D, and C versus D. The set of four odors with the lowest total similarity score was retained for the main experiment.

Next, we gave subjects further instructions and reminders regarding the main experiment. We also provided them with an actiwatch to monitor their sleep-wake activity. Subjects were instructed to wear the actiwatch on either wrist, and to continue wearing it until they returned to the lab for the main experiment. Subjects were also instructed to mark their sleep and wake times by pressing and holding a button on the actiwatch, as an additional measure of self-report. The purpose of actiwatch monitoring was to increase the chances that subjects would adhere to sleep requirements (i.e., go to sleep at their regular bedtime, wake up three hours early) the night prior to the main experiment.

Notes on development and rationale. During piloting, we observed that subjects were highly variable in their ability to discriminate between odors. For instance, some subjects perceived minty and citrusy odors (e.g., spearmint and lemon) as very similar, while others did not. Thus, because subjects already had to come to the lab to pick up the actiwatch the day before the main

experiment, we implemented this task to minimize perceptual overlap between odor stimuli. In retrospect, we do not feel that odor selection was very reliable. On a post-experiment questionnaire, four out of 18 subjects reported having difficulty distinguishing between odors in the final set (e.g., cedar and rose, cinnamon and lemon), despite careful selection. It likely would have been more effective (and more efficient) to identify a stable set of four easily-distinguishable odors based on a small independent sample of subjects.

3.6 Main experiment

The main experiment lasted approximately 6.5 hours, and included nine task blocks: odor ratings, initial learning, odor-category association, learning to criterion, EEG setup, pretest, sleep, interference learning and test, and posttest (**Fig. 3.3**[N]). Initial learning and sleep task blocks took place in the MRI scanner. Critically, data for the main experiment was collected at night, such that the sleep phase aligned with each subject's habitual bedtime, to further increase the likelihood that subjects would fall asleep (and stay asleep) in the fMRI scanner during reactivation. Because subjects' habitual bedtimes were highly variable, the main experiment began anywhere between 7pm and 10:30pm, and ended anywhere between 1:30am and 5am. Immediately following the main experiment, subjects completed a brief questionnaire before leaving the scanner facility[O].

[N] Since the odor ratings task took less than five minutes to complete, it is not indicated on Figure 3.3.

[O] Given that the experiment ended late at night, subjects were given the option to return home in an Uber (paid for by the lab), to mitigate safety concerns.

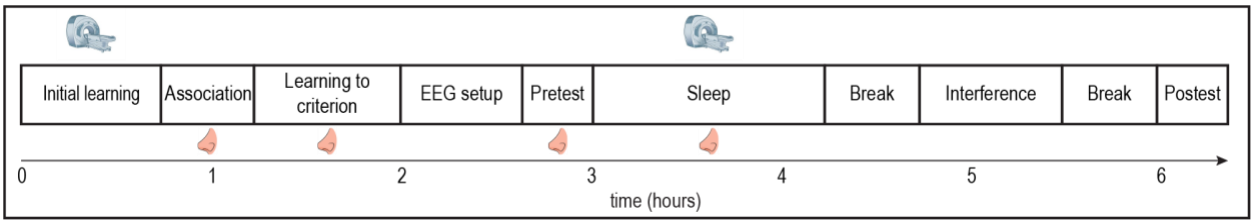


Figure 3.3. Main experiment timeline. Subjects completed several task blocks over the course of approximately 6.5 hours. MRI scanner symbols indicate scanned study phases, and nose symbols indicate study phases during which odors were presented.

3.6.1 Odor ratings

When subjects arrived at the MRI facility, we first re-familiarized them with the four odors that were chosen for the main experiment, again by prompting them to smell each odor from an amber bottle, and specifying the odor label. Then, subjects completed a computer task where they made ratings for the four selected odors. On each trial, subjects were cued to sniff upon odor presentation, and then they rated the perceived odor in terms of intensity (from 'barely detectable' to 'extremely strong') and valence (from 'extremely unpleasant' to 'extremely pleasant'). Subjects made two intensity ratings and two valence ratings for each of the four odors. Odors were generally perceived as moderately strong (*mean intensity rating* = .71/1 ± 0.02 SEM), and moderately pleasant (*mean valence rating* = .67/1 ± 0.02 SEM). Moreover, odors that were selected as within-sleep reactivation cues versus those not selected did not differ in terms of perceived intensity ($t_{(17)} = 0.60$, $p = 0.56$) or valence ($t_{(17)} = 0.18$, $p = 0.86$).

3.6.2 Initial learning

Next, subjects completed an MRI safety form, and changed into MRI-safe surgical scrubs provided by the facility. Then, we provided subjects with a description of the MRI environment as well as task instructions. Subjects were told to learn the locations of visual stimuli (object

images and scrambled images[P]), which appeared on a 4-x-4 greyscale grid (**Fig. 3.4**) while undergoing fMRI scanning. Each of the 160 images appeared a total of three times over the course of the task, which was divided into 12 2.25-minute runs. Each run consisted of five blocks (animals, buildings, faces, tools, scrambled) presented in a random order, with 12 s between blocks. Each block lasted 14 s, and consisted of a series of eight images presented on the grid for 1 s each, with 0.75 s between consecutive image presentations. Grid locations were balanced such that two objects per category were presented in each of the 16 grid spaces. During this task, subjects were not required to make any kind of response. Rather, they simply observed the visual objects on the grid. To motivate subjects to attend to the task, we informed them that the better they learned object locations during this phase, the less time later phases of the experiment would take.

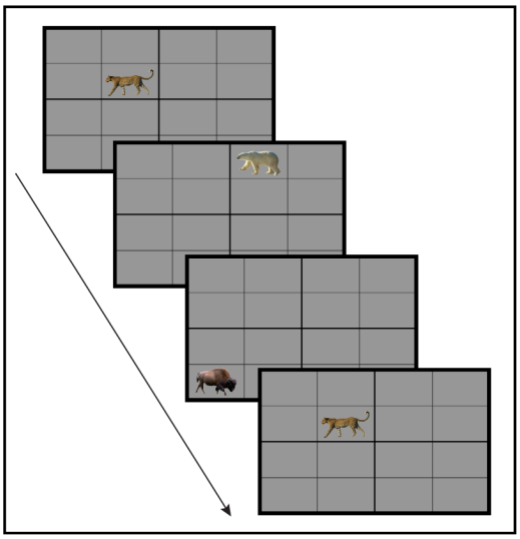


Figure 3.4. Initial learning task. Objects appeared in one of 16 possible grid spaces.

Notes on development and rationale. The purpose of this phase of the experiment was two-fold. First, subjects began to learn object locations. Second, and more importantly, we could use the fMRI data from this task to define the unique templates of MVPA activity in response to each of

[P] Since scrambled images were only incorporated to allow for identification of object-selective voxels, they did not appear in later phases of the experiment. However, subjects were still instructed to learn their locations to avoid memory confounds. On the post-experiment questionnaire, we probed whether subjects expected to be tested on scrambled image locations. Of the 18 subjects, 13 expected to be tested, 4 did not have expectations either way, and only one expected not to be tested.

the four object categories on a subject-by-subject basis. For this reason, it was important that subjects learned object locations prior to the introduction of odor cues, to ensure that fMRI templates exclusively reflected visual category information (and not odor information).

Prior to running the study, we collected data from five pilot subjects that completed this initial learning phase in the fMRI scanner (during the day). Pilot analyses revealed robust decoding of the four object categories in several ROIs (e.g., fusiform cortex) for all five subjects (**Fig. 3.5**). Moving forward, we felt confident that our task would evoke distinct MVPA ensemble patterns – a critical prerequisite to detecting replay of the same patterns during sleep.

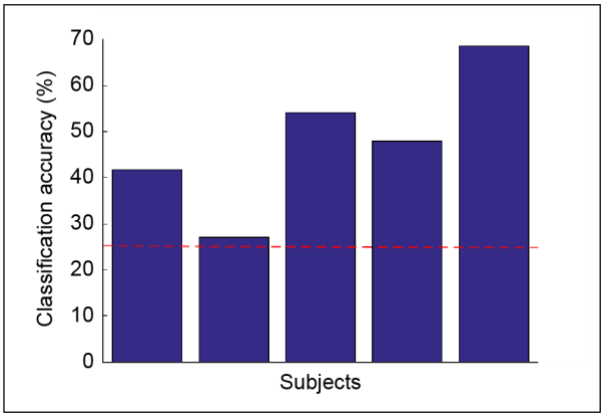


Figure 3.5. MVPA of fMRI pilot data from initial learning phase. All five pilot subjects demonstrated above-chance four-way classification accuracy in a ROI in bilateral fusiform cortex. Chance is 25% (dashed red line). Note that pilot MVPA analyses employed an SVM-based approach, while later analyses employed a correlation-based approach.

3.6.3 Odor-category association

Following initial learning, I led subjects from the scanner to a nearby testing room, where they completed a computer task to learn associations between each of the four odors and each of the four object categories. Odor-category pairs were randomly assigned for each subject. On each trial, subjects were cued to sniff upon odor presentation, and then immediately afterwards one object from each of the four categories appeared in the four different quadrants of the screen. Category objects were identical to those presented during initial learning. Subjects were

instructed to select the object that belonged with the presented odor as quickly and accurately as possible, and then a green box appeared around the correct choice for 2 s, as feedback. Trials were spaced at least 6 s apart, to avoid habituation and odor cross-contamination. The task continued until each odor-category pair was correctly identified 16 times (number of trials for perfect performance = 64), to ensure robust odor-category associations. Subjects learned these associations rapidly (*range* = 66-78 trials to reach criterion, *mean* = 69.44 ± 0.74 SEM).

Notes on development and rationale. Initially, we were not going to include an explicit odor-picture association task as part of the study. Rather, we felt that subjects would pick up on odor-category associations naturally, because odors would be paired with object categories while subjects learned object locations to criterion, and during the pretest (see Sections 3.6.4 and 3.6.6). However, during behavioral piloting, we found that subjects were not internalizing odor-category associations (or at least not consciously), perhaps due to the cognitive demands of the object location task, and because the background odor was irrelevant to task performance. So far, it is not clear from the literature whether a conscious link is required between sensory cues and associated memories for reactivation to work. However, olfactory reactivation paradigms often employ a single contextual odor cue, while auditory reactivation paradigms usually deliver multiple sound cues with inherent links to the paired information[Q]. In these cases, the cue-memory relationship is likely obvious to the subject, even when it is not pointed out. Thus, we included the association task so that subjects would forge a conscious link between odors and

[Q] See Fuentemilla et al., 2013[76] for a counterexample, where experimenters used sound-word pairs that were not semantically related (e.g., word "milk" + sound of a harp, word "museum" + sound of a person laughing). However, in this case, subjects were trained explicitly to associate the word-sound pairs prior to reactivation.

categories, in case this was needed, or would enhance reactivation effects. Ideally, future work will address whether explicit knowledge of cue-memory associations is necessary to observe memory enhancement. Although such a study would probably be considered incremental, it would be very practically informative.

3.6.4 Learning to criterion

Subjects then continued to learn the same object locations as in the initial learning phase, with two key differences: (1) objects appeared in the presence of category-specific odors, and (2) subjects were actively tested on their knowledge of object locations. During each trial, an object appeared in the center of the screen for 0.5 s, and then subjects attempted to select the grid space where the object belonged (16 grid spaces, chance = 6.25%). Then, the object appeared in the correct grid space for 0.5 s, as feedback. The task continued until subjects placed each object in the correct grid space once. For most of the task, objects were presented in category blocks of eight objects per category during continuous presentation of the associated odor. Near the end of the task, as the number of objects per category dwindled to less than eight, the length of category blocks varied depending on the number of remaining objects. Blocked presentation of objects allowed for efficient delivery of associated odors, as blocks were spaced 12-s apart to avoid habituation and odor cross-contamination. Because task performance was tied to subjects' ability to memorize object locations during the initial learning phase, there was considerable variability in the number of trials needed to reach criterion (*range* = 178-367 trials, *mean* = 250.22 ± 12.64 SEM).

To ensure continued attention to odor-category associations, subjects performed 16 "catch" trials (four per odor-category pair) over the course of the task. During catch trials, subjects were cued to sniff, and then they selected the category that belonged with the presented odor. Subjects then received feedback (green box, identical to that in the odor-category association task described in Section 3.6.3). Subjects retained a strong knowledge of odor-category associations during these catch trials (*mean* = 94.79% correct, ± 1.69% SEM), and there was no significant difference across categories in terms of mean trials correct (repeated-measures ANOVA: $F_{(3, 68)} = 1.06$, $p = 0.37$) or reaction time (repeated-measures ANOVA: $F_{(3, 68)} = 0.67$, $p = 0.52$).

3.6.5 EEG cap

After learning to criterion, subjects were led to the MRI control room, where the EEG system and recording computer were located. We briefly explained the EEG capping procedure, and then subjects were fitted with an MRI-compatible EEG cap (BrainCap MR, Brain Products). See Section 3.8.2 for more details. Next, we collected EEG baseline measures (blink 10x, grind teeth 5 s, eyes open 30 s, eyes closed 30 s), which allowed us to confirm signal quality, and to observe patterns in the subjects' data (e.g., presence or absence of alpha activity) prior to the introduction of MRI artifacts (see Section 3.6.7). Finally, subjects were fitted with an MRI-compatible breathing belt, to record respiration during the sleep phase.

