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# Memory engram storage and retrieval

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A great deal of experimental investment is directed towards questions regarding the mechanisms of memory storage. Such studies have traditionally been restricted to investigation of the anatomical structures, physiological processes, and molecular pathways necessary for the capacity of memory storage, and have avoided the question of how individual memories are stored in the brain. Memory engram technology allows the labeling and subsequent manipulation of components of specific memory engrams in particular brain regions, and it has been established that cell ensembles labeled by this method are both sufficient and necessary for memory recall. Recent research has employed this technology to probe fundamental questions of memory consolidation, differentiating between mechanisms of memory retrieval and the true neurobiology of memory storage.

## Addresses

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## Introduction

Memory refers to the storage of learned information in the brain, and is crucial for adaptive behavior in animals [1]. Understanding the material basis of memory remains a central goal of modern neuroscience [2]. The hypothetical material basis of learned information, the memory *engram*, was first conceived by Richard Semon who theorized that learning induces persistent changes in specific brain cells that retain information and are subsequently reactivated upon appropriate retrieval conditions [3\*,4,5]. However, experimental searches for specific memory engrams and memory engram-bearing cells using brain lesions proved inconclusive due to methodological limitations and the

likely distributed nature of a memory engram throughout the brain [6\*\*]. Here we review recent experimental studies on the identification of memory engram cells, with a focus on the mechanisms of memory storage. A more comprehensive review of recent memory engram studies is available elsewhere [7\*\*].

## Memory function and the hippocampus

The medial temporal lobe (MTL), in particular the hippocampus, was implicated in memory of events or episodes by neurological studies of human clinical patients, where its direct electrophysiological stimulation evoked the recall of untargeted episodic memories [8]. Subsequent study of humans lacking large regions of the MTL showed dramatic amnesia for episodic memories [9]. Rodent behavioral studies have since established that the hippocampus is a central brain region for contextual memory storage and retrieval [10,11]. Much is now known about brain structures, neural circuits, and molecules involved in memory encoding and consolidation [12\*\*,13,14], but comparatively few studies have attempted to investigate how individual memory engrams are stored in the brain [15].

## Synaptic plasticity as a mechanism of memory

Lasting memories have long been hypothesized to be encoded as structural changes at synaptic junctions of sparse neuronal assemblies [16]. Ramón y Cajal originally proposed that the strengthening of synaptic connections of existing neurons might be a mechanism of memory storage [17], but it was Donald Hebb's theoretical integration of neurophysiology and psychology that created the modern paradigm for memory research [16]. Hebb proposed that neuronal assemblies linked by adaptable synaptic connections could encode informational content in the brain. Empirical research into the physiological nature of memory storage has been dominated by various versions of Hebbian synaptic plasticity [18]. The typical experimental model of synaptic plasticity is long-term potentiation (LTP) [19], most studies of which rely on *in vitro* experimental paradigms where synaptic stimulation patterns are substituted for behavioral training. It is clear that memory and synaptic plasticity have many properties in common [20\*]. NMDA receptor function is necessary for the encoding of many types of memory, as well as for the induction of synaptic plasticity [13,21]. Moreover, both memory consolidation and LTP have a late, protein synthesis-dependent phase [20\*,22]. Despite these biological commonalities, and many serious theoretical efforts to integrate memory storage and synaptic

plasticity [23–27], it remains a controversial subject without a clear consensus [28–30].

### Limitations of standard methodology

Two confounds have hindered progress towards a satisfactory synthesis of synaptic plasticity and memory. First, behavioral studies of memory have relied on the disruption of brain regions, circuits, or molecules [12<sup>••</sup>,13,14], and have thus addressed the importance of these structures and signaling pathways to the *capacities* of memory storage or retrieval, rather than the storage of individual memory engrams themselves. Second, typical conceptions of memory conflate the properties of memory storage and memory retrieval. But it is a fundamental premise of psychology that successful memory function presupposes not only the retention of learned information, but also its successful retrieval [1]. Therefore a given case of apparent memory loss (amnesia) may in principle be due to a damaged memory engram, or an inability to retrieve that particular engram [31–34]. Both of these confounds have recently been overcome through the development of memory engram technology [35<sup>••</sup>].

