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Iterative Expansion and Contraction of Memory Engrams Across NREM-REM Cycles

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Abstract. Many theories have proposed sleep's role in memory, with the consensus that sleep strengthens memories and transfers them from the hippocampus to neocortical networks for long-term storage. Memory representations are not fixed, because cells have been shown to enter and leave memory engrams post-learning. However, while different theories account for different phenomena, no theory has yet explained how memory engrams can change post-learning, especially during sleep. This review examines synaptic, ensemble, network, and human representational evidence to propose the engram sculpting hypothesis, in which sleep-dependent memory consolidation may change which neurons a memory engram contains. We hypothesize that non-rapid eye movement (NREM) expands a memory's engram through disinhibited, replay-associated recruitment, whereas rapid eye movement (REM) does the opposite by selectively pruning and refining it. We speculate that repeated NREM-REM cycles increase engram selectivity, keeping the most strongly involved neurons and removing the more weakly involved ones, so that the engram ends up larger than before sleep but more selective. What this does to the memory itself is still unclear, since current evidence supports both context discrimination and generalization. If supported, our engram sculpting hypothesis could revolutionize our understanding of sleep-dependent memory consolidation by recognizing engram reorganization as another role sleep plays in memory.

Keywords: sleep-dependent memory consolidation, NREM sleep, REM sleep, memory engram, ensemble selectivity

1. Introduction

Sleep supports the long-term storage of declarative, procedural and emotional memories, and the main explanation for this process is the active systems consolidation framework [1]. According to this framework, memories formed during learning are repeatedly reactivated in the hippocampus during NREM sleep and gradually transferred to the neocortex for long-term storage. This NREM memory consolidation process is coordinated by slow oscillations, spindles and hippocampal sharp-wave ripples, while unnecessary synapses are weakened through processes such as the removal of AMPA receptors [2]. There has been strong experimental evidence supporting the active systems consolidation framework. For example, hippocampal place cells firing together during spatial learning tended to fire together again during post-learning NREM, while cells that were inactive or

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represented different locations did not show the same pattern [3]. Moreover, blocking sharp-wave ripples after learning impairs spatial memory without disrupting overall sleep [4]. These findings suggest that memory reactivation during NREM is necessary for consolidation and integration into cortical networks, and is likely facilitated by slow oscillations, spindles and ripples [5].

Whilst sleep research has focused mainly on how and when memories are reactivated, a parallel field of engram research has investigated the neurons that physically represent those memories. Engram cells are neurons activated during an experience that undergo lasting physical change and whose reactivation is both necessary and sufficient for recall [6]. However, engrams are not stable cell populations and can change dynamically. Over roughly two weeks, prefrontal engram cells generated at learning mature from a silent to an active state while hippocampal engram cells undergo the opposite process, meaning that the engram representing a specific memory differs largely between short-term and long-term recall [7, 8]. However, although this form of engram change (systems consolidation) requires weeks, engram composition within the dentate gyrus (DG) alone can change within just hours of encoding, a much faster timescale. Activity-dependent labelling, longitudinal calcium imaging and optogenetic manipulation within the mouse DG showed that some neurons were dropping out whilst others were joining in, and that inhibitory synaptic plasticity was necessary for the engram itself to be reactivated more strongly in the learning context than in a different one [9]. However, the role of sleep in this faster process of engram turnover has not been explored, which is a gap that this review aims to fill.

Sleep studies have explained in detail the oscillations and neural activity that accompany memory consolidation but generally do not track how individual neurons enter or leave the memory. Engram studies, meanwhile, have shown that engrams can change within hours of learning, but have rarely recorded and associated these changes with sleep. The lack of overlap between engram and sleep studies — no single study has measured both engram expansion and contraction in the same experiment, let alone during sleep — therefore raises a central research question that this review paper answers: do NREM and REM sleep affect engram composition differently after learning, and if so, how?

Given their differences in physiological and neurochemical properties, it is reasonable to hypothesize that NREM and REM sleep differentially regulate engram turnover. NREM has strongly reduced cholinergic activity, whereas REM has high, wake-like cholinergic tone with minimal noradrenergic activity [10]. A biophysical network model has shown that this single neurochemical difference is enough to give the two states opposite effects on a memory trace: low acetylcholine during NREM disinhibits inactive excitatory cells, allowing them to be recruited into an engram; and high acetylcholine during REM suppresses all cells except the most strongly associated, thus pruning the expanded population [11]. However, REM does not just globally downscale all activity; instead, chronic two-photon imaging has shown that REM sleep prunes the majority of newly formed dendritic spines, but selectively strengthens and maintains a minority of them, which are the ones that predict behavioural improvement [12].