Notes on development and rationale: We fit subjects with an EEG cap prior to the pretest for two reasons: (1) to allow time for the EEG gel to soak in prior to the sleep phase, thus improving impedance, and (2) to minimize time between the pretest and sleep phase.

3.6.6 Pretest

While wearing the EEG cap and breathing belt, and immediately prior to the sleep phase, subjects were led back to the testing room. They were then tested on their knowledge of object locations. During this phase, each object appeared in the center of the screen for 0.5 s, and subjects selected the grid space where they believed the object belonged, without receiving feedback. Rather, the object appeared in the selected grid space, whether or not it was the correct one. Each object only appeared once during the task. As in the previous phase, objects were presented in category blocks of eight objects per category during continuous presentation of the associated odor, and blocks were spaced 12-s apart.

Again, subjects performed 16 catch trials (four per odor-category pair) over the course of this pretest session. Catch trials were identical to those from the previous task, except that subjects did not receive feedback (no green box). During catch trials, subjects continued to demonstrate excellent retention of odor-category associations (*mean* = 95.83% ± 1.13% SEM), again without any significant differences across categories in terms of mean trials correct (repeated-measures ANOVA: $F_{(3, 68)} = 0.39$, $p = 0.73$) or reaction time (repeated-measures ANOVA: $F_{(3, 68)} = 0.92$, $p = 0.43$).

3.6.7 Sleep

Prior to the sleep phase, we instructed subjects to relax and try to fall asleep during scanning. We also assured them that we could use their data regardless of whether they slept (although this was not the case), to relieve any anxiety they might have about falling asleep in the scanner. We also told subjects that they may or may not receive odors during scanning, depending on which

experimental group they were randomly assigned to. Although the goal was to deliver odors to every subject, we wanted to decrease subjects' expectation that they would necessarily receive odors during the sleep phase. Finally, subjects were further instructed to press an MRI-compatible button during the nap if they perceived an odor, although we emphasized that there was no need to actively search for odors.

Subjects then returned to the MRI scanner. Immediately prior to the scan, we collected the same EEG baseline measures as previously, to observe the perturbation of the EEG signal in the B_0 magnetic field. Then, subjects relaxed and tried to fall asleep during approximately 75 minutes of continuous scanning A the start of the scan, subjects were given the option to take part in a monotonous reaction time task for approximately five minutes. Subjects who chose to participate pressed an MRI-compatible button each time a central crosshair changed from white to red (approximately once every 30 s), and they were encouraged to disengage from the task and close their eyes at any time if they felt drowsy. The purpose of this task was to help subjects re-acclimate to the scanner environment, and it was intentionally dull to induce sleepiness.

During this session, we monitored subjects' EEG data for signs of sleep from the adjacent control room. During sleep stages 2 and 3, two of the four task-related odors were presented in alternating 16-s on/16-s off blocks (to prevent habituation), as category-specific reactivation cues. Odor were selected strategically as cues, to ensure that category reactivation was counterbalanced across subjects (six possible category pairs, each presented to three of the 18 subjects). To decrease the chances of subjects waking up during odor presentation, we waited to observe at least two minutes of continuous stage 2 sleep prior to initiating the odor delivery

protocol. Since odor presentation depended critically on the duration and depth of sleep for each subject, there was considerable variability across subjects in terms of the number of reactivation cues presented (*range* = 30-105 total odor presentations, *mean* = 50.61 ± 4.61 SEM).

After the sleep phase, subjects exited the scanner and took a quick shower at the scanner facility, to rinse EEG gel from their hair and overcome sleep inertia. For the remainder of the main experiment, subjects did not receive odors.

Notes on development and rationale: The sleep phase was by far the most technically difficult part of the study to carry out. EEG data collected in the scanner is severely contaminated by two artifacts: (1) the gradient artifact, and (2) the BCG artifact. The gradient artifact completely obscures the EEG signal (**Fig. 3.6a**), due to the voltage imparted by rapidly fluctuating magnetic field gradients and radiofrequency pulses during scanning[103,104]. The BCG artifact resembles an EKG waveform, and arises from the interaction between the B_0 magnetic field and tiny movements caused by blood pulsing through arteries embedded in the scalp[105,106]. During the sleep phase, it was essential to remove these artifacts from the EEG data online (**Fig. 3.6b-c**), at least to the extent that we could visually identify sleep stages 2 and 3. The gradient artifact can be determined by scanning parameters. It is uniform and predictable, and thus it can be easily subtracted from the EEG data. Unlike the gradient artifact, the BCG artifact is highly variable across heartbeats, and manifests to different degrees in different subjects, which makes it much more difficult to remove consistently.

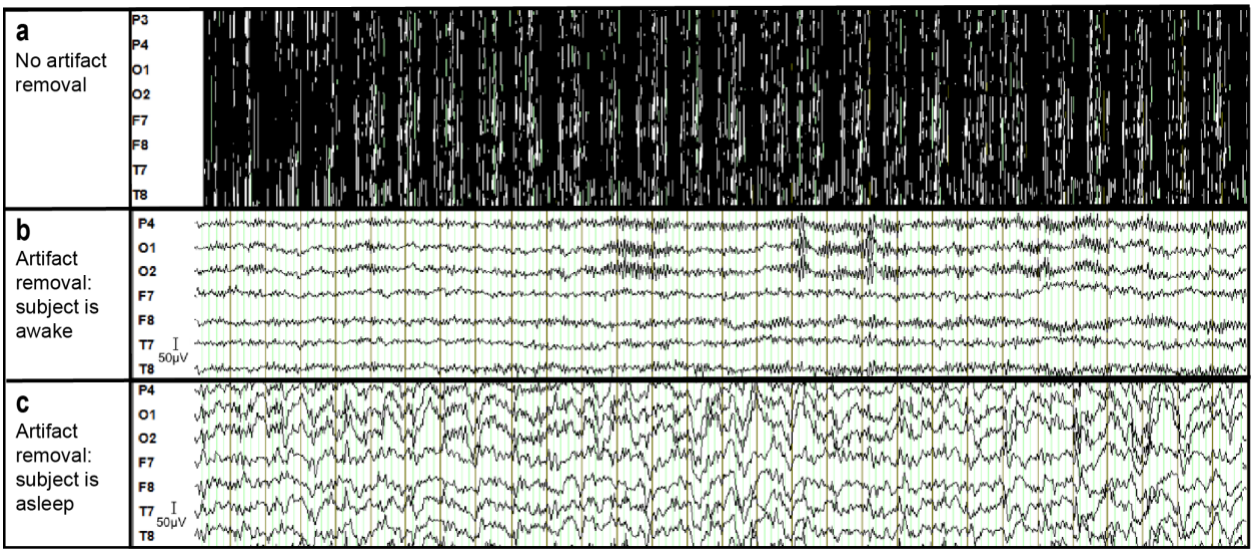


Figure 3.6. EEG data collected in the MRI scanner. Without artifact removal, the signal is completely obscured by the gradient artifact **(a)**. With artifact removal, it is typically possible to observe alpha activity during wake **(b)**, and SWA during SWS **(c)**[R]. EEG traces depict signals as observed online, without post-processing.

In a previous collaboration with visiting graduate student Julia Rihm, we collected EEG data in the MRI scanner with a Neuroscan system (MagLink Real Time) that was available at the scanning facility. The purpose of our collaboration was to collect additional data for Julia's project, where the goal was to determine the effects odors on brain activity during sleep versus wake states. Our efforts to collect data were mostly unsuccessful for two reasons: (1) the EEG-fMRI equipment we were using was not optimal for removing artifacts online, and (2) subjects were having trouble falling asleep during scanning.

To combat these issues, we applied for an internal grant (Core Facilities equipment funding) to purchase state-of-the-art EEG-fMRI equipment (BrainAmp MR Plus, Brain Products) for the

[R] This is a particularly clean example of EEG data collected in the MRI scanner, since the subject exhibited an extremely mild BCG artifact. For many subjects, EEG data was not as clean following online artifact removal.

scanner facility. The grant was successful, and the new equipment proved to be superior to the Neuroscan system. One essential feature of the new system was its ability to interface with the MRI scanner, to exactly synchronize the EEG sampling rate with the scanner clock. This allowed for more precise removal of gradient artifacts. Although visually scoring sleep online was difficult (especially when data was contaminated by robust BCG artifacts that could not be effectively removed online), we felt reasonably confident in our ability to present odors in stages 2 and 3 of sleep, where K-complexes and slow-waves typically stood out above scanner-induced noise.

Moreover, we developed a comprehensive strategy to make it easier for subjects to fall asleep in the MRI scanner. Various aspects of the strategy appear previously, but it is outlined in full in the table below.

Approach	**Rationale**
Only recruited subjects that had been in MRI scanner previously, and who felt they could relax and fall asleep in scanner environment	First-time scanner subjects would likely be too anxious to sleep
Subject went to bed at their usual bedtime the night before the main experiment, and woke up three hours early the next morning	Sleep deprivation ensured subjects would be especially drowsy during the sleep phase
The main experiment occurred at night, such that the sleep phase aligned with subjects' habitual bedtimes	Sleep phase occurred during a natural circadian dip
Subjects refrained from drinking caffeine or alcohol, and from taking naps during the day of the main experiment	These measures ensured subjects would be especially drowsy during the sleep phase

Subjects were told to try to fall asleep during the sleep phase, but were also told that we could use their data either way	Reduced pressure on subjects to sleep, thus reducing anxiety
Subjects had option of performing crosshair task at start of sleep phase	Gave subjects something to focus on (besides trying to fall asleep), and slow pace of task was soporific
Lined scanned platform with foam mattress topper, and used same material to cushion subjects' head in coil	Ensured subject comfort
Opted to use Teflon tube to deliver odors, rather than odor mask	Reduced constraint (and therefore discomfort) in scanner coil

This approach proved to be successful, as over 50% of subjects that participated in the study were usable (see Section 3.3). Although we determined sleep stage online to inform delivery of reactivation cues, we later formally scored sleep offline in accordance with standard criteria[11] (**Table 3.1**). See Section 3.8.2 for more details.

Sleep Stage	Time (min ± SEM)	Percentage (% ± SEM)
wake	9.92 ± 2.61	13.11 ± 3.48
stage 1	8.69 ± 1.82	11.39 ± 2.34
stage 2	26.5 ± 3.41	34.95 ± 4.26
stage 3	30.17 ± 4.12	40.54 ± 5.56
total sleep time	65.36 ± 2.79	86.89 ± 3.48
total length (wake + sleep)	75.28 ± 1.25	100

Table 3.1. Time spent in each sleep stage. Offline sleep scoring revealed that 99.45% of odors were presented during stages 2 and 3 of sleep, and most cues (77.56%) were presented during stage 3 of sleep.

After scoring the sleep data, we ran two follow-up analyses to compare EEG signals for (1) sleep stage 1 versus SWS, and (2) odor-on periods versus odor-off periods. Specifically, we calculated

EEG power spectral density for each condition by performing a fast Fourier transform analysis of frontal electrode 'Fpz' using the "pwelch" function in Matlab. We then ran a 2-factor (condition-x-frequency) repeated measures AVOVA to determine effects of odor cues and sleep stage on EEG power spectral density between 0.5 and 20 Hz. We found that SWA (0.5 to 2 Hz) was enhanced during SWS compared to sleep stage 1 (repeated-measures ANOVA, condition by frequency interaction: $F_{(39,17)} = 5.90$, $p < 0.001$; **Fig. 3.7a**). This was expected, as scoring of SWS requires visual identification of SWA. Still, given the challenges of scoring EEG data acquired in the scanner, we conducted this analysis as proof of concept, and the results were reassuring. Perhaps more critically, there were no significant spectral differences between odor-on and odor-off periods (repeated-measures ANOVA, condition by frequency interaction: $F_{(39,17)} = 0.31$, $p = 1.00$; **Fig. 3.7b**), suggesting that odor delivery was not associated with physiological arousal.

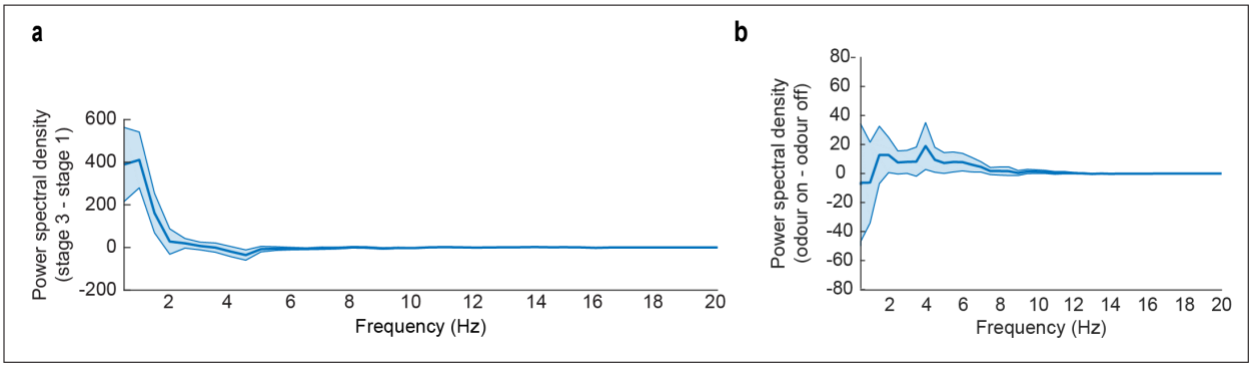


Figure 3.7. EEG spectral power analysis. An analysis of EEG power spectral density ($\mu V^2/Hz$) revealed elevated SWA during stage 3 sleep, as compared to stage 1 sleep **(a)**, and there were no spectral differences when comparing odor-on and odor-off periods **(b)**. Error bars depict mean ± SEM across subjects.