## Sea change: memory engram technology

### Identification and functional activation of engram cells

In order to progress in memory research it is crucial to identify the engrams and engram cells for specific experiences. The challenge of identifying individual memory engrams and engram cells amidst the complexity of the brain becomes less daunting if we co-opt natural brain activity during learning to point us to the relevant brain cells. This concept has been realized through the development of memory engram technology, which allows the labeling and subsequent manipulation of engram-bearing cells [35<sup>••</sup>]. Engram technology is based on the experimental fusion of immediate early gene (IEG) labeling and optogenetics. The expression of IEGs, such as *c-fos* or *arc*, is a marker of neuronal activity [36]. Thus the promoters of IEGs can be co-opted to tag neurons that are active during a given learning experience with an exogenous target protein (Figure 1) [37<sup>••</sup>]. Temporal specificity of labeling is achieved by engineering the labeling mechanism to be inhibited by administering doxycycline (DOX). When engram cells of the hippocampus dentate gyrus (DG) are labeled during contextual fear conditioning with channelrhodopsin-2 (ChR2) [38], their subsequent stimulation with blue light is sufficient to elicit retrieval of a target contextual fear memory, as measured by conditioned freezing behavior [35<sup>••</sup>]. Crucial control experiments, where engram cells for neutral contexts were stimulated in fear-conditioned mice, demonstrated that the information stored in labeled engram cells is specific to the target experience [35<sup>••</sup>]. Importantly, memory recall by natural cues reactivates the same engram cells [39<sup>••</sup>] satisfying another important criteria, the ephoric nature of an engram [4].

### Physiological characterization of engram cells

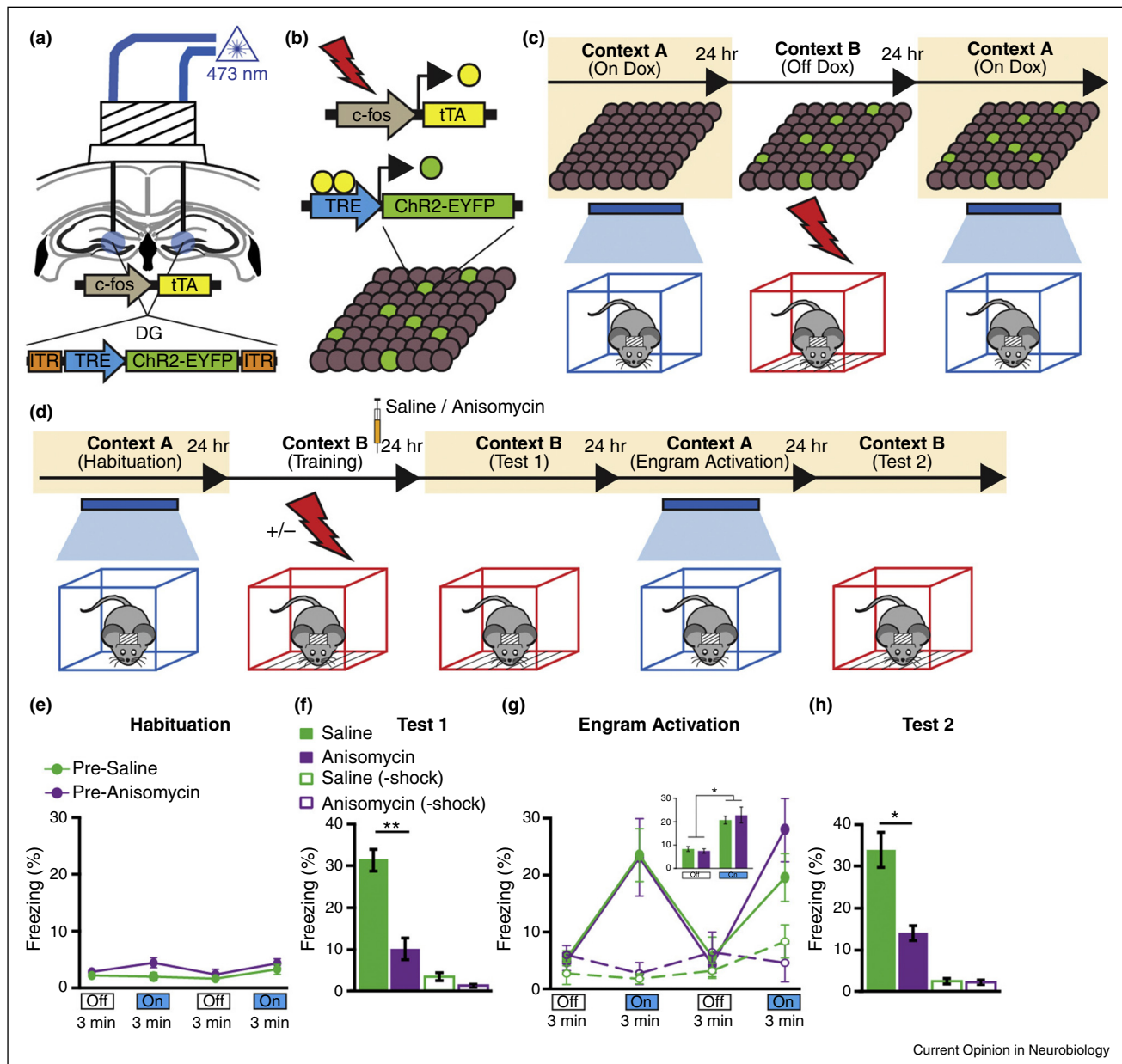
The storage of lasting memory in the brain must involve persistent plasticity of engram cell structure and/or physiology. Indeed, *ex vivo* characterization of DG engram cells revealed two engram cell-specific properties [40<sup>••</sup>]. First, engram cells showed significantly increased dendritic spine density relative to non-engram cells. Second, patch clamp recordings of excitatory postsynaptic currents in paired engram and non-engram cells elicited by presynaptic stimulation of perforant path axons showed substantially higher synaptic strength in engram cells.