This review hypothesizes that NREM and REM work together to reshape memory engrams across repeated sleep cycles. During NREM, reactivation of a memory recruits' additional neurons and synaptic connections into the engram. During the following REM stage, some of these newly recruited neurons may be removed, while the more strongly involved ones are maintained (Figure 1). REM contraction here means removal relative to the population that NREM has just added, not relative to the original engram. Repeated NREM-REM sleep cycles could therefore leave an engram that is larger than it was before sleep, but also more selective, with a higher proportion of strongly involved neurons.

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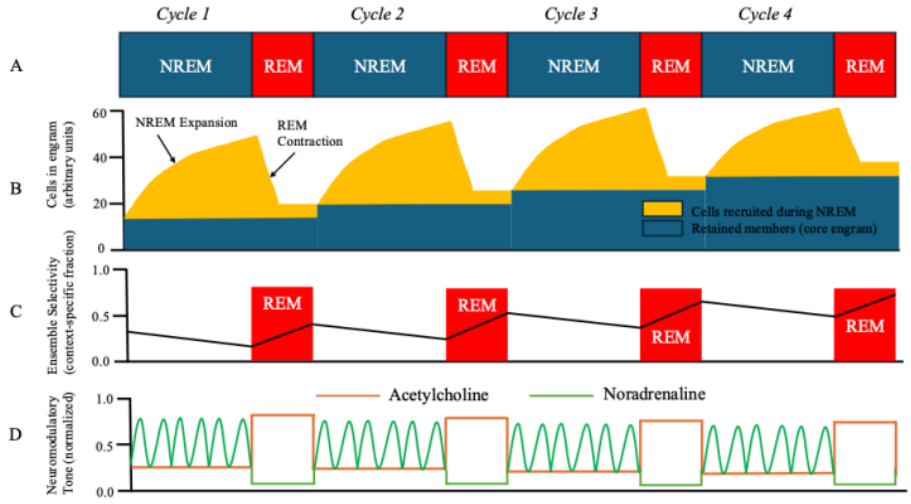

Figure 1. Our engram sculpting hypothesis's proposed NREM expansion-REM contraction cycle. (A) Four cycles of NREM-REM sleep throughout the night. (B) Ensemble membership: NREM recruits cells (yellow) into the core engram (blue), most of which are removed during the following REM. (C) Ensemble selectivity falls during expansion and rises during contraction, accumulating across cycles. (D) Neuromodulatory differences between NREM and REM: acetylcholine levels are low in NREM but high in REM, whereas noradrenaline levels oscillate on an infraslow timescale during NREM but become very low during REM

2. Evidence for engram sculpting across biological scales

Our engram sculpting hypothesis, suggesting that NREM and REM affect engrams oppositely, carefully considers evidence at the synaptic, ensemble, network and human representational levels.

2.1. Synaptic level: REM-dependent synaptic pruning is a possible mechanism for engram contraction

2.1.1. REM's role in synaptic pruning

At the synaptic level, existing literature supports our engram sculpting hypothesis by establishing REM-dependent synaptic pruning as a possible mechanism and logical basis for engram contraction. The clearest structural evidence supporting REM's role in synaptic pruning comes from chronic imaging of dendritic spines on layer 5 pyramidal neurons in the mouse motor cortex. After motor learning, many new dendritic spines are formed, but a large proportion of them are pruned later during REM sleep [12]. However, whilst many newly formed spines are removed, a smaller group associated with stronger memory and better behavioural performance is actually preserved and strengthened; these two processes of spine elimination and strengthening both depend on dendritic calcium spikes during REM [12]. Findings from other paradigms also support REM's role as a selective filtering and elimination process. Monocular deprivation and auditory fear conditioning both resulted in rapid spine elimination in the visual and frontal association cortices. This elimination was reduced by total sleep deprivation and REM deprivation, and was prevented completely when REM dendritic calcium spikes were blocked, showing that REM contributes causally to synaptic pruning [13]. In contrast, REM deprivation does not significantly affect the initial formation of new spines after motor learning [12].

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Although REM's role in synaptic pruning does not automatically mean it can also prune the individual neurons representing a memory, it provides a reasonable logical base: If REM is also selective about connections in an engram, then neurons carrying weaker or less relevant information will be removed while those carrying core features are preserved, just like synapses. Besides providing a logical basis, synaptic pruning may also be a lower-level mechanism for engram contraction. During REM sleep, selective loss of synaptic connections between engram neurons could cause some neurons to receive less input from neighbouring neurons. As a result, these neurons may no longer reach the threshold needed to fire an action potential even when other neurons fire, reducing their participation in the memory trace and causing their dropping out of the engram.

