Shining a Light on the Mechanisms of Sleep for Memory Consolidation

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Abstract

Purpose of review This paper reviews all optogenetic studies that directly test various sleep states, traits, and circuit-level activity profiles for the consolidation of different learning tasks.

Recent findings Inhibiting or exciting neurons involved either in the production of sleep states or in the encoding and consolidation of memories reveals sleep states and traits that are essential for memory. REM sleep, NREM sleep, and the N2 transition to REM (characterized by sleep spindles) are integral to memory consolidation. Neural activity during sharp-wave ripples, slow oscillations, theta waves, and spindles are the mediators of this process.

Summary These studies lend strong support to the hypothesis that sleep is essential to the consolidation of memories from the hippocampus and the consolidation of motor learning which does not necessarily involve the hippocampus. Future research can further probe the types of memory dependent on sleep-related traits and on the neurotransmitters and neuromodulators required.

Keywords Theta · Sharp-wave ripples · Delta · Slow oscillations · Memory · LTP · Depotentiation · Timing · Rats · Mice · Optogenetics · Consolidation · Hippocampus · Motor learning · Spatial learning · Spindles · Replay

Introduction

Sleep is a period of heightened vulnerability for an organism—an extended stretch of time during which an animal is oblivious to its surroundings, cut off from environmental cues that would alert it to a potential food source, an approaching mate, or an impending danger. And yet, despite all of the potential survival-related drawbacks that such a state entails, every animal sleeps. From drosophila to laboratory rats to humans, evolution has conserved this unguarded state across time. Clearly, the function of sleep is important enough to outweigh the

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danger of being in such an unguarded state. Researchers have proposed a range of potential roles for each sleep state, including recovery from oxidative stress in the brain [1, 2], energy conservation [3], a period of heightened protein synthesis [4, 5], and the stimulation of a sleeping brain to facilitate transitions into waking [6]. A somewhat more controversial proposition, however, is that sleep is an important stage of time for the consolidation of memories created during waking.

Sleep is divided into multiple stages (Fig. 1a); each of which is identified by stereotyped behavior, electrical activity, and neurochemical composition. The broadest distinction splits sleep into two stages, rapid eye movement (REM) and non REM (NREM) sleep. REM is characterized by muscle atonia, desynchronized waking-like EEG activity, and a 5–10 Hz hippocampal theta rhythm. NREM sleep displays 0–4 Hz slow oscillation / delta activity, accompanied by fast sharp-wave ripple (SWR) events in the hippocampus. Determining the role of each sleep stage and its signatures in the consolidation process is an area of intense investigation within the field of sleep research.

Experimentalists have been investigating the role of sleep in learning and memory storage for years, finding that depriving animals of normal sleep nearly always results in adverse outcomes in their ability to store and retain new information. For example, early studies of REM deprivation in rodents
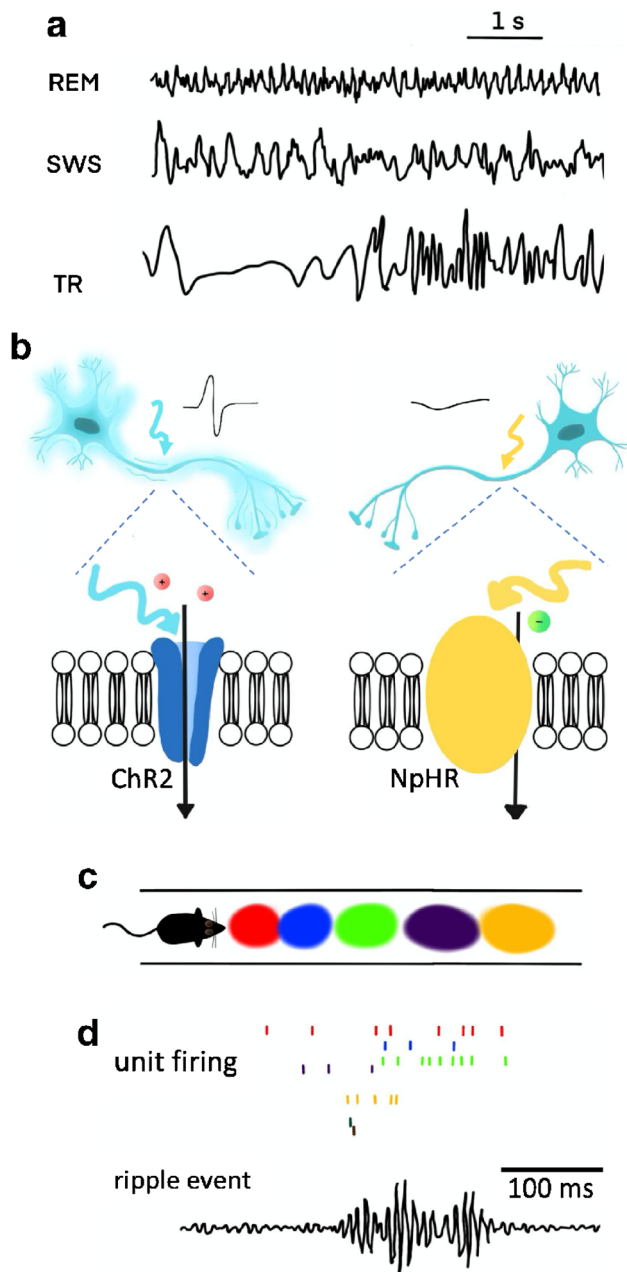
Fig. 1 A) Sample traces of rodent hippocampal EEG during sleep. Top: the 5-10 Hz theta of rapid eye movement (REM) sleep. Middle: Characteristic 2-4 Hz delta rhthym of slow wave sleep. Bottom: Examples of 10-14 Hz sigma in the form of sleep spindles that occur in brief periods of transition to REM (TR) sleep. **B)** Light-activated channels inserted into the neuronal membrane regulating activity. Stimulating channels such as ChR2 allow for the influx of cations when activated via blue light, resulting in membrane depolarization and action potential generation. To inhibit neural activity, light-triggered ion pumps are typically used, such as NpHR, which pumps in CI- ions to hyperpolarize the membrane. **C)**. As an animal (left, from above) moves along its path, place cells (represented by different colors) encode the spatial context. **D)** An example of hippocampal replay during SWS. During SWS, ripple events in the hippocampus are generated by unit, firing of different place cells that at high frequencies, often in the same order as places encountered during waking.