The above two properties are clear cases of plasticity occurring exclusively in engram cells, and are reminiscent of Hebbian plasticity. If this plasticity is representing mnemonic information then it should be encoded by the specific training experience. Protein synthesis is necessary for late phase synaptic plasticity and memory consolidation, and indeed when the protein synthesis inhibitor anisomycin was administered to animals immediately after fear conditioning, retrograde amnesia was observed one day later. Analysis of engram cells one day after anisomycin treatment showed that the anisomycin abolished engram-cell specific increases in both dendritic spine density and synaptic strength, but did not alter either property in non-engram cells. Importantly, anisomycin treatment one day post-training (outside the consolidation window) impaired neither the dendritic spine density increase nor the synaptic strength augmentation of engram cells [40<sup>••</sup>]. Therefore engram cell-specific structural and synaptic plasticity is protein synthesis-dependent and consolidated with the target training experience.

### Retrieval of lost memory from amnesia: dissociation of engram cell plasticity and memory

Surprisingly, direct optogenetic activation of amnesic engram cells in mice resulted in successful retrieval of the ostensibly lost contextual fear memory. The generality of the memory retrieval finding was tested in a range of experimental conditions [40<sup>••</sup>]. First, lost memory was retrieved by optogenetic stimulation of ChR2-labeled engram cells in hippocampal CA1. Second, amnesia for tone fear memory was generated with anisomycin, and the memory was retrieved by optogenetic stimulation of lateral amygdala (LA) engram cells. Third, lost memory was retrieved from amnesia due to impaired reconsolidation by activation of DG engram cells. Fourth, an alternative protein synthesis inhibitor, cycloheximide, was used to generate amnesia and subsequent activation of DG engram cells again retrieved the target memory. Finally, a contextual updating protocol [39<sup>••</sup>] was used to show that amnesic engram cells retained information about context specificity, and could be restored to a condition where they could be retrieved by natural contextual cues [40<sup>••</sup>].

Figure 1



**Engram Labeling Technology and Memory Retrieval in Retrograde Amnesia.** **(A)** Basic composition of the engram labeling system. Virus expressing TRE-ChR2 and optic fibers are targeted to the dentate gyrus of c-Fos-tTA transgenic mice. **(B)** In the absence of DOX, DG neurons that are active during the formation of a memory are labeled with ChR2. **(C)** Basic behavioral schedule for labeling and activation of engram cells. Animals are habituated to Context A with light stimulation while on DOX, trained by contextual fear conditioning in Context B while off DOX, and tested again in Context A with light stimulation while on DOX. **(D)** Behavioral schedule for generating amnesia by disrupting memory consolidation. Saline or anisomycin was injected into the mice after training. **(E)** Habituation to Context A with Light-Off and Light-On epochs. Blue light stimulation of the DG did not cause freezing behavior in naïve, unlabeled mice. **(F)** Memory recall in Context B 1 day post-training (Test 1). The anisomycin group showed impaired memory recall relative to the saline group as measured by conditioned freezing behavior to Context B. No-shock groups did not display freezing upon re-exposure to Context B. **(G)** Memory recall in Context A 2 days post-training (Engram Activation) with Light-Off and Light-On epochs. Freezing for the two Light-Off and Light-On epochs are further averaged in the inset. Freezing levels did not differ between groups. **(H)** Memory recall in Context B 3 days post-training (Test 2). The anisomycin group displayed significantly less freezing than the saline group.