2.1.2. Synaptic homeostasis hypothesis does not contradict REM's proposed role in selective filtering

Our proposed idea of REM as a selective filtering process (strengthening some synapses and neurons in the engram whilst weakening the majority) seems to conflict with the synaptic homeostasis hypothesis at first glance, which proposes that sleep broadly downscales *all* synaptic strength after wakefulness to maintain synaptic homeostasis [14]. As evidence for synaptic homeostasis, high noradrenaline has been shown to keep Homer1a away from excitatory synapses during wakefulness, whereas reduced noradrenaline during sleep allows Homer1a to enter synapses and promote weakening [15]. Electron microscopy also shows a net reduction in axon-spine interface size across sleep [16]. However, electron microscopy shoes a *net* reduction, suggesting that synaptic homeostasis supports net downscaling, not global downscaling; the majority of weak synapses undergo larger reductions, while stronger synapses are relatively preserved [16]. Therefore, synaptic homeostasis is actually compatible with our engram sculpting hypothesis, as both allow some connections to be removed or weakened while others are preserved.

In summary, synaptic-level evidence shows that REM selectively remodels connections, removing many synapses while preserving a smaller, relevant subset. This gives our hypothesis a possible synaptic mechanism for REM-dependent engram contraction, but not direct evidence for it. The next section examines whether engram-level evidence even supports engram expansion, contraction and change in the first place.

2.2. Ensemble level: can engram composition actually change

For our engram sculpting hypothesis to be possible, three things must be true at the engram ensemble level: engrams must be able to change at all, engrams must be able to gain neurons, and they must be able to lose them.

2.2.1. Engram composition can change within hours post-learning

Firstly, as mentioned before, engram composition can change dynamically in a short timeframe. Other than long-term systems consolidation [7], there is also short-term DG engram modification within hours after learning [9]. This change is functional instead of random, since the engram became increasingly selective for the learning context as neurons dropped out of and into it, and blocking inhibitory synaptic plasticity during consolidation prevented engram selectivity [9]. Importantly, it was the changing engram whose activity predicted how well the mice discriminated the learning and neutral contexts, not the fixed set of cells initially tagged at learning [9]. Engram

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turnover in the hours post-learning therefore seems to be functional instead of random, although this study did not record sleep.

2.2.2. Engram expansion

Secondly, engrams can also gain neurons, and sleep is involved. Simultaneous calcium imaging and engram labelling in mouse CA1 revealed that some engram cells already tend to fire together during sleep even before learning. After learning, synaptic depression and scaling allowed these preconfigured populations to become active during memory reactivation and thus join a memory engram [17]. Besides the hippocampus, engram expansion has also been demonstrated causally in the sensory cortex. Targeted recombination was used in active populations to label primary visual cortex neurons active during visual fear cue encoding, which were then optogenetically reactivated in anaesthetized mice. This artificial reactivation of the neurons was enough to increase the representation of the visual cue's preferred orientation among surrounding V1 neurons, whereas inhibiting reactivation reduced this expansion recruitment [18]. Therefore, engram reactivation during sleep can expand the engram by recruiting surrounding neurons and preconfigured cell populations. Moreover, a further result from the same study shows that this sleep-associated recruitment matters for behaviour: optogenetic inhibition of the labelled population during post-conditioning sleep (thus preventing memory reactivation) resulted in mice freezing more to both the conditioned and the neutral orientation and failing to discriminate between them, indicating memory overgeneralization [18]. However, this behavioral evidence cannot settle whether engram expansion ultimately supports discrimination or generalization, as the inhibition was applied across both NREM and REM, meaning that engram contraction could also have been disrupted.

2.2.3. Engram contraction

Finally, evidence for engram contraction is slightly weaker; the clearest evidence is simply that neurons drop out of the engram over the hours after learning [9], but no study has yet watched them drop out during a defined sleep stage. It is also not obvious that losing neurons should help. Tracking c-fos-labelled dentate populations across learning and recall showed that larger reactivated engrams supported better contextual discrimination, whereas smaller engrams resulted in greater generalization [19]. However, we are not confident to use this finding as a conclusion, due to two features of that study: engram size was changed indirectly, through how long the animals spent learning rather than through any offline process, and the study did not test sleep at all. Moreover, the authors' own analysis clarifies that context discrimination actually depends more on the proportion of the conditioning-context engram that is also reactivated in a similar context, rather than on absolute engram size [19]. Therefore, contraction can still be beneficial if it removes the unselective neurons that respond in many/both contexts rather than simply making the engram smaller, potentially explaining why sleep can result in both memory discrimination and generalization (see Section 2.4 for further discussion on what sleep actually does to memory on the human representational level). Without contraction, excessive overlap between large engrams formed close together in time may also cause related memories to merge [20].

In summary, the ensemble-level evidence supports the possibility and usefulness of both expansion and selective contraction, but does not establish which sleep stage performs which operation. Our engram sculpting hypothesis posits that expansion occurs mainly during NREM and selective contraction happens during REM; the next section aims to examine the network-level evidence that supports this hypothesis.