3.6.8 Interference learning and test

After taking a brief shower (and approximately 30 minutes after waking), subjects were led to the testing room, where they learned new grid locations for the same objects as presented in initial learning. Task structure was identical to that of initial learning, except that intervals

between category blocks were limited to 4-s (longer intervals were unnecessary in the absence of odors), and subjects advanced the task between blocks (rather than waiting for the experimenter). Subjects were allowed a 5-minute break after interference learning, and then they were tested on their knowledge of the new object locations (without feedback). The interference test was identical to the pretest, except that intervals between category blocks were limited to 4-s, and there were no odors or catch trials.

Notes on development and rationale. The purpose of including a memory interference task was twofold. First, sleep is thought to have a "stabilizing" effect on memory, and introducing memory interference has been shown to provide added sensitivity when assessing effects of sleep on memory performance[107]. More practically, during piloting, and prior to introducing the interference phase, subjects' memory for object locations did not decline substantially from pretest to posttest (**Fig. 3.8a**). Since it was critical to induce forgetting from pretest to posttest to observe changes in memory retention for cued versus non-cued objects, the interference phase was a necessary component (**Fig. 3.8b**).

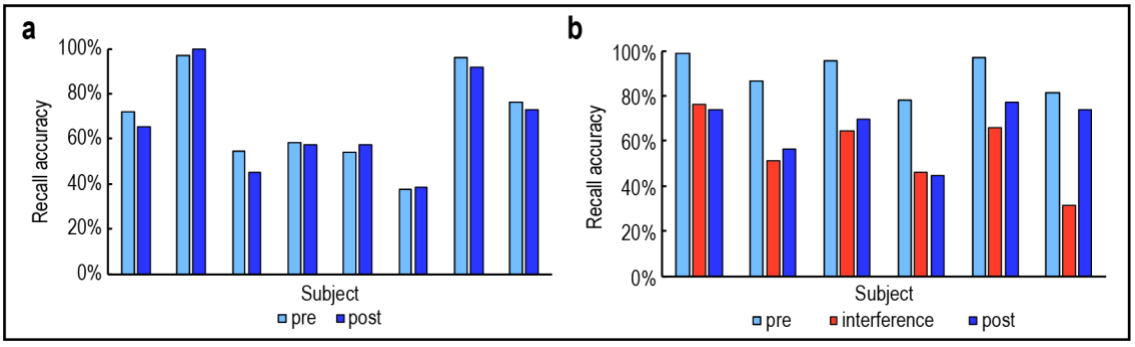


Figure 3.8. Memory performance with and without interference component. (a) Prior to introducing memory interference, recall accuracy did not decline substantially from pretest to posttest. **(b)** Interference introduced substantial forgetting from pretest to posttest.

3.6.9. Posttest

Next, subjects took a 30-minute break in the testing room. They could choose how to spend their time during the break[S], except that they were not allowed to sleep. Then, subjects completed a posttest to assess their knowledge of the original (non-interference) object locations from initial learning. Task structure was identical to that of the interference test.

3.7 Follow-up visit

One week after the main experiment, subjects returned to the lab for a series of follow-up memory tests. Although the follow-up visit was scheduled in advanced, subjects were not given information regarding the nature of follow-up tasks. First, subjects completed a free recall test, in which they were given two minutes per category to list as many objects from that category as they could remember. Category order was randomized, and subjects recorded their responses on a paper form. We told subjects when to switch categories. If subjects could remember an object, but were unable to recall its label, we encouraged them to do their best to describe it in on the form (e.g., Bruce Willis = actor in Sixth Sense and Diehard). Subjects were instructed to try to keep remembering objects until the end of the 2-min period.

Next, subjects were tested on their retention of the original object locations (from initial learning) in a task that was identical to the posttest from the main experiment. Finally, subjects were verbally instructed to sniff each of the four odorants, which were contained in amber bottles, and they recounted which category they thought each odor was paired with during the

[S] Subjects with long hair often opted to use a hair dryer during the break. Subjects also chose to use electronic devices, study, or chat with experimenters.

main experiment. Seventeen of 18 subjects were still able to remember odor-category associations during the follow-up visit.

Notes on development and rationale. Although the reinforcing effects of sensory cues on associated memories are well-established, memory testing in reactivation studies typically occurs immediately before and after the sleep period. It is not known whether reactivation effects persist beyond the scope of the experiment, so we implemented the follow-up session one week after the main experiment to address this question. Ultimately, the free recall test was not a very sensitive measure, and free recall data was difficult to score. When subjects could not remember an object's label, they occasionally provided a description that was difficult to interpret (e.g., "fortress in China which is colorful"). Moreover, subjects sometimes recorded objects that never appeared during the study (e.g., "mouse", "Statue of Liberty", "Robert De Niro", "chain"). Most critically, subjects' ability to recall objects was highly variable across categories (see Section 4.2), and we could not control for cross-category variability since we opted not to collect a free recall baseline measure during the main experiment. Thus, the free recall test may not have been sufficiently sensitive to detect reactivation effects.

3.8 Technical details

3.8.1 MRI data acquisition

During the initial learning and sleep sessions, MRI data were collected with a 3-Tesla scanner (Siemens PRISMA) equipped with a 64-channel head coil, using T2-weighted echoplanar imaging. Each volume comprised 40 slices covering the whole brain (field of view, 210-x-203 mm; matrix size, 124-x-120 voxels; slice thickness, 3mm; in-plane resolution, 1.69-x-1.69 mm;

repetition time, 2500-ms; echo time, 25-ms; flip angle, 80°). An additional whole-brain anatomical T1-weighted MRI scan was acquired immediately following the initial learning session, for coregistration purposes (GRAPPA; voxel size, 0.8mm^3).

Notes on development and rationale. Although the scanner was capable of multiband imaging, it was not possible to use a multiband protocol due to the MRI-compatible EEG equipment. The EEG software was not equipped to remove multiband artifacts online, and the hardware had not been tested during multiband scanning to ensure subject safety.

3.8.2 EEG data acquisition and processing

During the sleep session, EEG data were collected with an MRI-compatible EEG system (BrainAmp MR Plus, Brain Products). The 32-channel EEG cap contained 26 scalp electrodes[T] and two EOG electrodes, as well as wires to connect electrodes for chin EMG and ECG. EEG gel (Abralyt HiCl, Easy Cap) was applied to each electrode, and then impedance was minimized (< 20 kOhms). Special care was taken to minimize impedance for the ECG electrode (< 10 kOhms), since obtaining a strong ECG signal was particularly important for BCG artifact removal. The EEG cap was secured with mesh netting, to keep electrodes close to the scalp.

EEG data were sampled at 5 kHz, and gradient and BCG artifacts were removed online using Brain Products software (Rec View). At a later stage, EEG data were post-processed offline, also using Brain Products software (Analyzer). The following processing steps were implemented:

[T] The EEG cap contained flat electrodes, such that they were flush with the fabric of the cap. This cap design was optimal for subject comfort, since electrodes could not poke into the back of the scalp while subjects were lying prone on the scanner platform.

gradient artifact removal, BCG artifact removal (in semiautomatic mode), segmentation (to omit data recorded before and after the scan), re-referencing (to left mastoid), filtering (scalp electrodes: 0.1 Hz to 30 Hz, EMG electrodes: 10 Hz to 62 Hz, ECG electrode: 0.3 Hz to 70 Hz), and edit channels (eight channels were retained for sleep scoring: “Fp1”, “FZ”, “PZ”, “OZ”, “C3”, “EMG1”, “EOG1”, and “EOG2”).

Chapter 4: Odor cues boost consolidation of category-specific memories

4.1 Overview

The first aim of this thesis work was to address whether more than one olfactory cue could be deployed in sleep to reactivate discrete components of a memory task. To address this aim, we analyzed the behavioral data from 18 subjects that successfully completed the olfactory reactivation paradigm, which is outlined in detail in Chapter 3. This involved assessing recall accuracy and RTs at each of four test phases (pretest, interference test, posttest, and follow-up test), and free recall performance at the follow-up test. Critically, behavioral measures were compared across cued and non-cued object categories. In this chapter, we report findings from these analyses, and briefly discuss their implications.

4.2 Methods and results

4.2.1 Task structure

Briefly, during each of the four memory tests, we assessed subjects' knowledge of the object locations that they had learned previously during the experiment. In the pretest, posttest, and follow-up test, subjects reported object locations from initial learning, whereas in the interference test, they reported object locations from interference learning. On each trial, an object appeared in the center of the screen, and then subjects selected the space on a 4-x-4 grid where they believed the object belonged (16 grid spaces, chance = 6.25%). Each of the 128 category-specific objects appeared once on each test, and objects were presented in category blocks (8 objects per block). This allowed for efficient odor presentation during the pretest, although none of the other tests included odors. Subjects did not receive feedback during test

phases. Rather, objects appeared in whichever grid space subjects selected (regardless of accurate placement). For each test, recall accuracy and RTs were recorded as behavioral measures[U]. Recall accuracy refers to subjects' ability to place objects in the correct grid space, while RT refers to the amount of time subjects took to select a grid space after the object disappeared from the center of the screen.

Finally, subjects completed an additional free recall test prior to visuospatial testing during the follow-up visit. During the free recall test, subjects wrote down as many objects as they could remember from the main experiment for each object category in turn, with a 2-m time limit per category (see Section 3.7 for more details).

Overall memory outcomes

Recall accuracy: Recall accuracy was defined as the overall percentage of objects that subjects placed in the correct grid space at each of the four tests. For each test, we used t-tests to address the hypothesis that recall accuracy was greater than chance level (6.25%). We found that subjects demonstrated robust visuospatial recall at the pretest (73.17% correct ± 2.71% SEM, $t_{1\text{-}tail(17)} = 24.68$, $p < 0.001$), interference test (41.11% correct ± 4.02% SEM, $t_{1\text{-}tail(17)} = 8.66$, $p < 0.001$), posttest (56.70% correct ± 2.92% SEM, $t_{1\text{-}tail(17)} = 17.27$, $p < 0.001$), and follow-up test (38.24% correct ± 2.92% SEM, $t_{1\text{-}tail(17)} = 10.96$, $p < 0.001$). Moreover, we also used a paired t-test to evaluate the prediction that recall accuracy decreased from pretest to posttest, and found that

[U] During the memory pretest, we also assessed subjects' ability to retrieve odor-category associations. Further discussion of association performance is outside the scope of this chapter, but see Chapter 3 for more details.

memory performance declined significantly across tests ($t_{1\text{-}tail(17)}$ =10.06, $p < 0.001$), which was consistent with behavioral piloting (**see Fig. 3.8**). These findings are summarized in **Figure 4.1a**.

RTs: RT was defined as the average time that subjects took to place an object in its grid space across all 128 trials (regardless of accuracy). On average, subjects took less than 2 s to respond at the pretest (*mean* = 1.40 s ± 0.07 s SEM), interference test (*mean* = 1.63 s ± 0.16 s SEM), posttest (*mean* = 1.60 s ± 0.15 s SEM), and follow-up test (*mean* = 1.53 s ± 0.15 s SEM). These results are shown in **Figure 4.1b**.

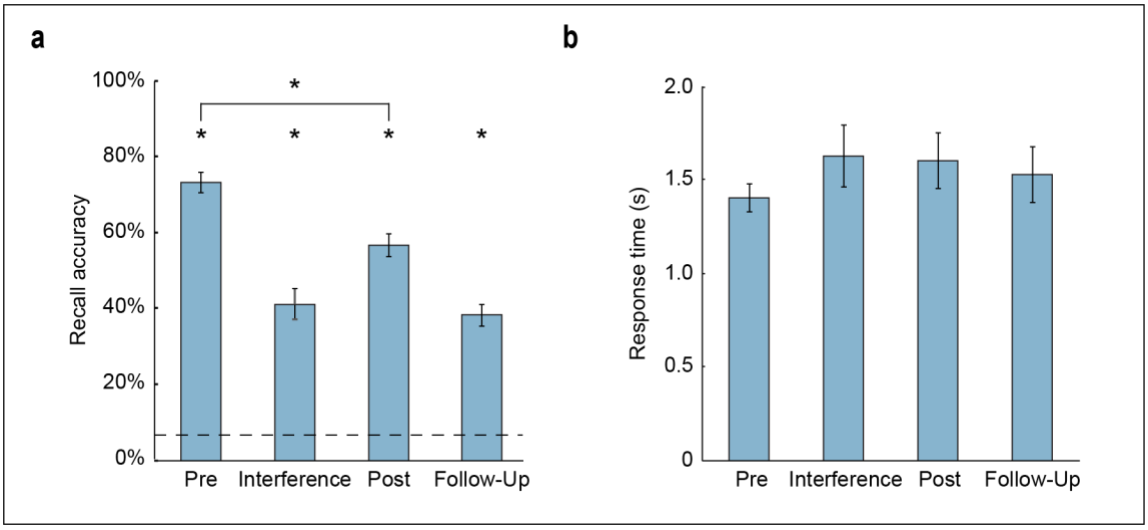


Figure 4.1. Overall memory performance. (a) Subject demonstrated above-chance recall accuracy across object location tests, with a significant decrease from pretest to posttest. **(b)** Average RTs were below two seconds across object location tests. Error bars depict mean ± SEM.