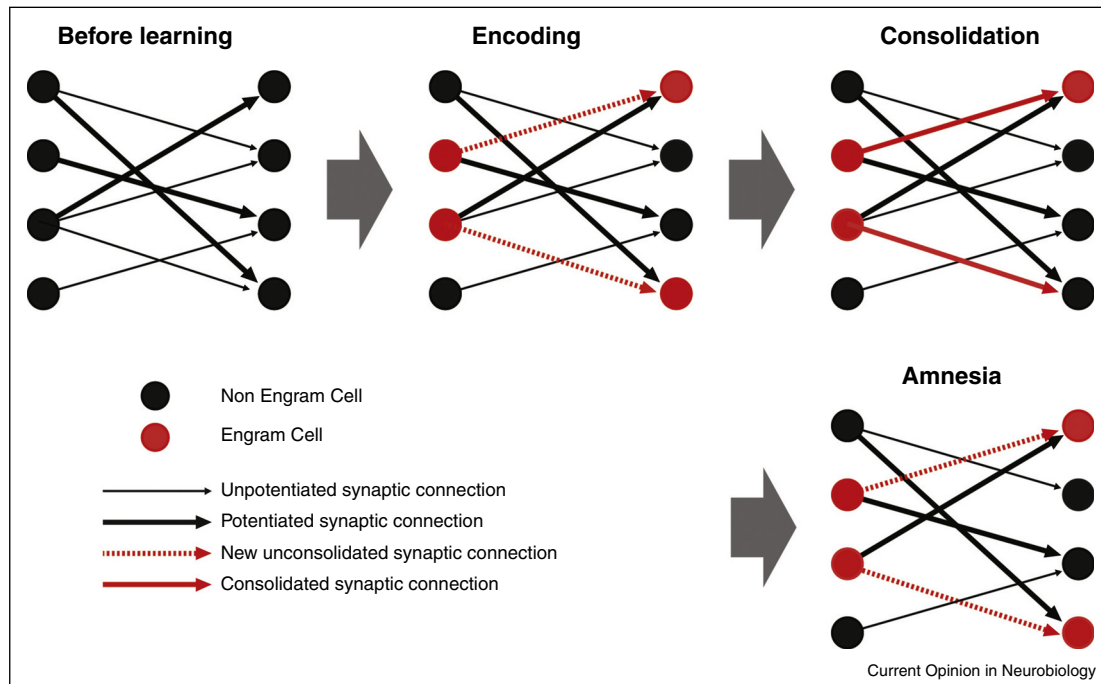
Taken together, the behavioral and physiological results clearly show that engram cell-specific structural and synaptic plasticity is strongly correlated with normal memory function, since both engram cell plasticity and memory expression are sensitive to protein synthesis inhibition during the consolidation window. Nevertheless, these findings showed a stark dissociation between synaptic plasticity and memory content, since engram cells retained memory information even in the absence of engram cell-specific increases in spine density and synaptic strength.

### Connectivity between engram cells as the mechanism for retained memory

The dissociation of engram cell plasticity and memory prompted the question; how can the consolidated memory be stored? One hypothesis would be that memory may be stored in a specific pattern of connectivity between engram cell ensembles distributed in multiple brain regions and this connectivity pattern is established during encoding and retained during consolidation in a protein synthesis-independent manner (Figure 2).