showed decreased performance on hippocampal-dependent working memory tasks, especially contextual/spatial mazes that require established cognitive maps of the animals' environment [7–10]. Physiological studies indicate that REM deprivation results in a loss of excitability in the rodent hippocampus [11, 12] and reduces long-term potentiation (LTP) [13]. Accumulated evidence demonstrates that NREM sleep is beneficial for declarative memory in humans and may provide a time for communication between disparate brain areas, particularly the hippocampus and prefrontal cortex through the generation of sleep spindles, which are correlated to memory recall [14, 15]. Conversely, REM sleep may be an important period for learning procedural tasks [16, 17].

While the body of evidence supporting an important role for sleep in memory consolidation grows, a number of critiques have plagued the field since its inception and proven themselves difficult to fully resolve. Limitations in the specificity of past methods as well as incongruous and conflicting study results have led some prominent sleep researchers to altogether reject any role for sleep in memory processing, instead viewing sleep solely through the lens of homeostatic regulation and systemic physiological function [6, 18, 19]. New techniques such as optogenetics enable researchers to target light-activated ion channels to control the activity of select brain areas, neural populations, or specific memory traces (Fig. 1b). Using light-activated channels such as channelrhodopsin (ChR2) and halorhodopsin (NpHR) allows for precise spatial and temporal neural activation or inhibition in order to tease apart the function of specific structures and cell populations [20]. Modulating neurons at such a granular level during sleep can tell us how activity outside of waking encoding (offline) shapes the connections and circuits of a memory ensemble. In this review, we aim to look at the ways in which optogenetics has allowed us to more specifically delineate the role of sleep in the consolidation of waking experiences (Table 1). We find that optogenetic techniques enable the modulation of various facets of sleep, while avoiding many of the confounding variables obscuring the results of earlier studies.

Stress Is Not the Culprit: Sleep Feature Disruption Using Optogenetics Causes Learning and Memory Deficiencies Without the Stress of Behavioral Sleep Deprivation Methods

One of the biggest critiques of early sleep studies was the difficulty in dissociating sleep loss from the stress induced by methods of sleep deprivation as playing the causal role in subsequent learning deficits. Methods to disrupt REM sleep can result in hypothalamic-pituitary-adrenal (HPA) axis activation and increased cortisol levels, which have been shown to negatively impact hippocampal dependent memory consolidation [36–38]. These methods led researchers to argue that

Table 1 Comparison of studies using optogenetics to investigate the role of sleep in memory consolidation