Free recall: To measure free recall performance, we tallied the number of items that subjects remembered correctly for each object category. Subjects were able to recall a substantial number of objects overall (*mean* = 52.50 ± 2.15 SEM), and for each category individually (animals:

mean = 14.61 ± .97 SEM; buildings: *mean* = 11.67 ± 0.71 SEM; faces: *mean* = 13.67 ± 0.67 SEM; tools: *mean* = 12.56 ± 0.53 SEM)[V]. Interestingly, there were significant differences in free recall performance across object categories (repeated-measures ANOVA: $F_{(3, 68)} = 4.85$, $p = 0.01$; **Fig. 4.2**). Post-hoc paired t-tests revealed the following significant comparisons: animals > buildings ($t_{2\text{-}tail(17)} = 2.92$, $p = 0.01$), animals > tools ($t_{2\text{-}tail(17)} = 2.66$, $p = 0.02$), and faces > buildings ($t_{2\text{-}tail(17)} = 2.62$, $p = 0.02$).

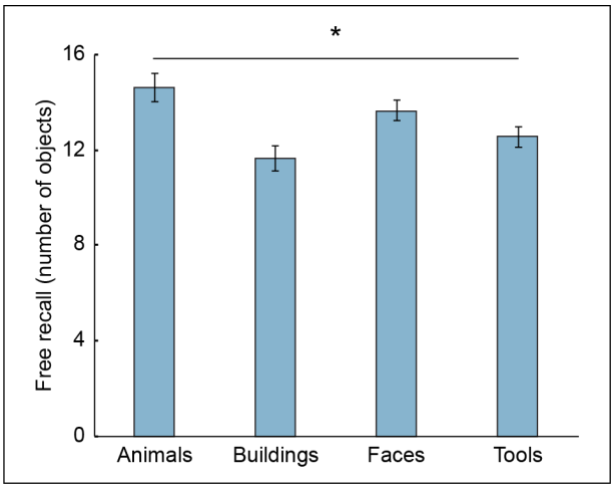


Figure 4.2. Overall free recall performance. Average number of items recalled differed significantly across object categories. Error bars depict mean ± within-subjects SEM.

Comparing memory outcomes across reactivated and non-reactivated objects categories

Recall accuracy: First, we compared pretest recall accuracy between those objects that were later reactivated during sleep and those that were not using a paired t-test. Critically, there was no significant difference ($t_{2\text{-}tail(17)} = 0.62$, $p = 0.54$; **Fig. 4.3a**), suggesting a level baseline prior to reactivation. There was also no significant difference in recall accuracy between cued and non-cued objects at the interference test ($t_{2\text{-}tail(17)} = 0.04$, $p = 0.97$). The critical test for reactivation effects was the posttest, where subjects recalled the original object locations, half of which had been cued during the sleep phase. Prior to performing statistical analyses, we divided posttest

[V] Items most frequently recalled, by category: dog and lion (tied), John Hancock, Barack Obama, hammer. Items least frequently recalled, by category: beaver, Bellagio and Marina City (tied), Tom Cruise, safety glasses.

recall accuracy by pretest recall accuracy on a subject-by-subject basis (to account for baseline performance). Then, we used a paired Wilcoxon signed-rank test to evaluate the prediction that subjects would demonstrate superior memory for cued information at the posttest. Indeed, subjects forgot a smaller percentage of reactivated object locations from pretest to posttest, when compared to non-reactivated object locations ($Z_{1\text{-}tail}$ = 1.70, p = 0.04; **Fig 4.3b**). Notably, 15 out of the 18 subjects retained a higher percentage of object locations from cued versus non-cued categories (p = 0.003; Binomial test). Finally, we conducted the same test to evaluate recall accuracy at the follow-up test (again, compared to a pretest baseline), and effects of odor cues were no longer evident ($Z_{1\text{-}tail}$ = 0.17, p = 0.43).

RTs: To compare RTs across cued and non-cued object categories, RT values were log transformed[W] on a trial-by-trial basis prior to averaging, to diminish the contribution of outliers. Although there was no difference in recall accuracy for cued versus non-cued object locations at the interference test, odor cues did have an influence on RTs for new object locations. Specifically, a paired t-test revealed that RTs were significantly slower for cued versus non-cued objects during interference testing ($t_{2\text{-}tail(17)}$ = 2.76, p = 0.01; **Fig 4.3c**), possibly reflecting increased competition between the new object locations and the original object locations that were reinforced by odor cues during sleep. There was no difference in RTs when comparing the same object categories at the pretest ($t_{2\text{-}tail(17)}$ = 1.24, p = 0.23), posttest ($t_{2\text{-}tail(17)}$ = -0.12, p = 0.91), or follow-up test ($t_{2\text{-}tail(17)}$ = -0.46, p = 0.64).

[W] For another example where measurements were log transformed prior to comparison, see Fill et al., 2012[108].

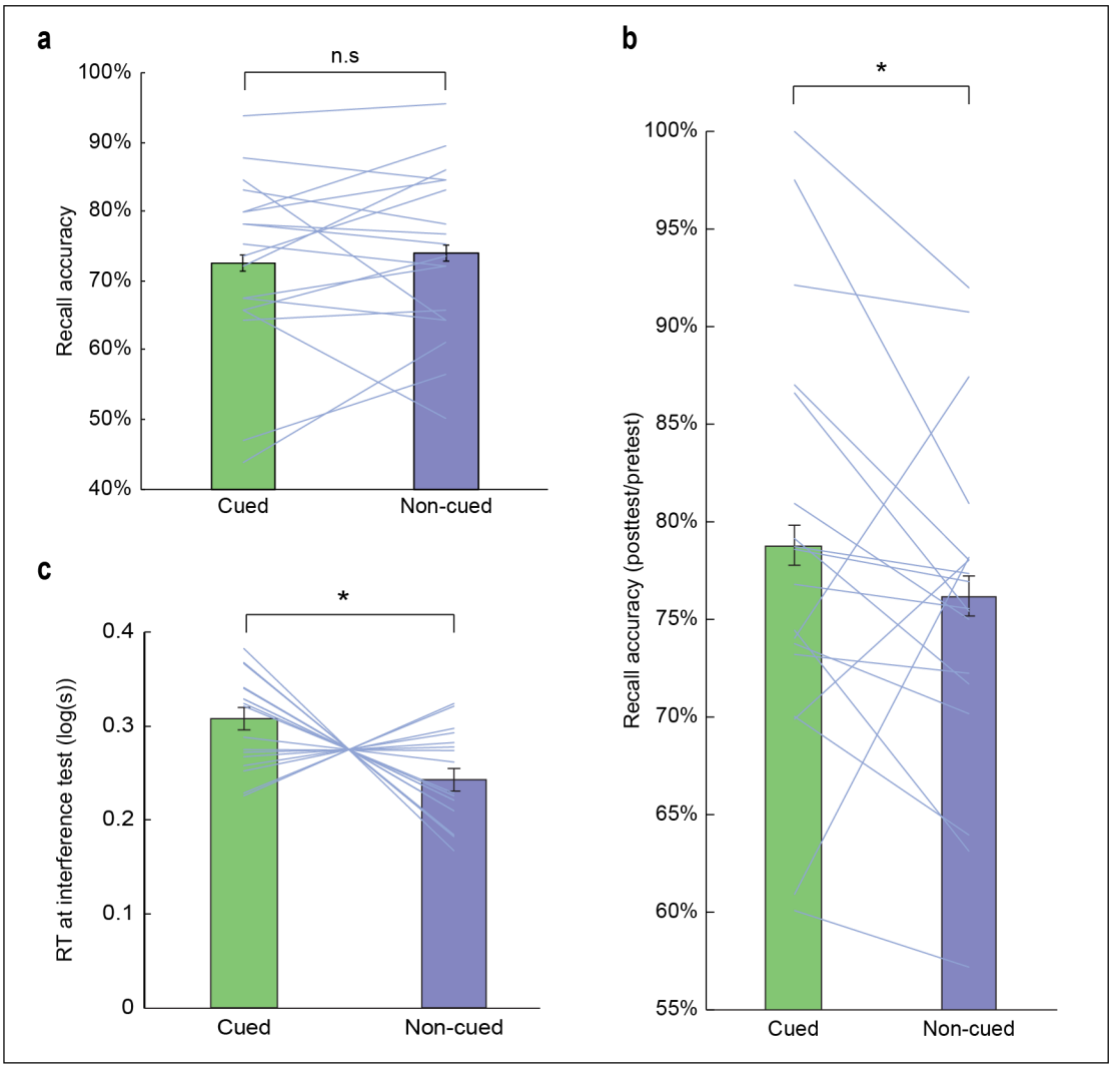


Figure 4.3. Memory performance for cued versus non-cued object categories. (a) At pretest, there was no significant different in recall accuracy between those object categories that were later reactivated during sleep, and those that were not. **(b)** At posttest, recall accuracy (percentage of object recalled at posttest, compared to a pretest baseline) was enhance for reactivated object locations, compared to non-reactivated object locations. **(c)** During interference testing, subject RTs were significantly slower for objects that were reactivated in the preceding sleep period, compared to those that were not. Error bars depict mean ± within-subjects SEM.

Free recall: To test the hypothesis that subjects would demonstrate superior performance for reactivated objects compared to non-reactivated objects during free recall, we conducted a paired t-test. Contrary to our hypothesis, results were not significant ($t_{1\text{-}tail(17)} = 0.20$, $p = 0.42$). See

Section 3.7 for a discussion of why the free recall test may not have been sufficiently sensitive to detect reactivation effects.

4.3 Discussion

Our behavioral findings confirm the utility of the novel olfactory reactivation paradigm described in Chapter 3. First, subjects demonstrated strong recall accuracy at all four test points, which indicates robust encoding of object locations during learning phases. Next, recall accuracy declined substantially from pretest to posttest, which was critical given that the paradigm was designed to identify subtle differences in memory retention across categories. Had there been ceiling effects, it would have been impossible to detect an influence of reactivation cues on memory performance. Moreover, prompt RTs (< 2 s) increase our confidence that subjects were able to focus their attention during tests, even during those that were completed following sleep deprivation (i.e., pretest, interference test, posttest).

More importantly, when we compared memory measures across cued and non-cued object categories, we observed a reactivation effect. Namely, within-sleep odor cues enhanced memory retention from pretest to posttest for reactivated objects, when compared to non-reactivated objects. This finding is in line with those from previous studies that have shown that both olfactory and auditory cues improve recall for associated memories (see Section 1.4). However, the results described here extend those from prior work, by demonstrating that two unique odor cues can be presented in sleep to successfully reactivate content from two discrete categories. Finally, we found that subjects' RTs were prolonged when placing cued objects (when compared

to non-cued objects) on the grid during interference testing, which further suggests an implicit effect of odor cues on memory. Collectively, these findings thoroughly address Aim 1.

Overall, the results described in this chapter convincingly show that odor cues presented during sleep had a significant impact on memory consolidation in our paradigm. The next step was to investigate the neural mechanisms underpinning these behavioral changes (see Chapter 5).

Chapter 5: Odor-evoked memory replay in vmPFC during sleep promotes memory consolidation

5.1 Overview

The second aim of this thesis work was to elucidate the neural mechanisms through which reactivation cues promote the directed memory consolidation observed in Chapter 4. Two address Aim 2, we analyzed MRI data collected from 18 subjects that completed the olfactory reactivation experiment (see Chapter 3 for a detailed account of the paradigm). Specifically, we wanted to investigate memory replay. We first employed MVPA techniques to define the unique patterns of fMRI activity in response to the four picture categories (animals, buildings, faces, tools) during the initial learning phase. Next, we studied the reinstatement (or "replay") of these patterns in response to category-specific odors during sleep. We were interested in identifying the main effects of odors on memory replay, as well the relationship between memory replay and visuospatial memory performance. In this chapter, we outline our findings from these fMRI analyses, and reflect on their implications.

5.2 Methods

5.2.1 Task structure

Briefly, during initial learning, subjects studied the locations of objects from four categories: animals, buildings, faces, and tools[X]. There were 32 objects per category, and object images were presented on a 4-x-4 spatial grid in category blocks of 8 objects during fMRI scanning. Subjects

[[X]] As discussed previously, subjects also learned locations of scrambled images. See Section 3.4 for further details.

were instructed to memorize the location associated with the objects, each of which appeared three times over the course of the scan. Prior to the sleep phase, subjects completed four additional task sessions (odor-category association, learning to criteria, EEG setup, pretest). During the sleep phase, subject returned to the scanner, where they were instructed to try to relax and fall asleep during approximately 75 minutes of fMRI scanning. During sleep stages 2 and3, two of the four odors were presented (16-s on, 16-s off), to reactivate objects from two of the four object categories. For a more detailed description of the initial learning and sleep phases, see Sections 3.6.2 and 3.6.7.