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2.3. Network level: cholinergic control of expansion and contraction

2.3.1. Biophysical model supporting NREM expansion-REM contraction

A network-level mechanism may explain why engram expansion is more likely during NREM, whereas selective refinement may be favoured during REM. A simplified biophysical network model based on in vivo CA1 recordings proposes that the difference is largely controlled by acetylcholine and its effects on inhibition [11]. During NREM, low acetylcholine reduces the activity of inhibitory interneurons, causing overall network disinhibition. This lowers the threshold for recruitment of neurons into the engram, allowing originally inactive (slow-firing) excitatory neurons that were only weakly connected to the original engram to become synchronized with existing engram cells and be recruited through spike-timing-dependent plasticity [11]. During REM, high acetylcholine suppresses the M-current and increases network inhibition, effectively raising this threshold. As a result, only a number of strongly associated cells will survive the inhibitory threshold and remain in the engram [11]. This biophysical model therefore provides a direct mechanism for our hypothesis: NREM creates conditions that favour engram expansion, whereas REM creates conditions that favour selective refinement and contraction of the population that NREM has just added (Figure 2). Across repeated cycles, the same model predicts that engrams end up larger than they began but also more selective and better separated from one another [11].

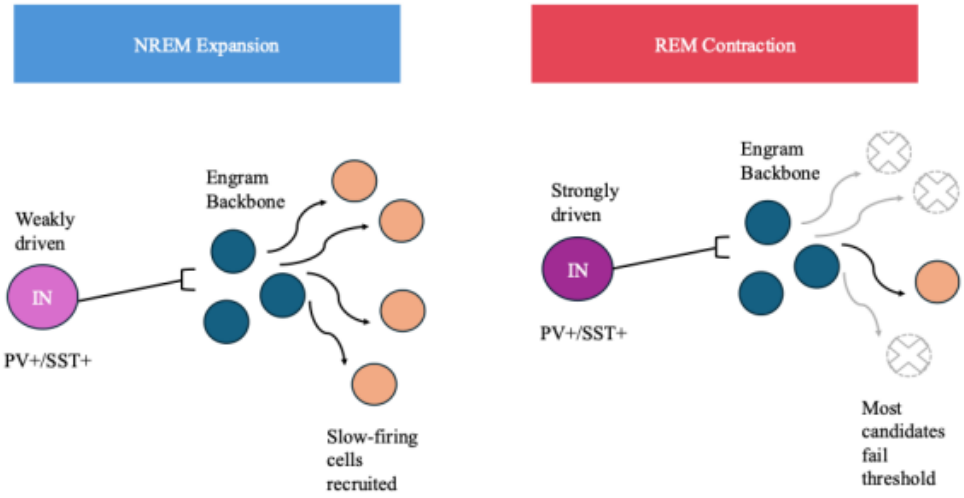

Figure 2. NREM Expansion and REM Contraction. In NREM sleep, due to low cholinergic tone, the inhibitory network (consisting of PV+/SST+ inhibitory neurons) is weakened, allowing slow-firing excitatory cells to sync with core engram neurons when ripples occur and hence join the engram. In REM sleep, the high cholinergic tone leads to high levels of inhibition from PV+/SST+ inhibitory neurons and a high threshold. Theta rhythms and dendritic calcium spikes choose the neurons that remain and those removed from the engram

2.3.2. Experimental evidence supporting NREM expansion

Besides the evidence provided by the model, there are also experimental results consistent with the hypothesis that expansion happens during NREM. Hippocampal CA1 pyramidal cells differ widely in how fast they fire at baseline, and this predicts how plastic they are. After mice explored a novel environment, the slow-firing cells, which are the most plastic and modifiable, developed strong place fields and were increasingly drawn into sharp-wave ripple events during post-learning NREM

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[21]. The faster-firing cells, which change less with experience, kept the activity patterns they already had before learning [21]. Post-learning NREM therefore recruits cells that were barely participating beforehand, which is exactly what our expansion hypothesis predicts.

Besides observations about which neurons get recruited, a second observation is about firing activity. Across a night of sleep, hippocampal firing rates increase during NREM and then fall much larger and faster during the following REM stage [22]. Firing rate cannot directly show neurons entering or leaving an engram, but it describes a network that gains activity during NREM and loses it during REM, which is part of the alternating cycle our hypothesis proposes.