Study authors, date	Species	Optogenetic manipulation, brain region	Sleep state	Physiological results	Behavioral results
Kumar et al., 2020 [21•]	Mouse	Inhibition & excitation of adult-born neurons (ABN) in DG	REM	Inhibiting ABN ↑ dendritic spines w/o Δ in spine density, head diameter, & neck length	Inhibition & excitation both impaired CFC memory consolidation
de Sousa et al., 2019 [22•]	Mouse	Post-learning activation of RSC	Iso; sleep	↑ 100 Hz in ACC and HPC in sleep or iso	↑ freezing within fam & new context despite HPC inactivation
Davis & Vanderheyden 2020 [23]	Rat	Excitation of MCH neurons	24 h	Initial ↓ REM & NREM in dark phase; then daily ↑ REM & NREM	↓ cued fear freezing
Izawa et al., 2019 [24]	Mouse	Inhibition & excitation of MCH neurons & terminals in HPC	REM	Stim of MCH inputs ↓ CA1 pyramidal activity & ↑ inhibitory currents	Inhibition ↑ memory consolidation; excitation ↓ memory consolidation
Kim et al., 2019 [25•]	Rat	Inhibition of primary motor cortex neurons	SWS	Inhibition during delta ↑ SO-spindle nesting ↑ reactivation in SO-nested spindles	↑ motor performance after delta ensemble inhibition; ↓ neuroprosthetic motor performance after inhibition during SO
Swift et al., 2018 [26•]	Rat	Inhibition of primary motor cortex neurons	Sleep	↓ delta power; ↓ theta power; ↓ sigma power	↓ spatial encoding; ↓ reversal learning
Lu et al., 2018 [27]	Mouse	Activation of GABA neurons of parafacial zone to induce SWS	SWS	Induced slow waves; ↓ EMG tone; ↑ delta power. When stim w/in 30 min of learning:	↑ 6 h novel arm Y maze discrimination; ↑6 h CFC; ↑ 6 h NOPR discrimination
Ognjanovski et al., 2018 [28]	Mouse	Inhibition of PV neurons	SWS	↓ delta power; ↓ theta power	↓ freezing following CFC
Gulati et al., 2017 [29]	Rat	Inhibition of primary motor cortex neurons	SWS	Inhibition during SO upstate = ↓ rescaling of indirect neuronal activity	↓ BMI task performance
Latchoumane et al., 2017 [30•]	Mouse	Induced spindles in PV inhibitory neurons in TRN	SWS	↑ ripples co-occurring with spindles induced in SO upstate	↑ CFC; ↑ NOPR performance
Boyce et al., 2016 [31]	Mouse	Silence medial septal neurons	REM	↓ theta power in HPC	↓ NOPR performance, ↓ CFC recall
Kovacs et al., 2016 [32]	Mouse	SWR-timed silencing of CA1 pyramidal neurons for 3 h	sleep/rest	↓ pyramidal cell activity;↓ SWR; ↑rebound on inhibition release; No D in place cell dynamics	Passive exploration. No learning tests.
Miyamoto et al., 2016 [33]	Mouse	Inhibition of M2 fibers in S1	NREM	↓ M2-S1 causality; reactivated S1 neurons	↓ texture recognition
van de Ven et al., 2016 [34]	Mouse	Inhibition of SWR in principal hippocampal neurons	sleep/rest	↓ assembly pattern reactivation during novel environment re-exposure	No Behavior Tested
McNamara et al., 2014 [35]	Mouse	Activation of dopaminergic neurons in VTA or CA1	Waking	↑ ensemble reactivation strength during SWR in sleep	↑ maze performance
Rolls et al., 2011 [36]	Mouse	Activation of hypocretin neurons	All stages	↑ sleep fragmentation; TR-specific ↓ delta power and ↑ theta power	↓ NOR performance in light phase (sleep phase)

Abbreviation Key: *ABN* adult-born neurons, *DG* dentate gyrus, *REM* rapid eye movement sleep, *CFC* contextual fear conditioning, *RSC* retrosplenial cortex, *Iso* isoflurane, *ACC* anterior cingulate cortex, *HPC* hippocampus, *MCH* melanin-concentrating hormone, *NREM*, *CA1* cornus ammonis 1 region, *SWS* slow wave sleep, *SO* slow oscillation, *NOPR* object place recognition, *PV* parvalbumin, *TRN* thalamic reticular nucleus, *SWR* sharp wave ripples, *S1* primary somatosensory cortex, *M2* secondary motor cortex, *VTA* ventral tegmental area, *TR* transition to REM, *NOR* novel object recognition, *MS* medial septal nucleus, *LC* locus coeruleus,

the behavioral deficits seen were a result of impaired hippo-campal function due to stress rather than sleep loss. Indeed, stress does impair hippocampal neurogenesis just as chronic sleep disruption and sleep deprivation does, and perhaps the two mechanisms are the same [37, 38]. With the use of optogenetics, the studies discussed herein target specific sleep stages to modulate sleep features in a manner that does not introduce stress as a confounding variable.