5.2.2 MRI data preprocessing

We preprocessed and analyzed the MRI data using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/) and custom Matlab scripts. Functional images were realigned to the mean of the images, motion corrected, and coregistered to the T1-weighted image. Multivariate searchlight analyses were conducted in each subject's native space, and images were minimally smoothed with a 2-mm Gaussian kernel. Searchlight analyses were restricted to grey matter voxels by generating a grey matter mask from the SPM12 tissue probability map, and then warping that mask to each subject's native space using the transformation parameters from the standard T1 template to the subject's individual T1-weighted image. For univariate and PPI analyses, images were normalized and then spatially smoothed with a larger 6-mm Gaussian kernel.

5.2.3 Multivoxel pattern analysis: identification of category-sensitive voxels from initial learning

To detect category-sensitive voxels, a GLM was constructed for each subject from initial learning scans, where category images were modeled as five separate regressors of interest (animals, buildings, faces, tools, scrambled) in a blocked design (**Fig 5.1**). We included the six motion parameters generated from realignment as nuisance regressors, and beta estimates were calculated for each condition.

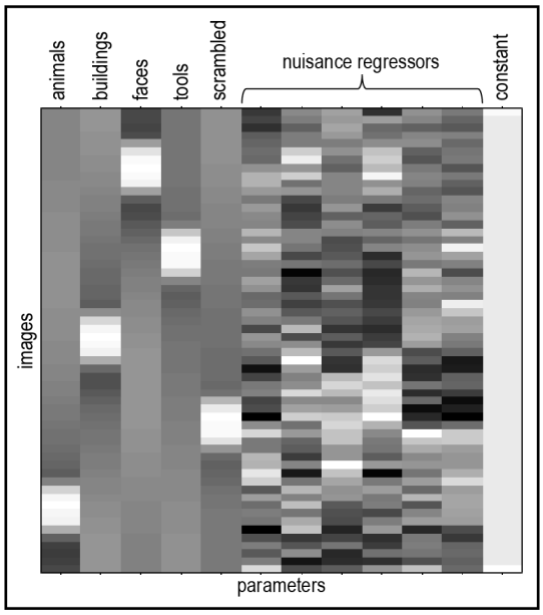


Figure 5.1. Design matrix for initial learning GLM.

To quantify pattern discrimination of the four object categories, we implemented a whole-brain searchlight-based correlation analysis. At each search sphere (radius = 3.7 voxels, maximum total = 203 voxels), we extracted 48 beta pattern vectors (four object categories[Y] X 12 runs). In a leave-one-out approach, we averaged beta patterns within each object category from 11 "training" runs, and then compared them to beta patterns from the remaining "test" run (**Fig. 5.2**). In each iteration, we first subtracted the mean activity across all four conditions from training and test beta patterns separately. Next, we calculated linear correlations between each combination of training and test pattern vectors[Z], and the resulting correlation coefficients were

[Y] Although the scrambled images condition was included as a regressor of interest in the GLM, we only considered the four object categories during pattern analysis.

[Z] I.e., training animal pattern versus test animal pattern, training animal pattern versus test building pattern, training animal pattern versus test face pattern, training animal pattern versus test tool pattern, and so on.

Fisher's *Z* transformed. This procedure was repeated a total of 12 times, so that each of the 12 runs could be left out in turn as a test run. The resulting Z-values were averaged across iterations. We then calculated the average Z-value across both within-category pairs (training patterns versus the category-congruent test pattern), and between-category pairs (training patterns versus the three category-incongruent test patterns). This procedure was repeated at each search sphere, and then we subtracted the two resulting correlation maps (within-category correlations – between-category correlations) to arrive at a final category specificity map, which was normalized and then smoothed with a 6-mm Gaussian kernel prior to group comparison. Category-sensitive voxels were designated at the group-level ($p < 0.001$). Critically, sleep pattern analyses were restricted to those brain regions that demonstrated category-selectivity during learning, as this was a prerequisite to identifying replay of the same category information in sleep.

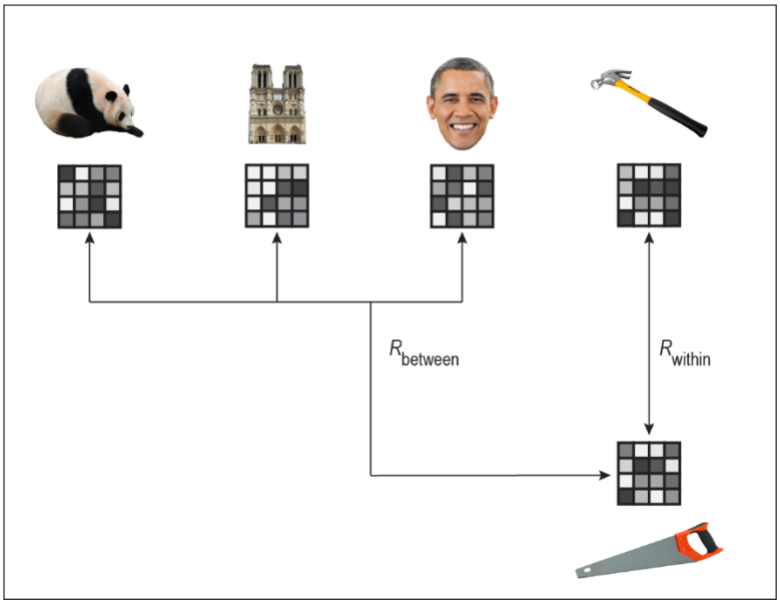


Figure 5.2. MVPA approach to determine category specificity. Category ensemble patterns (depicted as 4-x-4 greyscale grids of voxels) were correlated across fMRI runs, both within categories (e.g., tool pattern versus tool pattern) and between categories (e.g., animal pattern versus tool pattern). To be considered category-selective, a brain area would need to demonstrate higher within-category correlations than between category-correlations.

In a follow-up analysis, we determined the selectivity for each object category individually. In this analysis, the same steps were repeated as previously, with one exception. At each search sphere, rather than averaging indices of pattern discrimination across all four object categories, we compared within-category correlation values to an average of the three between-category correlations values for each object category separately. This resulted in four category specificity maps per subject. As previously, these maps were normalized, smoothed, and then considered at the group level.

5.2.4 Multivoxel pattern analysis: replay of category information during sleep

Main analysis. To determine whether category information re-emerged in response to olfactory reactivation cues during sleep, we used the GLM constructed from fMRI initial learning data (see Section 5.2.3) to define pattern templates for each of the four object categories. An additional GLM was also constructed for each subject from the fMRI sleep data, where each of the two odor cues was included as a separate

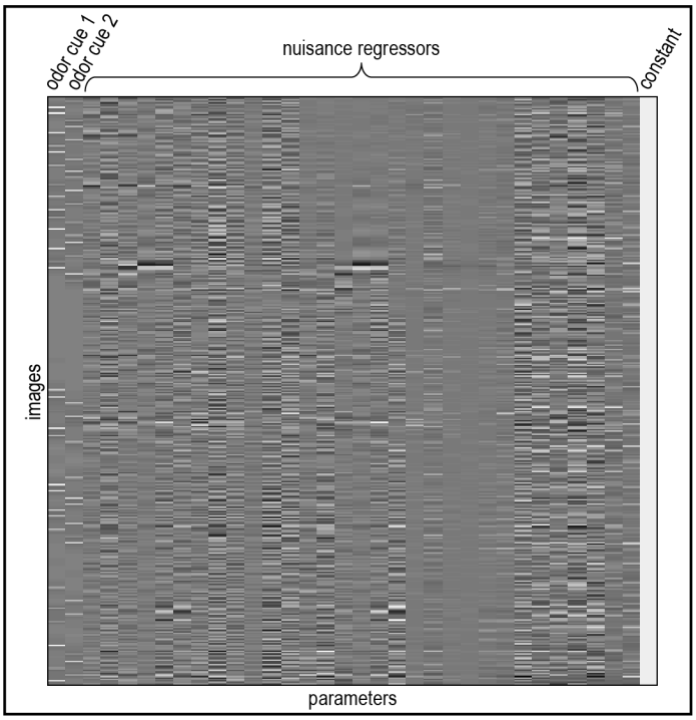


Figure 5.3. Design matrix for sleep GLM.

regressors of interest, and odor presentations were modelled as events of 0-s duration[AA] (**Fig 5.3**). Odor onset times were adjusted to align with the first point of inhalation (i.e., rising slope on the breathing trace) after odor presentation (**Fig 5.4**). To minimize signal contributions induced by head motion during the sleep scan, we discarded all volumes prior to the first odor presentation and following the last odor presentation (except for six additional volumes on either end). Given the large number of volumes remaining (*range* = 401-1416 volumes, *mean* = 948.61volumes), there were sufficient degrees of freedom in the sleep-based GLM to incorporate extra nuisance regressors without overfitting. This allowed us to account for head motion more rigorously given the long duration of the sleep session[BB]. Nuisance regressors included the six motion parameters generated from realignment, and their squares, derivatives, and squared derivatives (24 parameters total). To account for within-scan motion, we also included the signal difference between even and odd slices, the within-volume variance across slices, and derivatives of both parameters as nuisance regressors. Additional nuisance regressors were included when necessary, to capture individual volumes demonstrating excessive head motion. Finally, the respiration trace was post-processed and downsampled to the scanner repetition time frequency, so that it could be included as an additional nuisance regressor. Finally, beta estimates were generated for each condition.

[AA] Although odors were presented in 16-s on/16-s off blocks (except when the odor delivery protocol was interrupted due to a potential change in the sleep/wake state), odors were modelled as events to capture the maximal fMRI response to each reactivation cue.

[BB] In contrast, we could not apply this more rigorous motion correction approach to the wake data without overfitting, due to the smaller number of fMRI volumes (58 volumes per run), and thus fewer degrees of freedom.

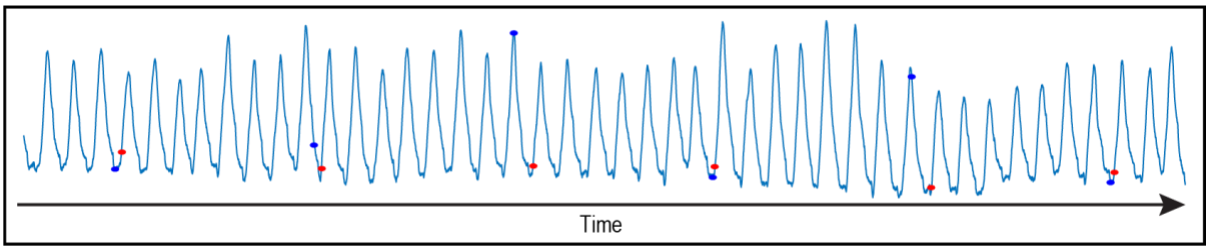


Figure 5.4. Odor onset adjustment. Odor onset times (blue dots) and modified odor onset times (red dots) are overlaid on a respiration trace (blue line).

To investigate replay of pattern-based information during sleep, we again implemented a whole-brain searchlight-based correlation analysis. At each searchlight sphere (same parameters as previously), we used the beta pattern vectors extracted from the initial learning GLM to construct pattern templates for each object category. More specifically, each category template was computed by averaging beta patterns across runs for each category separately, and then subtracting the mean activity across the four conditions from each pattern template. Next, two beta pattern vectors were extracted from the sleep GLM, each corresponding to one of the two odor cues[CC]. We then calculated correlation coefficients between the four category templates, and each of the two odor-cued beta pattern vectors[DD], and the resulting *r*-values were converted to Fisher's *Z* scores. As an index of memory replay, we compared the cued correlation (odor-evoked pattern in sleep versus reactivated category template) to an average of the non-cued correlations (odor-evoked pattern in sleep versus the three non-reactivated category templates) for each odor condition separately. The resulting Z-values were then averaged across the two odor conditions. This procedure was repeated at each search sphere, and ultimately generated a

[CC] Here, mean activity was not subtracted from beta pattern vectors. Since the test set was limited to two conditions of interest (two odor cues), doing so would have created two anticorrelated pattern vectors, essentially reducing two data points to a single data point.
[DD] I.e., animal pattern versus odor 1 pattern, animal pattern versus odor 2 pattern, building pattern versus odor 1 pattern, building pattern versus odor 2 pattern, and so on.

memory replay map (cued correlations – average of non-cued correlations) for each subject. First-level replay maps were normalized and then smoothed with a 6-mm Gaussian kernel prior to group comparison. Finally, we conducted a group-level analysis, where cued memory benefit was included as a covariate of interest, and category-selective voxels (defined from MVPA of initial learning phase, see Section 5.2.4) were designated as an explicit mask. Cued memory benefit was based on recall accuracy at the visuospatial posttest (expressed as a percentage of objects remembered from pretest baseline), and was defined as recall accuracy for reactivated object categories, minus recall accuracy for non-reactivated object categories. This second-level model was used to assess both the main effect of odor cues on memory replay, and the relationship between odor-evoked replay and memory consolidation.

Follow-up analyses: main effect of odor cues on memory replay. We ran an additional analysis to determine whether there were spatial differences in odor-evoked replay maps when considering each of the four object categories separately. The procedure was identical to the main analysis described above, except that correlation values were not averaged across the two odor conditions. Rather, two replay maps were generated for each subject, one per odor cue. Then, we considered replay of each object category individually, by building four separate group-level models (one per category). Since each of the 18 subjects received two of the four odor cues during sleep, this meant that each category was reactivated for a total of nine subjects. Thus, nine replay maps per category were included in each group-level model. Given the limited number of data points included in each model, the resulting category maps were not constrained by a mask.