Now that we have explained the evidence for expansion during NREM, it is important to establish the nuances in NREM reactivation mechanisms. First, although NREM is probably responsible for expansion, there are still parvalbumin-expressing CA1 inhibitory interneurons that set an inhibitory threshold to determine which additional neurons enter the engram; suppressing these neurons after conditioning impaired fear memory consolidation [23]. The NREM recruitment process seems to depend on specific forms of replay rather than all NREM activity equally. A closed-loop study identified a subset of large sharp-wave ripples associated with memory reactivation in both the hippocampus and prefrontal cortex, which became more frequent during post-learning sleep [24]. Artificially increasing these large ripples enhanced ensemble reactivation in both regions, improved later memory retrieval, and strengthened waking hippocampo-cortical coordination; in contrast, normal-sized ripples did not have such a big effect [24]. Together, these findings suggest that NREM expansion relies on carefully regulated inhibition and strictly controlled time windows (large sharp-wave ripples) to decide which new neurons are recruited.

2.3.3. Experimental evidence supporting REM contraction

Whilst the various properties of NREM make it compatible for engram expansion, the characteristics associated with REM make it more compatible for engram contraction. Contraction requires a way of telling strongly from weakly involved neurons apart instead of a general reduction, so that some are kept and others are not. REM provides three such means.

Firstly, inhibiting medial septum GABAergic neurons during REM reduced theta activity and impaired novel object-place recognition and contextual fear memory. This result suggests that inhibitory networks are important for memory consolidation processes during REM, one of which could be engram contraction, which should intuitively rely on inhibitory coordination. Inhibiting MS GABAergic neurons outside of REM did not produce the same effects [25]. Next, REM reactivation must be extremely precisely timed so that only cells firing within the designated window can be kept. Reactivation of engrams containing as few as three adult-born DG neurons was necessary for fear-memory consolidation, and this activity had to happen during a particular phase of the REM theta oscillation [26]. Silencing the same neurons during wakefulness had no effect, however [26]. A phase window like this works as a filter, because cells that fire within it are reinforced and cells that fire outside it are not, which is the exact strict criterion that contraction needs. Additionally, besides being precisely timed, REM reactivation also happens across a longer timescale [27], unlike the strongly time-compressed replay associated with NREM sharp-wave ripples [28]. Over these longer periods, only neurons whose activity is actually correlated will keep firing together, whereas weakly related ones will fall outside of each other's plasticity windows; the same plasticity rules that recruit cells during NREM also fail to maintain them during REM. Furthermore, inhibitory circuits provide another possible mechanism for REM selective refinement. During REM, inhibitory melanin-concentrating hormone neurons in the lateral hypothalamus project to the dorsal hippocampus and suppress CA1 firing [29]. Manipulating these neurons impaired hippocampus-dependent memory

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without affecting overall sleep architecture [29]. Together with the findings above, this supports the idea that REM provides an inhibitory and temporally precise environment capable of contracting previously active neuronal populations. The various properties of REM (e.g. theta oscillations, high cholinergic tone) and their stark differences with NREM properties (e.g. slow oscillation, low cholinergic tone) suggest that NREM is better positioned to expand engrams and REM is better positioned to contract engrams. However, current experimental evidence only shows that inhibition and selective reactivation during REM are important for memory, not that REM directly removes neurons from an engram.

2.3.4. Order matters

The biophysical model suggested that the order and repetition of NREM and REM also matter. For example, engram recruitment failed when REM was placed before NREM, and replacing repeated NREM–REM cycles with one prolonged cycle was predicted to cause simultaneously consolidating memories to merge [11]. Therefore, expansion must happen before contraction, and repeated cycles can help separate memories better. In terms of the specific mechanisms behind how NREM transitions to REM, decreasing noradrenaline levels during NREM creates spindle build-up, allows Homer1a-dependent synaptic weakening to happen and allows gradual transition into REM [30], the amplitude of these noradrenaline fluctuations is also associated with the memory-restorative effects of sleep [31]. Together, these are possible mechanisms for coordinating repeated cycles of NREM expansion and REM contraction during sleep.

In summary, evidence at the network level provides clear support for our NREM expansion-REM contraction hypothesis. Computational modelling directly predicts that low-acetylcholine NREM lowers the recruitment threshold and expands the engram, whereas high-acetylcholine REM raises this threshold and only keeps a smaller, more selective subset [11]. However, whilst current experiments support separate parts of our engram sculpting hypothesis, no experiment has directly tracked the same engram across NREM and REM to test if its composition first expands and then contracts.

2.4. How sleep affects memory at the human representational level is still disputed and not direct evidence for our cellular-level framework

Our hypothesis is about which neurons belong to an engram, and doing research on a human representational or behavioral level cannot serve as direct evidence tracing back to single neurons. Therefore, the human level cannot test engram expansion or contraction directly, but it can tell us the big picture: whether memory content changes across a night of sleep at all, and if so, how.