This hypothesis was tested by two different types of experiments using *ex vivo* electrophysiological and *in vivo* IEG technologies [40\*\*]. First, when both DG and hippocampal CA3 engram cell ensembles were simultaneously labeled and the presynaptic DG engram cells were activated optogenetically, the occurrence of the postsynaptic response of CA3 engram cells was significantly higher (~80%) than that of CA3 non-engram cells (~25%) and these proportions were not affected by anisomycin treatment. Second, engram cells were simultaneously labeled in the DG, CA3, and basolateral amygdala (BLA) during contextual fear conditioning. One day after training, re-exposure to the conditioning context preferentially activated engram cells in all three brain regions as measured by endogenous c-Fos<sup>+</sup> cell counts, and this phenomenon was significantly impaired by anisomycin treatment in the consolidation window when natural recall cues were used. Nevertheless, direct optogenetic activation of DG engram cells resulted in a greater than chance level of c-Fos<sup>+</sup> overlap with CA3 or BLA engram cells in both control and anisomycin-treated mice.

Figure 2



Engram Cell Connectivity. Schematic illustrating the dynamics of synaptic connectivity in a neural ensemble recruited during the formation of a new memory. Before learning the neural network presents a connectivity arrangement characterized by a mix of potentiated (thick black line) and unpotentiated (thin black line) synapses. During memory encoding, a sparse number of cells (engram cells, red) are recruited, giving rise to new connections or activating preexisting ones (dashed red line). Immediately after encoding the process of memory consolidation enables the stabilization of the new connections (thick red line). The stabilization is characterized by an enhancement of synaptic strength and is fundamental for memory retrieval. Disruption of the consolidation process by interventions such as protein synthesis inhibitors impairs the stabilization/potentiation of new synaptic connections (dashed red line) resulting in retrograde amnesia. Synaptic connectivity provides a substrate for memory storage whereas the potentiation of synapses is required for memory retrieval.

These results demonstrate intact functional connectivity among engram cell ensembles distributed in neural circuits encompassing multiple brain regions and reinforces the hypothesis that consolidated memory is stored by engram cell-specific connectivity formed in a protein synthesis-independent manner (Figure 2).

### Synaptic strengthening as a mechanism of memory retrievability

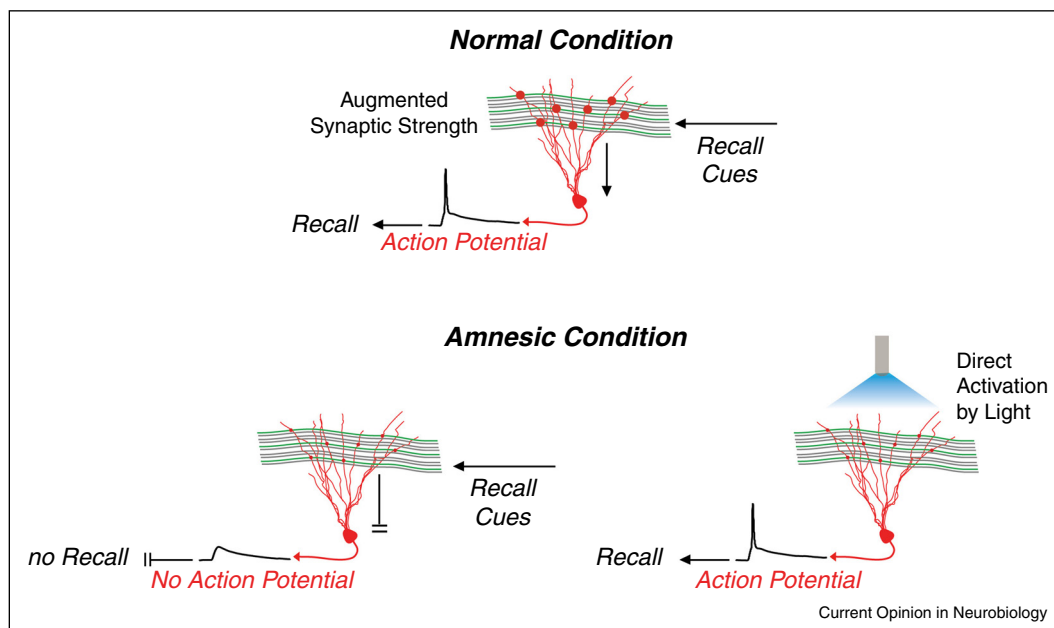
Based on these integrative findings, we propose that enhanced engram cell-specific synaptic strength is crucial for the retrievability of particular memory engrams [33], while the memory information content itself is encoded in a pattern of engram cell ensemble connectivity. Under amnesia, the impaired synaptic strengthening prevents effective activation of engram cells by natural recall cues and subsequent engram cell spiking (Figure 3). However, the information stored in engram cell ensemble connectivity can be retrieved by the optogenetic stimulation of various nodes in the engram cell circuit. The notion that synaptic strengthening is crucial for memory retrieval, but not for stable storage of memory *per se*, is consistent with a number of complementary studies. It was recently shown that optogenetically-induced long-term depression (LTD) of rat amygdala cells impaired existing conditioned fear responses [41]. However, subsequent optogenetically-induced LTP of the same cells restored optogenetic cue-evoked recall of the fear memory. Therefore, the memory information must have persisted