Follow-up analyses: relationship between odor-evoked replay and memory consolidation. We conducted multiple follow-up analyses, to further probe the relationship between odor-evoked replay and cued memory benefit. First, we repeated the main analysis for each of the two reactivated object categories separately. Thus, we utilized the same individual category replay maps as described in the previous paragraph. From those maps, we extracted *Z*-values from clusters of interest in vmPFC and posterior fusiform cortex (identified from the main analysis, where odor-evoked replay was correlated with cued memory benefit, $p < 0.001$), and took the average of *Z*-values across voxels. We then correlated the resulting values with recall accuracy for the corresponding object category at posttest (again, expressed as a percentage of items remembered from pretest baseline).

Next, we ran an additional analysis to investigate the time course of memory replay. To that end, we constructed a separate finite impulse response model based on the fMRI sleep data. For each of the two odors, the model included ten regressors of interest spaced 2.5-s apart, which spanned a 25-s time window. These regressors modeled responses starting three volumes prior to odor onset, a single volume aligned to odor onset, and six volumes following odor onset. The model also included the same nuisance regressors as were implemented for the sleep-based GLM. For regressors of interest corresponding to each time point, beta estimates were extracted for each of the two odors separately. Next, a searchlight analysis was conducted (as in the main analysis), which resulted in ten individual replay maps per subject (one for each time point). For each replay map, *Z*-values were extracted from clusters of interest in vmPFC and posterior fusiform cortex (as previously) and averaged. Finally, the resulting values for each respective time point were correlated with cued memory benefit.

5.2.5 Univariate analysis: odor-evoked activity in sleep

In an additional univariate analysis, our goal was to identify brain regions that were activated by odor cues during sleep, without considering pattern-based effects. To achieve this, we used the same sleep-based GLM as previously, except that the model was applied to images that had already been normalized and smoothed with a 6-mm Gaussian kernel. Then, we generated individual contrast maps for both odor cues (considered together) at the individual subject level, and then included those contrast maps in a group-level analysis.

5.2.6 Connectivity analysis: connectivity during within-sleep odor presentation

We used the gPPI toolbox[109] to assess connectivity between amygdala/hippocampus and clusters of interest in vmPFC and posterior fusiform cortex (as previously) during odor presentation. For each individual subject, we estimated a PPI model using images that had been normalized and smoothed with a 6-mm Gaussian kernel, and we included the same nuisance regressors as were included in the sleep-based GLM. The physiological factor was defined as fMRI activity in the seed region (5mm sphere surrounding the peak voxel in the amygdala/hippocampus cluster identified from the univariate analysis), and odor onset (for both odors) was the psychological factor. This resulted in a map of connectivity parameters at odor onset for each subject. From each map, we extracted beta values from clusters of interest on a subject-by-subject basis, and then took the average across voxels. At the group level, we compared the resulting values to zero using a one-sample t-test.

5.3 Results

5.3.1 Category-specific objects induce widespread discriminable patterns of fMRI activity during visuospatial learning

As we expected, and in line with pilot results (see Section 3.6.2), a multivoxel pattern analysis of fMRI data collected in the initial learning phase revealed category specificity in widely distributed brain regions. These included much of the visual pathway, as well as substantial parts of parietal and prefrontal cortices (**Fig. 5.5a**). Importantly, subsequent sleep-based pattern analyses were restricted to category-selective voxels identified here ($p_{unc} < 0.001$; **Fig. 5.6**). In a follow-up analysis, we also observed widespread pattern specificity when considering each of the four object categories separately (**Fig 5.5b-e**). Robust category discrimination was an important prerequisite for sleep-based pattern analyses. These data were used to define multivoxel representations of each object category, which were then used as reference templates to identify content-specific fMRI activity that might resurface in the sleep phase. Note that pattern templates were based solely on visual category information, as odors were not introduced as association cues until after initial learning.

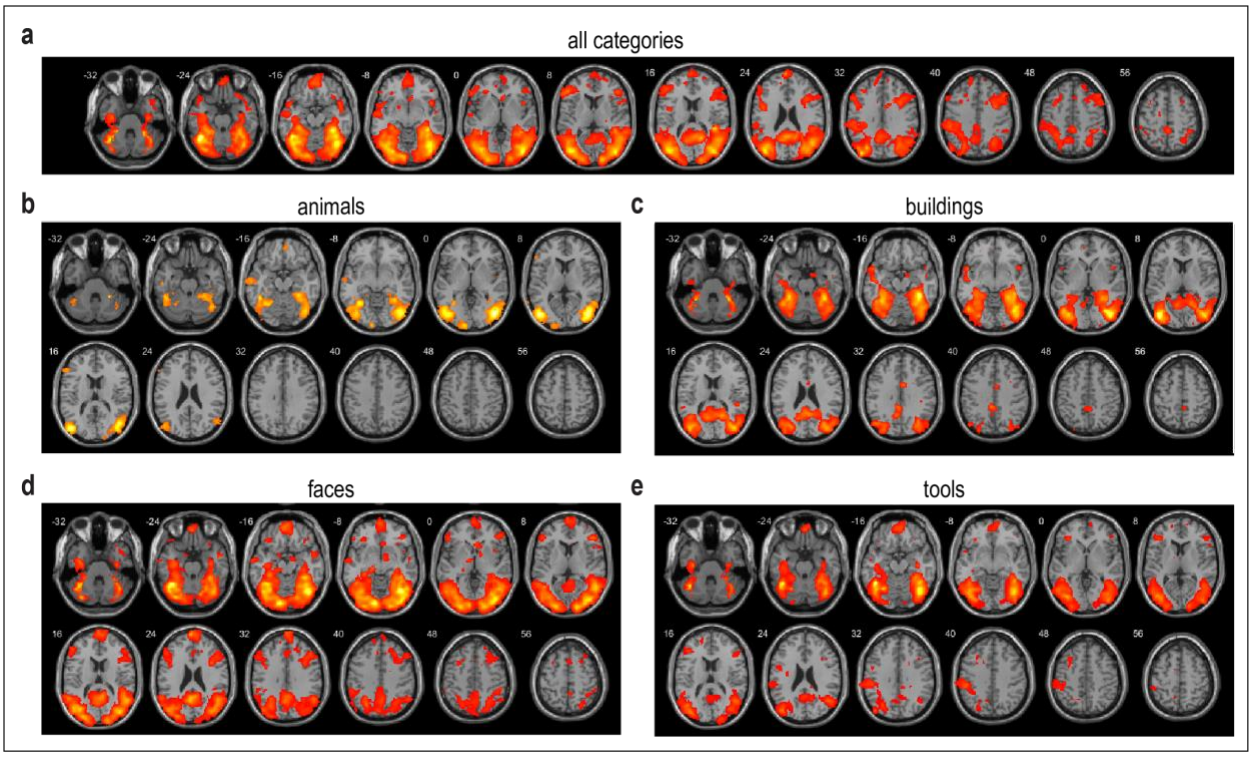


Figure 5.5. Brain regions demonstrating category selectivity during initial learning. Multivoxel pattern analysis of initial learning data reveals widespread category specificity across the four object categories **(a)**, and for each of the four object categories individually **(b-e)**. fMRI activity is shown at $p < 0.001$ uncorrected, and images are overlaid on a canonical single-subject T1-weighted MRI scan. This result was visualized using the xjView toolbox (http://www.alivelearn.net/xjview).

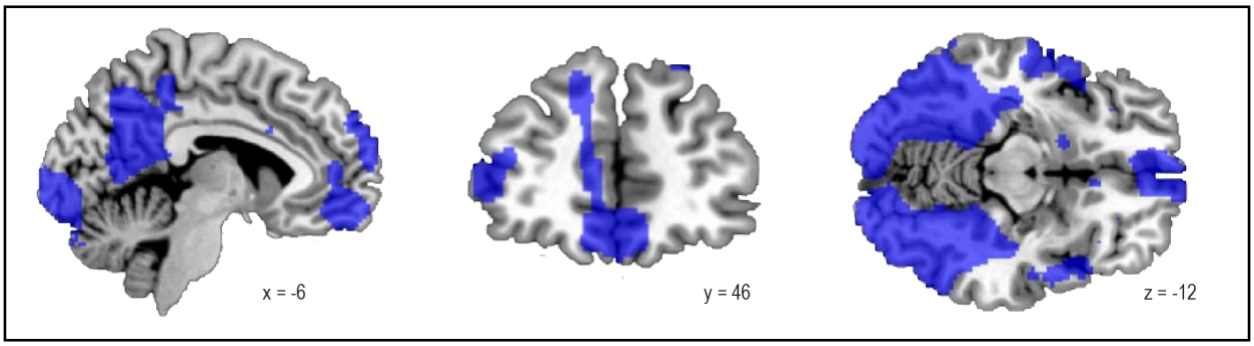


Figure 5.6. Category-sensitive brain mask. Binary mask is displayed at $p < 0.001$ uncorrected, and images are overlaid on a canonical single-subject T1-weighted MRI scan.

5.3.2 Main effect of odors on replay of associated category information during sleep

One of our central hypotheses was that reactivation cues would induce neural replay of the associated object category templates during sleep. For instance, if animal objects were paired

with banana odor, then presentation of banana odor during sleep would evoke a pattern of fMRI activity that was more consistent with the animal template (defined in the wake state), compared to the other three category templates. To this end, we first correlated odor-evoked fMRI ensemble activity in sleep with each of the four category-specific fMRI pattern templates from the initial learning phase of the task, and then compared the degree of pattern overlap between cued and non-cued category templates, yielding a measure of cue-specific replay strength for each subject (see Section 5.2.4 for methods details). As mentioned previously, this analysis was restricted to brain regions that demonstrated category specificity during prior learning. Given the visuospatial nature of the reactivated memory task, we reasoned that odor-evoked replay might emerge in visual associative brain regions[38,110].

This analysis revealed odor-evoked replay of cued category templates in lateral occipital complex ([-56, -62, 4], $t_{(16)} = 3.99$, $p_{unc} = 0.001$[EE]; **Fig. 5.7**) and inferior frontal gyrus ([-50, 30, 8], $t_{(16)} = 3.91$, $p_{unc} = 0.001$), although neither of these clusters survived correction for multiple comparisons. These results were not particularly strong, and we reasoned that memory replay might have manifested in different brain areas, depending on which object category was being reactivated. In a follow-up analysis, we considered replay of each object category in isolation, rather than collapsing replay measures across all four categories[FF]. We found that, at a threshold of $p < 0.001$ uncorrected, odor cues promoted replay of associated multivoxel patterns in discrete brain areas (**Fig. 5.8**). For instance, in response to odor cues, animal and tool information was

[EE] Throughout analyses, all fMRI p-values are defined at the peak voxel.
[FF] This exploratory analysis was not restricted to category-specific voxels, given the limited pool of subjects included in each model.

replayed in different parts of parahippocampal cortex (animals: [18, -40, -4], $t_{(8)} = 8.50$, $p_{unc} < 0.001$, **Fig. 5.8a**; tools: [32, -54, -10], $t_{(8)} = 9.38$, $p_{unc} < 0.001$, **Fig. 5.8d**), building information was replayed in lateral occipital complex ([50, -66, 16], $t_{(8)} = 5.76$, $p_{unc} < 0.001$; **Fig. 5.8b**), and face information re-emerged in occipital cortex ([-12, -72, 24], $t_{(8)} = 8.20$, $p_{unc} < 0.001$; **Fig. 5.8d, blue arrow**). However, it is important to note that our experiment was not designed to test individual category effects, because any given category was only cued for 50% of subjects (n = 9). As such, this analysis was underpowered, and these findings should be considered as very preliminary.

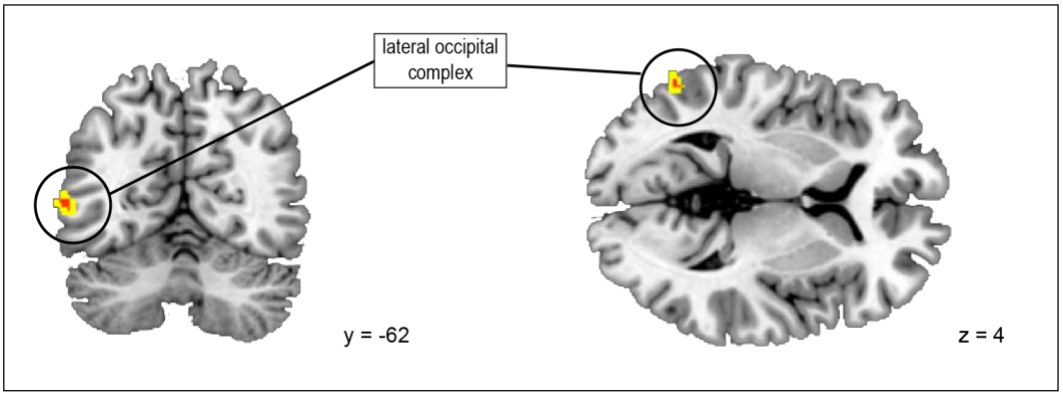


Figure 5.7. Main effect of olfactory reactivation cues on memory replay. Odor cues evoked replay of associated category templates in lateral occipital complex, although this effect did not survive statistical correction for multiple comparisons. fMRI activity is shown at $p < 0.001$ uncorrected (red) and $p < 0.005$ uncorrected (yellow), and images are overlaid on a canonical single-subject T1-weighted MRI scan.