Two gaps stand in the way of connecting this big picture evidence to our engram sculpting framework. The first gap is the amount of controversy surrounding what REM actually does to memory content, as there is evidence pointing toward both memory generalization and discrimination as possible consequences of REM memory consolidation. In terms of the evidence surrounding generalization, representational similarity analysis of overnight EEG found that item-level representations were greatly reduced across sleep while category-level representations were strengthened, and a higher ratio of REM to slow-wave sleep predicted both effects [32]. Read on its own, this finding partially corroborates our general theory that REM prunes the weaklier involved parts of a memory, and it fits the Refining and Rescuing hypothesis, which proposes that REM strengthens a minority of learning-specific representations while inhibiting the majority [33]. However, there is also abundant evidence for context discrimination, the opposite effect. A

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randomized study of 126 participants found that reducing REM through late-night sleep deprivation produced fear overgeneralization, implying that REM contributes to discrimination [34]. Moreover, a daytime nap resulted in a less discriminated fear response than wakefulness [35]. However, some evidence also implies that generalization and discrimination can happen simultaneously, with sleep seemingly improving category-level memory without discarding item-specific detail [36]. All these studies clearly do not point toward one clear direction of what REM actually does to memory on the human representational level. The second gap is that even a settled answer to the first gap could not be linked directly or causally to engram contraction, since human representation and individual neurons are operating on different levels, and there will therefore be multiple explanations. EEG measures the strength and format of a representation, not which individual neurons contribute to it, so there could still be a shift from item-level to category-level representation without any neuron leaving an engram; for example, one explanation for this shift could be simply that item-specific synapses are more vulnerable to general synaptic downscaling [14]. Human evidence therefore establishes that memory content changes across sleep and that REM is involved, but it doesn't tell us the direction of the change and cannot be directly linked to our framework, which works on the cellular and ensemble level. Both of these gaps will be addressed in the Discussion.

3. Discussion

Our engram sculpting hypothesis posits that memory consolidation relies on repeated engram expansion and contraction cycles across sleep: NREM recruits additional neurons and connections into the memory trace, while REM selectively refines this expanded population. Read together, the evidence discussed above provides support for different parts of this process across four levels of analysis. At the synaptic level, NREM supports the formation of new dendritic spines, while REM prunes many of them but preserves and strengthens a more relevant minority, thus providing a potential lower-level mechanism for engram contraction [12, 13]. At the ensemble level, sleep can recruit previously non-engram cells toward future engrams, while other studies show that neurons can enter and leave existing engrams as engram selectivity develops [9]. This establishes that engram membership is dynamic enough for the proposed sculpting process to occur. At the network level, low acetylcholine levels during NREM lower network inhibition and support engram expansion, whereas high acetylcholine levels during REM heighten the inhibitory threshold; biophysical modelling also suggests that the order and repetition of these two states are important for keeping memories distinct [11]. Finally, at the human representational level, the evidence is more scattered, as it is not clear what sleep specifically does to memory (Discrimination? Generalization?), let alone any causal relationship between engram composition and memory expressed as behavior. Some research shows that item-specific neural representations weaken whilst broader category-level representations are kept after a night of sleep, and this shift is associated positively with REM [32]. However, this does not directly prove that engram contraction resulted in context generalization; it only supports that REM overall plays a role in generalization, and there is evidence also supporting context discrimination or both at the same time. As Section 2.4 noted, the human literature does not point in one direction. Table 1 summarizes evidence and lists unanswered questions across these different levels.

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Table 1. Evidence for the proposed NREM-REM engram sculpting theory at multiple levels. Studies supporting NREM engram expansion and REM engram contraction are cited under five different analytical levels. The last column contains the main questions that need to be answered by future research in order to test the theory

Level of analysis	Evidence for EXPANSION (NREM)	Evidence for CONTRACTION (REM)	Unanswered questions
Synapse	Zhou et al. 2020: NREM supports the formation of new spines.	Li et al. 2017: REM removes many newly formed spines but keeps a smaller, stronger subset. Zhou et al. 2020: Spine elimination is specifically linked to REM sleep.	*Does REM specifically remove the new spines that were formed during the previous NREM period?*
Ensemble	Ghandour et al. 2025: Some neurons that later become part of an engram already become coordinated during offline periods. Clawson et al. 2021: Reactivating a memory-related ensemble can recruit more nearby neurons into the representation.	Feitosa Tomé et al. 2024: Some neurons leave an engram as the engram becomes more selective. Leake et al. 2021: Memory precision depends more on which neurons remain than simply on engram size.	*Are the engram cells pruned during REM the ones that were added in the previous NREM period?*
Network & oscillation	Grosmark & Buzsáki 2016: During post-learning NREM, previously slow-firing neurons become more involved in sharp-wave ripples, suggesting recruitment into memory-related activity. Girardeau et al. 2009: Sharp-wave ripples are important for memory consolidation.	Boyce et al. 2016: REM theta activity is necessary for normal memory consolidation. Srinivasan et al. 2025: Very small groups of neurons must reactivate at specific phases of REM theta -> highly selective processing.	*Does engram selectivity increase as the number of repeated NREM-REM cycles increases?*
Neuromodulatory	Satchell et al. 2025: A computational model suggests that low acetylcholine during NREM reduces inhibition, making it easier for additional neurons to join the engram.	Izawa et al. 2019: REM-active MCH neurons suppress hippocampal activity and contribute to forgetting. Satchell et al. 2025: The model suggests that high acetylcholine during REM raises the selection threshold, leaving only strongly associated neurons in the engram.	*Is it the cholinergic tone difference between NREM and REM that decides whether the engram expands or contracts?*
Human behaviour and representation	No human studies for expansion.	Liu et al. 2025: After sleep, detailed item-level information becomes weaker while broader category-level information is preserved, and this shift is associated with more REM relative to NREM sleep.	*Is this shift from specific details to broader information actually caused by neurons entering and leaving the engram, or by another process?*