One set of experiments used an optogenetic protocol to fragment sleep without inducing stress in the animal [36]. By selectively expressing ChR2, an excitatory optogenetic ion channel (Fig. 1b), in hypocretin/orexin (Hcrt) neurons in the lateral hypothalamus (LH), investigators were able to decrease latency to arousal in both REM and NREM, resulting in fragmented sleep periods. Stimulation of the LH at 60 s intervals with blue light resulted in Hrct neurons firing action potentials and was enough to fragment sleep, as measured by microarousal periods. Overall sleep amount, quality, and composition of sleep was unchanged, and no evidence of sleep debt occurred following the stimulation protocol, indicating that only the continuity of sleep was disrupted. Recent research suggests that this arousal effect might be mediated through extensive projections to the locus coeruleus (LC), an important structure for sleep and arousal regulation [39–41]. To ensure that the animal was not under stress due to stimulation, investigators tested cortisol levels in the plasma of both ChR2 and control mice following the 4-h stimulation protocol, finding no difference between stimulated and unstimulated animals. Similarly, animals that underwent the optogenetic stimulation did not show increased anxiety behavior in an open-field maze. Taken together, these findings show that the optogenetically induced sleep fragmentation protocol did not increase physiological markers of stress or behavioral indications of increased anxiety.

The Hcrt stimulation-induced sleep disruption paradigm allowed researchers to directly test the effect of discontinuous sleep on learning and memory. Using the novel object recognition (NOR) task as a measure of hippocampal- and sleep-dependent memory [42–44], they ran animals through the optogenetic stimulation protocol during sleep in the crucial 4 h window during the light period immediately following training on the task [9]. In animals with Hcrt neurons expressing ChR2, the blue light stimulation resulted in a significant impairment in NOR performance as compared to control animals. Importantly, this effect was only seen when stimulation occurred in the light period following task acquisition, not in the dark period hours later (outside the critical consolidation window), highlighting the role of Hcrt neurons in sleep-dependent consolidation rather than a result of aberrant Hcrt activity alone. Additionally, this effect was abolished when stimulation intervals were increased to 120 s apart, indicating that there is a minimum length (> 60 s) of sleep "quanta" necessary for adequate memory consolidation in the rodent. Another study found that 60-s long inductions of SWS

repeatedly for 30 min within 30 min of learning stabilized the memory such that discrimination was maintained for as long as 6 h after object place exposure and novel arm Y maze task [27]. They produced SWS by optogenetically stimulating the GABAergic neurons in the parafacial zone, thereby increasing delta and reducing EMG activity as is consistent with the induction of SWS. The 60-s long inductions of SWS also stabilized contextual fear memory and improved context discrimination in these animals.

It would be interesting to know what that minimum undisturbed sleep quanta for memory stabilization and consolidation is in the human. Even as few as 5 airway obstruction–related arousals per hour (equating to once every 12 min) is associated with memory deficits in humans [45].

The ability to separate the effects of sleep deprivation from the methods used to sleep deprive and the negative effects they can cause allows research to move toward understanding the role that each component of sleep plays in the memory consolidation process. Work in our lab has used optogenetics to change baseline activity in neuromodulatory systems during sleep without disrupting overall time spent in sleep or sleep architecture itself [26•]. Unlike the Hcrt study, we did not induce arousals from sleep, yet found similar memory consolidation deficits related to changes in the electrophysiological features of each sleep state. We optogenetically modulated the locus coeruleus (LC) activity during sleep, and provided correlational evidence for the role various sleep signatures play in sleep-dependent consolidation. Typically, the LC quiets during NREM periods and is silenced during REM sleep [46]. Maintaining LC activity at waking levels throughout sleep after learning resulted in a decrease in the 1–4 Hz delta band and in the 10–14 Hz sigma band corresponding to sleep spindles. Longer spindles, which increased in control learning animals, were nearly eliminated when LC activity was maintained during sleep. In REM sleep, theta power was significantly decreased during LC stimulation. These changes were correlated with poor performance in incorporating changes made in a spatial memory task. Greater reductions in delta and theta band power were directly related to an increase in switching away from reliance on the hippocampal map. Similarly, reductions in spindle-ripple coupling predicted confusion between the original and modified food positions on the maze. The ability to adjust the frequency of LC activation with optogenetics during sleep allowed us to keep normal sleep bout lengths, yet still observe changes in memory consolidation.