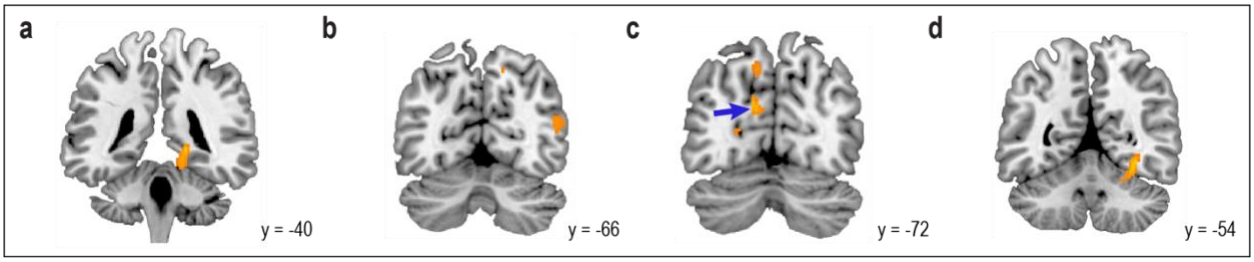


Figure 5.8. Main effect of odor cues on replay of individual object categories. fMRI activity is shown at $p < 0.001$ uncorrected for animals **(a)**, buildings **(b)**, faces **(c)**, and tools **(d)**. The blue arrow in panel c indicates the cluster in occipital cortex referenced in the main text. Images are overlaid on a canonical single-subject T1-weighted MRI scan.

5.3.3 Odor-evoked replay in the sleeping brain predicts post-sleep memory performance

Another primary goal of our study was to investigate the functional links between odor-evoked memory replay during sleep and memory consolidation. Indeed, we reasoned that among the brain areas that might replay category information during sleep in response to odor cues, only those areas that had a systematic effect on post-sleep recall accuracy would have behavioral relevance. Here, we hypothesized that memory replay in medial temporal and prefrontal brain areas known to participate in memory consolidation and retrieval[111-118] might predict subsequent memory performance in the wake state. To this end, we regressed our index of odor-evoked replay onto a measure of cue-specific memory performance (i.e., cued memory benefit, see Section 5.2.4) on a subject-by-subject basis. As previously, this analysis was limited to areas that were shown to be category selective during initial learning. In this way, we found that greater category replay in vmPFC was associated with increased recall accuracy at posttest for cued over non-cued objects ([-6, 46, -12], $t_{(16)} = 7.53$, $p_{FWE} = 0.01$; **Fig. 5.9a**). These effects were robust across both odor cues, as within-sleep replay strength in vmPFC was significantly correlated with post-sleep recall when each cue was considered independently ($r_{1(16)} = 0.53$, $p_1 = 0.01$; $r_{2(16)} = 0.48$, $p_2 = 0.02$; **Fig. 5.9c**). In a time-resolved analysis of stimulus-evoked activity during the sleep period, correlations between odor-evoked reactivation in vmPFC and recall accuracy increased at odor onset and persisted over several seconds, with a maximal effect size (r-value) of 0.70 (95% confidence interval = 0.42 to 0.86), returning to baseline prior to odor offset (**Fig. 5.9e**). Together these findings in vmPFC highlight the categorical and temporal specificity of odor-cued reactivation on memory retrieval.

Interestingly, we also found that replay in a visual associative brain region was predictive of memory performance, perhaps because our memory paradigm had a strong visual component. Specifically, across subjects, recall accuracy for cued versus non-cued object categories scaled with the degree of odor-evoked replay in posterior fusiform cortex ([-24, -70, 0], $t_{(16)} = 6.73$, $p_{FWE} = 0.04$; **Fig. 5.9b**). When considering each reactivated category separately, the correlation between replay in posterior fusiform cortex and posttest memory performance was evident, but only for one of the odor cues ($r_{1(16)} = 0.43$, $p_1 = 0.04$; $r_{2(16)} = 0.22$, $p_2 = 0.19$; **Fig. 5.9d**). Similar to the time-course profile in vmPFC, within-sleep replay in fusiform cortex also emerged following odor onset, but appeared more sustained throughout the duration of odor presentation (**Fig. 5.9f**).

To a lesser extent, memory replay in left precuneus was also correlated with memory performance upon waking, but this cluster did not survive correction for multiple comparisons ([-14, -48, 24], $t_{(16)} = 5.15$, $p_{unc} < 0.001$).

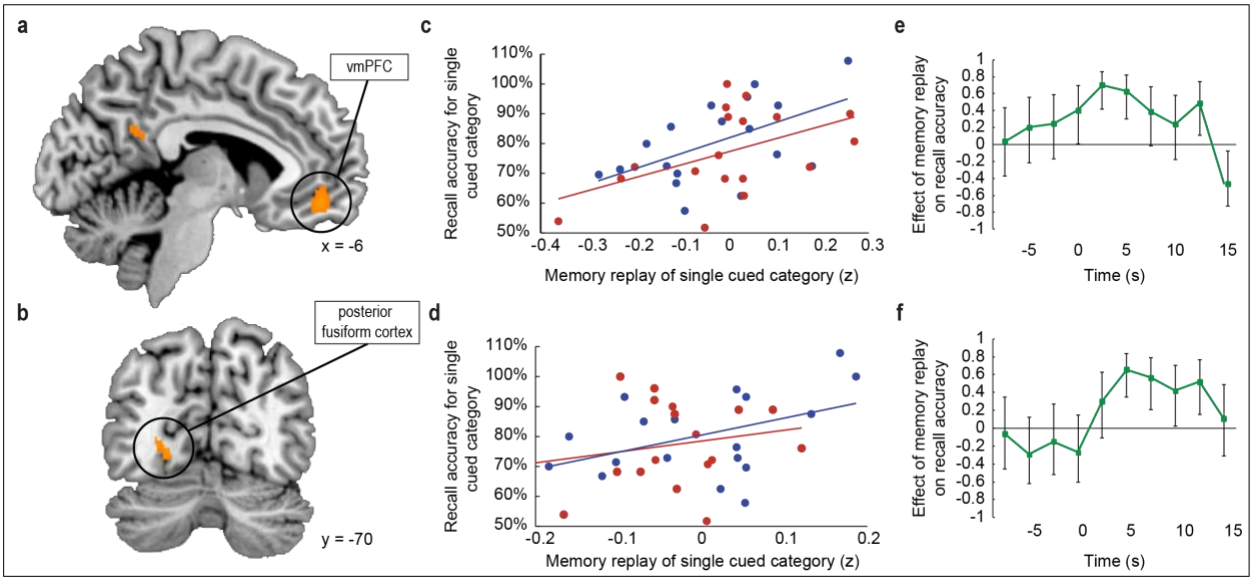


Figure 5.9. Correlation between odor-evoked memory replay and posttest memory retention. (a-b) During SWS, the extent to which odors evoked category-specific memory replay in vmPFC **(a)** and posterior fusiform cortex **(b)** was significantly correlated with cued memory benefit at posttest. fMRI activity is shown at $p < 0.001$ uncorrected, and images are overlaid on a canonical single-subject T1-weighted MRI scan. (**c-d)** Correlations between fMRI memory replay of an individual object category and recall accuracy for that category at posttest, for the two reactivated object categories taken separately in vmPFC **(c)** and posterior fusiform cortex **(d)**. Blue and red dots represent individual object categories, where category assignment is arbitrary. **(e-f)** Illustration of correlation depicted in panels a and b across time points in vmPFC **(c)** and posterior fusiform cortex **(f)**. Time 0 is aligned to odor onset. Error bars depict 95% confidence intervals.

5.3.4 Within-sleep odor cues activate limbic brain regions

The finding that an index of odor-cued replay in vmPFC and posterior fusiform cortex during sleep predicts memory recall necessarily implies that the olfactory system must communicate with extra-olfactory structures to consolidate visuospatial representations. To define potential pathways by which odors might induce cortical replay in sleep, we implemented a univariate analysis to characterize which brain areas were activated by odor cues, irrespective of their effects on memory performance. We identified an olfactory-related cluster in the left amygdala, extending into left hippocampus ([-28, 0, -24], $t_{(17)} = 4.18$, $p_{SVC}^{GG} = 0.02$; **Fig. 5.10**), which could plausibly serve as a conduit to cortical structures. A cluster in right amygdala was also observed in an almost symmetrical location to the left amygdala, but did not survive correction for multiple comparisons ([26, 0, -24], $t_{(17)} = 3.47$, $p_{unc} = 0.001$; **Fig. 5.10**) [HH].

[GG] Here, the p-value is small volume corrected for the family-wise error rate based on an olfactory ROI in left amygdala from the AAL Atlas.

[HH] It is worth pointing out that the identification of olfactory-related regions in sleep could arise because the odor cues were driving downstream activity in the olfactory system, or because they were reactivating previously associated memory content. Ideally, delivery of a control odor that had never been associated with category information would help resolve these two different possibilities. In our study, we opted not to deliver a control odor to maximize the number of odor trials available for pattern analysis. Therefore, subjects did not receive any odors that were not associated with prior learning during scanning. As such, it was not possible to disentangle

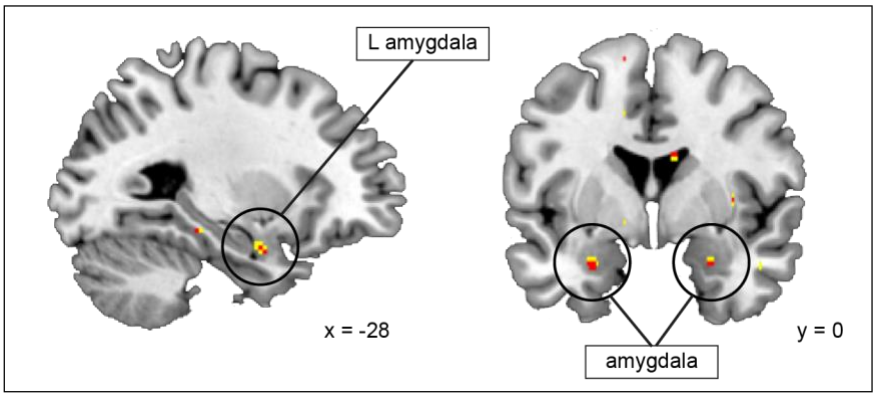
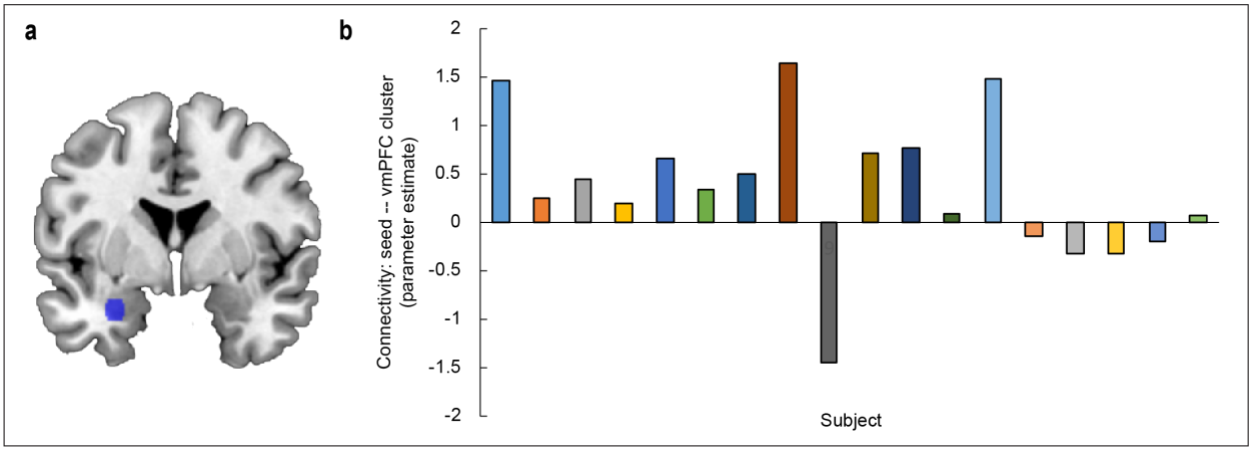


Figure 5.10. Odor cues activate amygdala. fMRI activity is shown at $p <$ 0.005 uncorrected (red) and $p < 0.01$ uncorrected (yellow), and images are overlaid on a canonical single-subject T1-weighted MRI scan.

5.3.5 Odor cues promote connectivity between limbic brain areas and vmPFC during sleep

To assess whether the amygdala-hippocampal cluster might be preferentially coupled with vmPFC or posterior fusiform cortex in the presence of reactivation cues, we used PPI analyses to test the functional connectivity between the amygdala-hippocampal cluster (**Fig. 511a**) and the downstream cortical areas. We observed significantly enhanced coupling during odor presentation between this olfactory-related region and vmPFC ($t_{(17)} = 1.95$, $p = 0.03$; **Fig. 511b**), but not between the same region and posterior fusiform cortex ($t_{(17)} = 0.85$, $p = 0.20$).



contributions of the odor *per* se, and those related to associated memories. Rather, odor-cued activity in this analysis likely reflects an amalgam of olfactory and reactivation-related influences.

Figure 5.11. Odor cues promote connectivity between amygdala seed and vmPFC cluster. **(a)** A seed region was designated in left amygdala (blue circle), based on findings from the prior univariate analysis. Images are overlaid on a canonical single-subject T1-weighted MRI scan. **(b)** Parameter estimates of connectivity between the designated seed region and a cluster of interest in vmPFC are shown for each subject individually.