in the brain of the rats even after the amygdala synapses were depressed, but the lack of synaptic potentiation prevented successful memory retrieval. Supporting this perspective is the demonstration that amnesia for a purely contextual memory can be overcome by direct engram activation paired simultaneous presentation of aversive shock [40]. Other correlative studies have shown that contextual fear memories formed during a certain period of adolescent development were not expressed in recall tests until the transition into adulthood, and this developmental change correlated with a delayed learning-specific synaptic potentiation of the BLA fear circuit [42]. Thus, the fear memory was present during adolescence, but its retrievability was temporarily impaired due to lack of BLA synaptic strength. In addition, reminder experiments in *Aplysia* showed that amnesia for gill withdrawal sensitization can be restored by extra puffs of serotonin, and that this response persisted despite significantly altered presynaptic varicosities [43]. Collectively these studies strongly support a dissociation of synaptic strength and memory persistence, and point to its crucial role in the reactivation of a memory engram and retrievability of a memory.

### Engram cell ensemble circuit

If engram cells are truly carrying memory information at the holistic level of an engram circuit, then inhibition of engram cells at various nodes of an engram circuit should inhibit retrieval of the target memory. This prediction has

Figure 3



Augmented Synaptic Strength for Recall. Under normal conditions, a consolidated engram cell is efficiently activated by recall cues from the animal's environment through potentiated synaptic inputs. Under amnesic conditions, engram cells are present but lack synaptic potentiation and are thus only weakly evoked by recall cues. Nevertheless, direct activation of engram cells is sufficient to overcome impaired synaptic potentiation and results in memory retrieval.

been satisfied by studies showing that individual fear memories require engram cells from multiple brain regions. Optogenetic inhibition of engram cells labeled with the IEGs *c fos* and *arc* in hippocampal CA1 [44\*] and DG [45\*] caused impairments both in downstream engram cell reactivation and contextual fear memory recall. Moreover, when CREB is artificially expressed in the LA, it biases certain LA cells to acquire the fear engram during tone fear conditioning [15]. Subsequent interference with these LA engram cells by either ablation or acute chemogenetic inhibition [46\*\*,47\*] impaired fear memory recall. In addition, optogenetic inhibition of BLA cells representing valence-specific unconditioned stimuli impaired memory recall elicited by associated tone and odor conditioned stimuli [48]. Taken together, these studies clearly show that a functional memory requires multiple nodes on an engram cell ensemble circuit.

Recently it has been demonstrated that retrieval of a positive memory by optogenetic activation of DG engram cells was impaired by simultaneous inhibition of downstream BLA engram cell projections to the nucleus accumbens [49\*]. Thus, the downstream connectivity of engram cells is crucial for the retrieval of memory. The converse scenario has also been investigated, where inhibition of upstream areas was optogenetically bypassed by direct activation of downstream engram cells. In an experiment where contextual memory retrieval was acutely impaired by pharmacological inhibition of AMPA receptors in the hippocampus, simultaneous optogenetic activation of downstream engram cells in the retrosplenial cortex successfully evoked memory retrieval [50\*]. These findings provide evidence for the encoding of memory across an engram cell ensemble circuit.

An important prerequisite of any putative memory storage mechanism is activity-dependency during encoding. This criterion has been tested by chemogenetic inhibition of CA1 neurons during encoding [40\*\*]. This procedure generated anterograde amnesia that was irretrievable even by

direct stimulation of upstream DG engram cells. Finally, any putative substrate of memory storage should hold the potential for plasticity following further relevant new learning. To this end, it has been shown that when the positive or negative emotional valence associated with a specific contextual memory was reversed in an optogenetic counter-conditioning schedule, the functional connectivity of DG and BLA engram cells was abolished [51].