The fact that changing the underlying physiology of sleep without disrupting other aspects of sleep affects memory consolidation suggests that the brain activity associated with each sleep state, and the particular neurochemical composition associated with each phase of sleep, is critical in supporting memory consolidation. Additional studies have found that

optogenetic modulation of neuronal subtypes during sleep can similarly disrupt memory consolidation. Researchers investigating the inhibition of parvalbumin interneurons (PV+) during NREM sleep found that this intervention abolished the typical post-learning sleep increase in delta and theta power in the hippocampus [28]. In another group of animals that were sleep deprived, a similar decrease in delta and theta power was observed. These delta and theta power decreases were concurrent with disrupted contextual fear memory in both groups. However, activating hippocampal parvalbumin interneurons rhythmically at theta frequency during sleep deprivation stabilized CA1 population activity measured during subsequent sleep and rescued appropriate post-sleep hippocampus-dependent fear behavior. These findings suggest the necessity of sleep at least partially lies in the population activity engendered during the state and provides evidence that this activity directs the consolidation of memories.

Optogenetics Allow Us to Target and Modulate Specific Sleep Signatures, to Test Their Causal Relationship to Learning and Memory

NREM Sleep

The phenomenon of hippocampal replay present in NREM sleep has long intrigued sleep researchers as a possible mechanism by which labile memory traces in the hippocampus are stabilized and sent to the neocortex for long-term storage. Replay consists of cells active during waking task acquisition (Fig. 1c) being reactivated during sleep (Fig. 1d) with conserved temporal order. Ripples (Fig. 1d) take place in the CA1 region of the hippocampus indicating fast, synchronous excitatory inputs from the principal cells of the CA3. Replay of waking sequences occurs at the peak of ripples [47, 48]. Recruitment into ripple activity is at least in part associated with the strength of connections between cells formed during wakefulness—cells that are significantly more likely to fire together during waking are more likely to fire together during ripple associated replay events [47, 49]. Thus cell participation in ripple events is heavily biased by waking patterns of activity. These findings served as the basis for the theory that SWR and replay activity therein are mechanisms for strengthening and storing memories.

Indeed, when SWR activity is electrically disrupted during sleep following acquisition of a new hippocampus-dependent task, a significant impairment is observed in an animal's ability to perform those tasks, likely due to aberrant consolidation [50, 51]. Whether this performance impairment was due to the loss of SWRs specifically, or simply the disruption of hippocampal population activity due to the electrical stimulation, was not testable until optogenetics became a common technique.

Studies utilizing optogenetics found that selectively inhibiting pyramidal cells of the CA1 once SWRs were detected resulted in a significant decrease in an animal's ability to reinstate assembly activity representing a novel environment [34]. Intriguingly, inhibiting SWRs did not result in a change in assembly activity representing already consolidated familiar environments, suggesting the necessity of hippocampal SWR activity for consolidating new information. In addition, novel assemblies that stabilized more rapidly than others during waking acquisition were also unaffected by the disruption of offline reactivation, perhaps because their original online activity was enough to result in synaptic stabilization, obviating the necessity of consolidation. These findings support the hypothesis that offline activity is important for resolving unfinished business, strengthening weak connections made during waking.

A second study using a similar paradigm in which principal CA1 cells express an inhibitory optogenetic ion channel investigated the effect of SWR suppression during the 3 h following passive exploration of a novel open field. They explored the ability of hippocampal place cells to subsequently encode the same open field environment after inhibiting pyramidal activity for 500 ms triggered after the detection of the ripple. No explicit learning task was given for the animals in the environment. Surprisingly, no significant differences were found between the place cell stability, coherence, or likelihood of remapping in the novel environment between animals with and without CA1 pyramidal activity during spontaneous SWR in this offline period [32]. Given the task impairment seen in Girardeau et al. [50], as well as the change in assembly activity in novel environments of the van de Ven [34] paper, one might conclude that disrupting the place cell code during the consolidation of novel memories underlies the spatial memory deficits seen following SWR inhibition. On the surface, this conclusion is not born out by Kovacs et al. [32]. However, they did observe a post-inhibition rebound in pyramidal cell activation that appeared to be coincident with strong local field potential (LFP) depolarization and, in their example, occurred in the ripple frequency. The apparent rebound depolarization-coupled ripple activity could itself have stabilized the memory code. Also, the authors point out that passive exploration may be relatively resistant to memory disruption by SWR inhibition as compared with active learning. For instance, a task in which learning is involved (and tested), such as an appetitive maze, should require the encoding and association of more information than is required when wandering in an open field. Another possibility is that ripples are involved primarily in the transmission of information to long-term cortical storage, and their disruption does not have an effect on spatial map stabilization within the hippocampus. Future experiments should be able to distinguish between these possibilities.