5.4 Discussion

The fMRI results outlined in this chapter contribute significantly to an understanding of the neural correlates of olfactory memory reactivation. First, we were able to show robust, widespread discrimination of the four object categories during the initial learning phase of the experiment, not only across categories, but also for each individual object category. These findings are in line with previous fMRI MPVA studies of visual category perception (see Section 1.6 for a summary), and establish feasibility for sleep-based pattern analyses. Namely, because we observed easily-distinguishable category-based patterns of fMRI activity in the wake state, we could reasonably search for re-emergence of the same category patterns during sleep in response to odors, as an index of memory replay.

When evaluating the main effect of odors on category replay (without considering behavior), we observed clusters in lateral occipital complex and inferior frontal gyrus. However, these findings were relatively weak, and did not hold up to statistical correction. One potential explanation for these lackluster results could be that replay might occur in different brain areas for the four object categories. Indeed, although individual category specificity maps from initial learning share significant overlap, there are regional differences. Thus, it is possible, and even probable, that category representations might manifest in disparate brain areas during sleep. Preliminary analyses of our own dataset support this hypothesis, but further work is needed to rigorously address the topic of category-localized memory replay.

Still, category-specific replay aside, it remains likely that there is a shared memory conduit – a brain region where replay leads to consolidation, regardless of category content. Along these lines, our most interesting finding was arguably that behavioral memory performance was significantly correlated with the degree to which odors promote category-specific replay in vmPFC and posterior fusiform cortex. That these effects hold even when considering the two cued object categories separately lends credence to these results. Given that mPFC has been heavily implicated in remote memory processing (see Chapter 6 for an in-depth discussion), this brain region is a conceivable candidate for the proposed memory conduit.

Finally, we found that during sleep, odors evoke neural activity in brain areas related to olfactory and limbic function. Interestingly, this region overlaps closely with previous findings showing odor-cued reactivation of left anterior hippocampus during SWS[54,75]. Moreover, the same brain area was more connected to a cluster of interest in vmPFC during odor presentation compared to odor-off periods. The implication here is that cued memory replay in the sleeping brain begins with odor-evoked activity in medial temporal brain structures, which may mediate the re-instantiation of visual categorical content in vmPFC.

Together, our findings demonstrate the effects of odor cues on memory replay, and especially highlight the functional significance of cue-evoked replay in promoting consolidation of declarative memories in sleep. In doing so, these results effectively address Aim 2.

Chapter 6: Conclusions and future directions

Here we used EEG-fMRI recordings combined with MPVA to investigate the neural mechanisms underlying memory outcomes in a novel olfactory cueing paradigm. First, we demonstrated an olfactory reactivation effect, namely, that within-sleep odor cues boost memory performance selectively for associated objects. In auditory reactivation studies, a multitude of sound cues are routinely presented in sleep[55,76,119]. These sound cues induce memories that are highly specific, and almost always semantically linked to the cue (e.g., cat picture + meow sound, kettle picture + whistle sound). By contrast, prior olfactory reactivation paradigms have largely employed a single arbitrary odor cue to reactivate an entire memory task[54,75,100]. A handful of studies that utilized two distinct odors during learning and a single olfactory cue during sleep have established that olfactory stimuli can influence behavior with some specificity[63,77,101], but to our knowledge ours is the first olfactory study to reactivate multiple task components during sleep (i.e., two odor cues associated with objects from two different categories).

Odors offer unique benefits over sounds as memory reactivation cues. Namely, olfactory stimuli are less likely to provoke arousal from sleep[120], particularly pure odorants lacking a trigeminal component[121,122]. In addition, the lack of a requisite thalamic relay for odor stimuli and the relative proximity of olfactory and limbic structures in the brain may confer an anatomical advantage[123,124]. Indeed, Rasch and colleagues site these reasons as motivation for choosing odors as associative stimuli in their first memory reactivation study[54], and additionally point out

the utility of odors as salient retrieval cues[II126-130]. Despite these advantages, sounds have been employed more frequently than odors as reactivation cues. This is likely due to practical limitations (i.e., auditory cueing studies can be carried out with headphones, while the olfactory equivalent requires an olfactometer), and because sound cues have been successfully implemented to reactivate highly specific memory traces. Our demonstration that odor cues can promote memory gains more selectively should increase confidence in their utility for more complex memory reactivation paradigms.

Still, in our study we chose to reactivate objects grouped into four categories, and we paired a single odor cue with objects from each category. Given that there were 128 objects, it would have been extremely difficult to reactivate individual exemplars in our paradigm, as it would have involved training subjects to associate each object with one of 128 unique odor cues. As the main experiment was already lengthy and involved, we never considered employing more specific odor cues here. However, we observed that subjects had no trouble internalizing odor-category associations, and almost all of the subjects still remembered which odor belonged with each category a week after the main experiment. Therefore, we certainly did not push the limits of associative learning, and subjects arguably could have learned far more than four odor-memory pairs, especially with more extensive training. Future experiments should implement a larger catalogue of odors as reactivation cues for individual memory traces, to test whether

[II] Along these lines, a recent study demonstrated that birds of prey could learn associations between biologically irrelevant odors and wrapped food items[125]. The study was conducted at the Bronx Zoo, and test subjects included the following species: Andean condor, bald eagle, golden eagle, cinereous vulture, and king vulture. The authors argue that odors are a valuable addition to enrichment activities for birds of prey in captivity.

olfactory stimuli can truly drive memory consolidation with the same precision as auditory stimuli.

Perhaps more critically, we show that the selective memory gains in our paradigm are strongly correlated with the extent to which olfactory reactivation cues drive replay of category information in vmPFC and posterior fusiform cortex. Moreover, during the sleep period, odor cues promote activity in left amygdala extending into hippocampus, and enhance connectivity between this brain region and vmPFC. Simultaneous recording of EEG and fMRI data during sleep is technically challenging (see Section 3.6.7), and for the vast majority of sensory cueing studies, the sleep period is not scanned. Thus, research exploring fMRI correlates of memory reactivation is scant. Two previous EEG-fMRI sleep studies have demonstrated that olfactory cues evoke activity in left anterior hippocampus during SWS, at similar coordinates as we observed here[54,75]. However, there was no non-reactivation condition (i.e., a control odor or odorless air presented in sleep) for behavioral comparison, preventing more nuanced conclusions regarding the relationship between brain activity and memory. One additional study found that auditory cues elicit fMRI activity in parahippocampal cortex during sleep, but did not demonstrate a behavioral effect of reactivation on post-sleep memory performance, perhaps due to subjects' reduced ability to process sound cues in the noisy scanner environment [102]. Recently, Berkers and colleagues re-analyzed the same data set using a graph-theory approach to evaluate connectivity, and found that auditory cues facilitate network integration of occipital cortex[131]. Although this prior work brings important understanding to the dynamics of reactivating memories during sleep, our study uniquely highlights its behavioral benefits, permitting us to relate memory performance to neural replay on a subject-by-subject basis. Moreover, by utilizing

ensemble pattern-based analysis of fMRI data, we were able to explore the contents of within-sleep memory replay with greater specificity than would be possible with the more conventional fMRI analyses employed in previous studies.

Until recently, the theory that sensory cues enhance memory by triggering replay of associated material during sleep has been implied, suggested, and assumed, but not thoroughly tested[132]. In the past couple of years, a number of labs have raced to address this gap in the literature. Two research articles have been published on this topic in the past year[79,80], with another in the pipeline[133]. All three of these studies used MVPA of EEG data to decode neural patterns, and demonstrated to some degree that learning-specific patterns of EEG activity emerged in response to auditory cues during sleep. That all three studies arrived at similar conclusions using different memory paradigms (i.e., testing procedural memory, recognition memory, or memory for lateralized judgments) and methodologies inspires confidence, and interestingly, two of the three studies linked replay to sleep spindles[80,133]. However, none of these studies demonstrated a behavioral reactivation effect in the traditional sense[JJ], perhaps because memory tasks deviated from well-tested paradigms to favor ease of pattern classification. Moreover, one of the studies included a potentially damaging confound, in that EEG learning data that included sound cues was used to train a pattern classifier[79]. Because the same pattern classifier was then used to identify re-emergence of learning-related patterns during sleep, it is possible that above-chance

[JJ] Specifically, Belal and colleagues reactivated 100% of sound-associated information during sleep, without providing a control to test for a behavioral cueing effect. The Staresina group found that sound cues improved recognition memory, but after an additional night of sleep (without cueing), and not immediately after the reactivation nap. Finally, Antony and colleagues found an almost-significant behavioral effect, but only after limiting their analysis to items that were correctly remembered at pretest (which is arguably an approach to take).

classification could be attributed, at least in part, to decoding of auditory stimuli *per se* rather than replay of associated memories. Although the other two studies were not complicated by the same confound, pattern classification in both cases was limited to the EEG sleep data. Thus, results cannot speak to replay of the same patterns that manifest during wake encoding. In our paradigm, we avoided these issues in that we observe an effect of odor cues on memory performance, and we waited to associate odors with mnemonic content until after fMRI training data were acquired. Also, by measuring replay of category-specific brain activity using fMRI, we could identify brain regions participating in neural replay with a level of regional and network specificity that EEG approaches cannot provide. Thus, our results extend those from previous studies, in what has quickly become a burgeoning field. Further work applying MVPA techniques to neural data – EEG, fMRI, and even intercranial EEG – will likely lead to more exciting discoveries regarding memory replay.

Moreover, additional research is needed to more fully understand the relationship between reactivation cues and memory replay. For instance, so far, most reactivation experiments aim to enhance declarative memory, although prior work spearheaded by Katherina Hauner in our lab demonstrated that odor cues can also dampen fear memories[63]. During my time in the lab, I contributed substantially to postdoctoral fellow Isabel Hutchison's study, where she adapted my EEG-fMRI methods to scan the reactivation period in a modified version of the Hauner paradigm. The goal of this work is to understand how neural replay might serve to extinguish fear memories during sleep, and analysis is ongoing. This study and others should lend to a broader understanding of how cue-evoked replay might influence memory consolidation in different situations.

Outside of the context of sensory cueing, consolidation is thought to involve the gradual integration of declarative memory traces within the neocortex, guided by the hippocampus. It has been proposed that the PFC may play a key role in integrating memories across cortical modules[134,135], and indeed frontal lobe damage has been shown to impair recollection[136-139]. Additionally, PFC (and especially mPFC) has been increasingly implicated in remote memory retrieval[111-118]. For instance, in a study by Takashima and colleagues in 2006, subjects memorized pictures of scenes, and then performed a recognition test during fMRI scanning at four time points after encoding: 1 day, 2 days, 30 days, and 90 days[112]. They found that, over time, correct confident recognition of scene pictures prompted decreased activity in hippocampus, and increased activity in vmPFC. To ensure that elevated vmPFC activity was not reflective of the growing struggle to recognize items as they became more remote, the same group conducted a follow-study, where they replicated their vmPFC findings while controlling for task difficulty[113, KK].

Given the established role of sleep in memory consolidation (see Section 1.2), it is perhaps unsurprising that sleep has been shown to influence the role of mPFC in remote memory retrieval. In 2007, the Peigneux group conducted a study where subjects learned word pairs, and then either returned home for a full night of sleep, or underwent 24-hour sleep deprivation[140]. Six months later, subjects returned to the lab, where they recalled the same word pairs during fMRI

[KK] Notably, in their follow-up study, the Takashima group identified precuneus as an additional region that responded preferentially during remote memory retrieval. We also identified a cluster in left precuneus at similar coordinates, where odor-evoked memory replay in that cluster was related to memory performance, although effects did not survive statistical correction for multiple comparisons.

scanning. The researchers found that recall evoked stronger activity in mPFC for the sleep group, when compared to the sleep deprived group. In a related experiment, subjects studied neutral and emotional pictures prior to a full night of sleep or sleep deprivation[141]. When subjects performed a recognition memory task during fMRI scanning six months later, recollection elicited stronger responses in vmPFC for the sleep group (versus the sleep deprived group), especially for negative pictures. Together, these studies suggest that post-encoding sleep has lasting influences on remote retrieval networks in mPFC.

Moreover, it has been suggested that sleep-based integration of memories into neocortical networks is aided by the slow oscillations characteristic of SWS[142]. In line with this concept, mPFC is thought to be a predominant generator of SWA[143], and cortical volume loss in mPFC has been linked to parallel deficits in SWA and sleep-dependent memory retention[144,145]. Our finding that odors presented during SWS may drive replay of associated mnemonic content in vmPFC to support recall meshes well with this prior work, providing robust mechanistic support that cue-evoked cortical replay promotes consolidation in the sleeping human brain.

Memory consolidation remains one of the greatest unsolved mysteries in neuroscience. How does it work? Why are some memories lost, while others are tagged for long-term storage? Where are those long-term memories exactly, and how do they change over time? And to what extent can we control memory consolidation, with sensory cues, or otherwise? Our work and the studies described in this thesis have only just begun to answer these fascinating questions. Further research in both humans and animal models is needed to more fully address the complex interplay between sleep, sensory reactivation, memory replay, and memory itself. Ultimately, a

better understanding of these relationships could lead to better control over what we remember and what we forget. This could have important implications, not only for healthy individuals, but also for patients suffering from memory disorders, such as Alzheimer's disease.

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