Our engram sculpting hypothesis is different from current theories of sleep's role in memory (e.g. synaptic homeostasis, active systems consolidation, refining and rescuing) by focusing specifically on dynamic engram membership changes, in which neurons are recruited into or removed from the memory trace during consolidation. As shown in Table 2, our framework provides predictions that can be experimentally distinguished from existing frameworks of sleep-dependent consolidation.

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Table 2. Comparison of the engram sculpting hypothesis with three main existing frameworks of sleep-dependent consolidation, and the observations that would distinguish them

Feature	Synaptic homeostasis	Active systems consolidation	Refining and rescuing	Engram sculpting
Theory's main focus	Changes in synaptic strength	Changes in memory strength and where the memory is stored	Changes in how strongly different parts of a memory are activated	Ensemble membership
Is subtraction targeted?	Yes; less-used synapses are weakened more	Not specified	Yes; depends on how strongly memory elements were activated before sleep	Yes; REM selectively refines neurons recruited into the engram
Role assigned to NREM	Weakens/down scales synapses	Replays and redistributes memories	Downscaling plus reactivation	Recruitment and expansion
Role assigned to REM	Continues synaptic downscaling	Supports consolidation, but has a less clearly defined role	Refines memories and rescues weaker traces	Selectively contracts/refines the expanded engram
Does cycle order or number matter?	No	Not specified	Repeated processing helps sharpen the memory	Yes; expansion should happen before contraction and engram selectivity increases with the number of cycles
Effect of REM deprivation on engram size	Smaller or unchanged	Unchanged	Not specified at cellular level	Larger but less selective

There are some remaining controversies and skepticism regarding our engram sculpting hypothesis. First, it is not experimentally supported that expansion happens during NREM and contraction happens during REM, especially the latter. Evidence from spine imaging suggests that REM has a specific role in pruning, given that REM deprivation reduced spine elimination but did not affect spine formation [12, 13]. Similarly, REM-specific optogenetic manipulations impair memory consolidation, whereas the same manipulations outside REM do not [25, 26]. However, synaptic weakening also occurs outside REM; for example, noradrenaline pathways associated with synaptic weakening already become active from sleep onset instead of only during REM [15]. Therefore, whether REM is actually solely responsible for engram contraction is not yet tested. Second, the benefits of engram contraction are still not clear. Pruning is not necessarily beneficial or harmful by itself; it depends on what gets removed. During REM, most newly formed spines are removed whilst a smaller, behaviourally relevant minority is preserved and strengthened [12]. Our hypothesis therefore predicts that moderate, selective contraction could improve memory by removing less relevant neurons from the engram, but too much contraction would weaken the memory. Therefore, keeping the balance between expansion and contraction is likely important. Thirdly, even though our engram sculpting hypothesis states that REM prunes engram size, a better-sculpted engram does not necessarily have to be smaller. As discussed in Section 2.2, the proportion of the engram that is selective is more important than absolute size [19]. This encourages us to clarify our hypothesis: REM's main goal could be raising the proportion of selective neurons in the engram, and it can do this by reducing engram size; reducing engram size is not the main goal itself. Fourth, the human findings cannot be linked directly to engram contraction, because EEG cannot

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tell us whether neurons are entering or leaving an engram. A possible explanation for the shift from item-level detail to category-level memory reported in [32] could just be that item-specific synapses are more vulnerable to general synaptic downscaling [14], and not have anything to do with engram contraction. Human findings therefore do not contradict our hypothesis, but they do not prove it either.