REM Sleep

The question of whether REM sleep has a role in learning and memory has been particularly controversial [6, 18]. In rodents, early sleep deprivation studies indicated that REM sleep is necessary for proper memory formation; however, as discussed above, the methods available at the time came with numerous caveats, leaving some sleep researchers skeptical of whether REM deprivation was truly causing memory impairments. Similar to our ability to optogenetically disrupt sleep as a whole, we now can design experiments selectively targeting REM sleep independently to isolate its role in memory processing without causing stress to the animal or affecting other sleep states. What we have learned about REM sleep thus far suggests that it serves several key functions in consolidation.

REM Sleep Is for Forgetting

REM sleep appears to be a time during which synapses are pruned, creating circuits with more sparse and more specific firing. Recent studies in mice show that the pruning of dendritic spines in the motor cortex (M1) during REM sleep is coincident with the improvement on a rotarod task [52, 53]. Similarly, the firing of adult-born neurons (ABNs) in the dentate gyrus becomes more sparse in response to contextual fear conditioning (CFC) following a period of REM sleep [21•]. These data provide evidence that one of the functions of REM sleep is to pare down excessive activity following learning, and suggests a role for REM sleep in "forgetting" or weakening memories.

Studies of melanin-concentrating hormone (MCH) neurons in the hypothalamus provide further evidence for the function of REM in forgetting [24]. Optogenetically inhibiting REM-active MCH neurons during REM periods following the learning phase of a novel object recognition (NOR) task improves the animals' ability to discriminate between new and familiar objects. The converse was also true—when MCH cells were optogenetically activated during REM periods, the animals' ability to discriminate between novel and familiar objects was diminished, as was their performance in the second task: contextual memory following fear conditioning. As this study also found that REM periods normally involve MCH cell activity, REM sleep appears to play a role in forgetting. It may be that the normal MCH activity during REM serves the function of schematic integration and extraction of gist, losing details as memories are moved to long-term storage or schematized.

REM Sleep Is for Remembering

To complicate matters further, however, evidence also exists that REM sleep promotes consolidation as well. The inhibition of theta activity by silencing medial septal GABAergic projections to the hippocampus during REM sleep significantly disrupted consolidation of CFC and novel object place recognition (NOPR) tasks [31]. This result raises the possibility that REM sleep is a time for both strengthening and weakening synapses due to the unique electrophysiological and neurochemical properties of the hippocampus in REM sleep. Hippocampal theta activity during REM sleep segregates the population activity into temporal cycles [54–57], which may be key to allowing both synaptic strengthening and weakening during this period of time. Previous studies from our lab show that spatial representations fire differently in relation to theta phase based on their novelty or familiarity [58]. New place cells fire at peak population activity, resulting in synaptic strengthening, while place cells encoding familiar spaces fire closer to the low point of activity, likely weakening the associated synapses through the principles of heterosynaptic depotentiation [58]. In addition to the theta rhythm, the neurochemical composition of the hippocampus during REM sleep, particularly the complete lack of noradrenaline and high levels of acetylcholine, could make it an optimal time for restructuring synaptic connections and integrating new information into old memory schema [25•, 59].

Population Activity and Timing Is Key in Sleep-Dependent Consolidation

Through the temporal precision afforded by optogenetic techniques, sleep researchers have been able to tease apart the function of various sleep signatures and oscillatory activity. What we are finding suggests that the effect that a particular sleep stage or signature has on a neural ensemble is highly dependent on the population activity surrounding the ensemble. We have a great deal of evidence that reactivation of an ensemble during sleep strengthens the circuitry involved [47, 48]; however, it was initially unclear if this strengthening was simply a result of activity-dependent plasticity that could occur during any brain state. Furthermore, activity within sleep stages must be tightly temporally regulated in order to properly consolidate information. Studies suggest that the timing of action potentials of cells in an ensemble in relation to oscillatory population activity is critical to whether the connections of a memory trace will be strengthened or weakened. In a series of studies utilizing a brain-machine interface task in rats, researchers determined that whether an ensemble reactivated in closer proximity to a slow oscillation (SO) or to a delta wave had an effect on whether the memory was consolidated or weakened [29]. Ultimately, they found that perturbing spiking activity during the upstates of SOs led to an impairment of memory consolidation, whereas disrupting spiking during the upstate of a delta wave actually boosted sleep-dependent memory consolidation. Further exploration suggests that one underlying mechanism of the changes in consolidation is dependent on nesting of spindles within SOs or delta waves. SO-spindle coupling appears to preserve memory reactivation, as

reactivation strength and duration increased with SO-spindle coupling, and changes to SO-spindle coupling correlated with task performance. Interestingly, when researchers optogenetically perturbed SO spiking activity, a rapid reduction in reactivation strength followed. The converse was true when delta activity was disrupted, resulting in significantly stronger and longer-lasting reactivation, supporting the idea that inhibiting delta waves increased the strength of a memory. Ultimately, inhibiting SO activity abolished any significant rescaling of the neural network involved in the task used, resulting in impaired performance. In another study, this group inhibited the specific neural ensemble controlling the task selectively during the upstates of NREM sleep, which also impaired task performance as well as the ability of the ensemble to develop sparse coding of the task [22•]. Finally, another group showed the same relationship between SO-spindle coupling and spindle-nested ripples that indicate cell activation. When they increased sleep spindles by activating parvalbumin interneurons in the reticular nucleus of the thalamus, they increased spindle-ripple coupling in the upstate of SO's and increased memory on both contextual fear memory and novel-object place recognition [30•]. These data all support the hypothesis that sleep is a time during which synaptic connections are both strengthened and weakened in order to efficiently and accurately encode an animal's environment.

Neural Ensembles Require Offline Processing to Accurately Consolidate New Information

Optogenetic techniques allow for further exploration into the circuit-level changes underlying disrupted memory consolidation following abnormal sleep dependent activity. Alteration of sleep state activity described in the experiments above broadly results in memory impairment suggesting a relationship between processing during sleep and integrity of memory ensembles. Although they provide invaluable insights, direct manipulation of neural ensemble activity during sleep is required.

Rhythmic Activity like the Theta Oscillation Is Important for Memory Consolidation

So far, studies suggest that ensembles undergo reshaping during offline processing that can lead to both strengthening and weakening of synaptic connections. The participation of these ensembles in the local rhythmic activity present in sleep appears to be crucial for proper memory formation, in a manner that is highly specific. In an experiment by Kumar et al., optogenetically modulating the activity of adult-born neurons (ABNs) elucidated the role of these cells in consolidating contextual fear memories during REM sleep [21•]. Their study shows that the tuning of ABNs representing the CFC memory during REM sleep is critical to the animal's ability to recall this memory the following day. Inactivating the ABN ensemble during REM sleep resulted in poor consolidation of the CFC memory, suggesting the necessity of ABN activity in the DG during REM sleep for memory retention. Silencing ABN activity during NREM sleep following learning did not produce the same effect, indicating a role for REM activity of ABNs in the processing of fear memories.

Non-ensemble Cells Are Not Involved

Intriguingly, when ABNs that were not part of the memory encoding process were randomly stimulated during REM sleep, consolidation was also impaired. This suggests that the integrity of the ensemble active during REM processing is critical to proper consolidation. Similarly, it provides evidence of the causal nature of sleep reactivation for memory consolidation—it suggests a role for the unique population activity of sleep stages in shaping ensemble circuits, which directly contributes to the retention of a memory.

Cortical Ensembles Also Participate in Offline Processing for Memory Consolidation

The necessity of ensemble reactivation during sleep appears to be critical to brain regions outside of the hippocampus as well. When looking at CFC in the retrosplenial cortex (RSC), researchers found a similarly important role for reactivation in learning and memory [22•]. Isolating stimulation to cells active during a CFC task enabled tightly controlled temporal and spatial modulation of the ensemble presumed to represent the CFC memory trace. To examine the necessity of activation during offline processing to memory integrity, researchers optogenetically stimulated the fear memory ensemble during both anesthesia and natural sleep; ultimately, both conditions produced similar results. This study in particular focused on examining the potential role of sleep-dependent processing in transferring memories to long-term storage. Predictably, animals with activity in their hippocampi blocked 24 h after learning did not form the proper association between the context and the shock, indicating an improperly consolidated fear memory. This was not the case in the animals that underwent optogenetic stimulation of the RSC memory trace during either anesthesia or sleep right after learning—these mice displayed normal freezing behavior despite the 24 h later hippocampal block, suggesting that high-frequency activation of the trace ensemble was able to create a hippocampus-independent memory of the fear trace, effectively accelerating the process of systems consolidation. Intriguingly, the optogenetic stimulation protocol was only effective when the animals were in a non-waking state. Stimulation of the fear trace while animals were awake and behaving did not protect against memory impairment following hippocampal

block, indicating that sleep state activity, or anesthesia plus artificially stimulated activity, is necessary for systems consolidation. Which aspects of sleep are crucial for this process, however, are uncertain, as these experiments did not differentiate between REM or NREM sleep. Additionally, as the researchers note, their stimulation protocol did not maintain the temporal patterns of activity. Given the difference in effectiveness of the optogenetic stimulation between wakefulness and sleep, future research into whether stimulation during only REM or NREM is still effective in inducing systems consolidation would provide more evidence into the role of each sleep stage in long-term memory storage.