The most important unresolved issue, however, is what the expansion-contraction cycle actually does to the memory. Even if engram selectivity increases across repeated NREM-REM cycles during sleep, this does not help us reliably predict what happens on the human behavioral level, which is the external portrayal of the memory. The memory's outcome depends on which neurons are weakly associated and thus removed from the engram, something that we may not be able to track easily. If the neurons removed are those that respond in many different contexts and are not context-specific, then we predict sharper discrimination. If the removed neurons are carrying item-level detail, then we predict that greater generalization should happen, given that the remaining neurons represent category-level information. Both outcomes are experimentally reported. On the discrimination side, contextual fear memories become selective over the hours after learning as engram composition changes [9]; preventing sleep-associated engram reactivation produces generalized freezing rather than amnesia [18]; selectively reducing REM in humans produces fear overgeneralization [34]; and the biophysical model suggests that engrams become larger than before sleep but are better discriminated from one another, rather than smaller [11]. On the generalization side, item-level memory weakens whilst category-level memory strengthens across a night of sleep in proportion to REM [32]; cueing memories during REM resulted in rule generalization and abstraction [37]; and a daytime nap broadened instead of narrowed fear responses [35]. Evidence supporting the co-existence of generalization and discrimination also exists; sleep can also seemingly improve category-level memory, without discarding item-specific information [36]. However, the discrimination side may have more evidence, as it includes causal manipulations in both rodents and humans, whereas the generalization side uses mostly correlational recordings and one cueing study. Nevertheless, no study has linked engram composition and a behavioral memory outcome (discrimination? generalization?) in the same animals within the same experiment, so the direction of the effect is currently unknown.

The central prediction of our engram sculpting hypothesis has not yet been directly tested by any existing experiments: does the same engram expand during NREM and contract during REM? Therefore, one of the most important future experiments would be to directly monitor the activity and composition of engrams across NREM-REM cycles. Researchers can combine longitudinal miniscope imaging of activity-tagged hippocampal engram cells with simultaneous EEG/EMG sleep stage monitoring, then track the same engram after learning and measure its membership after each NREM and REM cycle. Our hypothesis predicts that engram membership should increase across NREM and decrease across REM, while engram selectivity progressively increases across repeated cycles. Failure to observe this pattern would directly falsify our hypothesis. Next, we should also test whether REM acts specifically on the cells recruited during the previous NREM period, basically a narrower branch of the first experiment. Cells that are newly recruited during a specific NREM period could be labelled and followed through the immediately following REM period. If expansion and contraction are truly linked, REM should selectively remove or suppress some of the cells recruited during the preceding NREM stage [29]. Thirdly, we can disrupt REM and observe the resulting engram to establish a causation relationship. Blocking REM [25] should theoretically prevent contraction, producing a larger but less selective engram and behavioural overgeneralization (if the neurons REM removes are those that respond across many contexts). If REM disruption

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instead produces smaller engrams, this would argue against the contraction hypothesis. Last but not least, we should test whether repeated NREM-REM cycles matter. In animal experiments, we could manipulate the number of NREM-REM cycles allocated to each animal to test whether final engram selectivity is affected by the number of completed NREM–REM cycles; right now, our hypothesis is that engram selectivity should increase as the number of cycles increases. In humans, this can also be tested causally rather than only correlationally, by selectively depriving early-night or late-night sleep to shift the balance of NREM and REM, as in [34]. Most importantly, given the disagreement described above, the discrimination-versus-generalization question needs an experiment that measures both the engram and behavioral level. Cells recruited during a labelled NREM stage could be tagged and tracked through the following REM stage. The animals should then complete a memory behavioural test that separates the two possible outcomes: freezing in the training context, in a similar context, and in a clearly different context, so that discrimination and generalization can both be tested. Manipulating the expansion-to-contraction relationship, for example by REM restriction [25] or by closed-loop boosting of large ripples during NREM [24], should then shift ensemble composition and behaviour together if the two are causally linked. We would like to emphasize that these are proposed tests of our framework, not findings that currently support it.

4. Conclusion

Sleep-dependent memory consolidation cannot be fully explained as the strengthening of memory traces. Memory engram representations can change dynamically after learning, with neurons entering and leaving the engram during consolidation. Our engram sculpting hypothesis connects previously separate findings to propose that NREM and REM contribute differently to this process. During NREM, low acetylcholine, network disinhibition, and memory reactivation may recruit weakly connected cells into the engram, expanding it. During REM, increased inhibition thanks to high acetylcholine, theta-regulated reactivation, and dendritic calcium spikes may selectively prune this expanded population, only preserving a minority of strongly memory-associated neurons. Therefore, repeated NREM–REM cycles may iteratively reshape engram composition and increase engram selectivity. Because each REM episode removes only part of what the preceding NREM added, the engram that survives a night of sleep should be larger than it was before sleep, but with a higher proportion of strongly involved neurons. Our hypothesis is directly testable by tracking the same engram across sleep and seeing if it reveals expansion during NREM followed by contraction during REM. However, what engram sculpting eventually does to the memory itself is still debated, as there is evidence for both context discrimination and generalization. By predicting about how engrams change across sleep stages, our engram sculpting hypothesis offers a new framework for investigating how sleep shapes the content and fate of memories.

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