Similar work in the visual cortex assessed the effect of offline stimulation of the neural ensemble on memory consolidation [60•]. Rhythmically activating this trace while an animal was anesthetized biased surrounding neurons toward responding to the same cue during subsequent waking, suggesting a role for offline activity in modifying responsiveness of visual cortical neurons to allow visual discrimination. Inhibition of the cue ensemble during REM and NREM sleep resulted in a generalized behavioral response to all gradients. It will be interesting to observe in future studies what, if anything, distinguishes the roles of REM and NREM sleep in consolidation.

Conclusions and Future Directions

In summary, applying optogenetic techniques to sleep research has provided an exciting new avenue by which to understand the mechanisms involved in sleep-dependent memory consolidation. We are now able to say with confidence that the negative effects of sleep deprivation on memory consolidation are not solely due to the anxiety- and stress-inducing methods of sleep deprivation. Instead, we have begun to determine the specific functions of sleep signatures such as ripples coupled with spindles nested in slow oscillations and theta. The studies we covered here included demonstrations of sleep's involvement in hippocampus-dependent learning such as spatial learning and contextual fear learning, perceptual learning-like visual pattern and texture discrimination learning, motor learning, and even neuroprosthetic learning, the consolidation of all of which are disrupted by specific sleep feature manipulations: delta, theta, spindles, spindle-ripple coupling, and slow oscillation spindle coupling during REM and non-REM sleep. In fact, using optogenetics to selectively disrupt these features of sleep without altering overall sleep architecture or structure consistently finds learning and memory disruptions, unlike the gross sleep deprivation or disruption studies which have produced more variable results.

Despite these advances, the mysterious nature of sleep still remains, as do challenges to the sleep-memory hypothesis.

For example, we are still unsure of the degree to which experimental sleep studies done in rodents (where the vast majority of our knowledge of sleep's electrophysiology comes from) are applicable to humans. Because of the difficulty of obtaining human intracranial electrophysiological data, for example, we cannot be fully confident that sleep signatures important to rodent learning and memory, such as hippocampal replay or REM sleep theta, are present in human sleep, let alone critical for our sleep-dependent processing. In order to link sleep disturbances to disease and psychiatric illness, more studies will need to be done to demonstrate that sleep-dependent consolidation processes in laboratory animal models are analogous to human sleep. Without the ability to target specific neural ensembles in the human brain, as is possible when using optogenetics in animal models, researchers instead use targeted memory reactivation (TMR) as a method to modulate electrophysiological activity during sleep. TMR involves the re-exposure of sensory cues (odor or sound) during sleep that were present during wakeful learning. Unlike the use of light in rodent optogenetic studies, TMR uses cue presentations as a method to selectively reactivate neural ensembles associated with the stimulus. Since the discovery that TMR modulates neural activity in the sleeping brain at the time of cue presentation and improves memory of associated items at subsequent testing by Rasch et al. [61], researchers have sought to elucidate to what extent findings in rodent studies apply to humans. For a full review of recent publications related to this topic, see Cellini and Mednick 2018 [62] and Mankin and Fried 2020 [63]. The use of intracranial recordings to explore sleep in select patient populations as well as new non-invasive technology such as magnetoencephalography in healthy participants will help shed light on some of these questions.

The power of optogenetics as a technique comes from allowing researchers to design experiments that target neurons for modulation in a temporally and spatially specific manner. For sleep research in particular, this means being better equipped to test the underlying mechanisms of sleep without adding confounding variables such as stress or sleep architecture changes into experiments. From just the last few years of research into sleep using optogenetics, we have gathered convincing evidence that without sleep, memories are not processed correctly, largely due to the disturbance of activity patterns underlying sleep signatures such as ripples and spindles. We are beginning to see evidence of sleep-dependent processing occurring in various regions of the brain such as the visual cortex and retrosplenial cortex and, through optogenetic interruption, learning that when the electrophysiology of sleep is disrupted, memory consolidation is impaired as well. The specificity allowed by optogenetic techniques means we are able to narrow down our inquiries to single neuronal ensembles representing a memory trace and observe the impact of sleep-dependent consolidation on that memory alone.

Declarations

Conflicts of Interest The authors declare no competing interests.

Human and Animal Rights and Informed Consent This review article does not report any previously unpublished studies with human or animal subjects performed by any of the authors.

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•• Of major importance

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