## Conclusions and future directions

### Implications for memory research

The differentiation of synaptic plasticity and engram connectivity described here (Table 1) has significant implications for interpreting the neurobiology of memory consolidation and synaptic plasticity, because the conceptual and empirical framework introduced here can be used to attribute cellular signaling pathways to memory storage or retrieval. Molecular mechanisms that serve to potentiate or strengthen AMPA receptor transmission are parsimoniously attributable to memory retrievability [52–54].

What then would be molecular mechanisms for information retention in the substrate of engram cell connectivity?

It is known that NMDA receptor-dependent synaptic plasticity results not just in potentiated synapses, but also in the formation of new functional synaptic connections through synapse unsilencing [55\*]. The trafficking of a basal level of AMPA receptors into pre-existing silent synapses may facilitate the encoding of new functional connectivity. Nevertheless, LTP is known to be characterized by an early phase and a late phase, E-LTP and L-LTP, the latter sensitive to protein synthesis inhibitors [56]. The survival of engram connectivity upon protein synthesis inhibitors treatment suggests that the induction of engram connectivity may share mechanisms common to E-LTP. However, by impairing the late phase, it has been shown that the unsilencing can be prevented,

**Table 1**

### Comparison of putative plasticity mechanisms and suitability for memory storage or retrieval

Plasticity Mechanism:	Synaptic Strength	Engram Cell Connectivity
Locus:	Single engram cells or synapses	Engram Circuit
Extent:	Increases depending on active synapse and spine numbers involved, but essentially limited to single engram cells	Increases in complexity and computational capacity the more brain regions and neurons involved
Mechanism:	Changes in AMPA receptor trafficking and dendritic spine formation on engram cells	Changes in specific connectivity patterns of engram cell assemblies
Requirement for Protein Synthesis:	Yes, protein synthesis inhibitors impair engram cell synaptic plasticity	No, protein synthesis inhibitors have no effect on engram cell connectivity
Necessary for Memory Retrieval:	Yes, when synaptic plasticity is impaired, amnesia results	Yes, impairing engram cell or circuit activity prevents memory retrieval
Necessary for Memory Storage:	No, direct activation of target engram can retrieve memory	Yes, encoding of memory in circuit is necessary for memory formation, and valence reversal alters engram connectivity

suggesting that ‘silent synapses’ can only partially support the engram connectivity [57]. Alternatively, a subset of learning-induced dendritic spine formation may be responsible for novel connectivity patterns between engram cells. Under any of these scenarios, the retention of engram connectivity could conceivably be mediated by the homeostatic regulation of steady state AMPA receptor trafficking. Consistent with this perspective is a recent study showing that protein synthesis inhibitors, when administered before recall tests, transiently impaired AMPA receptor expression and memory retrieval [58<sup>\*</sup>]. Alternatively, the maintenance of memory engram connectivity might be mediated by specific molecular players that are yet to be fully characterized in the context of memory function, such as perineuronal net components or microRNAs [59<sup>\*</sup>,60].

It is currently unknown for how long engram cell connectivity persists, and whether it is permanent or reversible. Though it has been shown through engram overlap analysis that when the positive or negative emotional valence associated with a contextual memory is reversed, the functional connectivity of DG/BLA engram cells changes [51], a direct analysis of synaptic connections will be necessary to understand the true physiological nature of the plasticity of connectivity.

Regardless of the specific underlying molecular mechanisms, if engram cell connectivity is the substrate of memory information storage, then it will be necessary to fully explore the structure and function of the engram circuit. Such a task would require the comprehensive mapping of the entire engram circuit connectome for a given memory; the memory *engrime*. This could be achieved by combining engram labeling technology, whole brain IEG activity measurements [61], and three dimensional imaging of intact transparent brains [62]. The functional properties of engram circuits could be studied *in vivo* by calcium imaging of engram cell activity in multiple brain regions [63].

### Applications

Manipulation of engram circuits presents many opportunities for significant practical applications. The efficacy of this technology for artificially updating existing memories [39<sup>\*</sup>,64], as well as for reversing the emotional valence associated with contextual memories [51], has been established. Such interventions based on engram technology may have utility for the treatments of post-traumatic stress disorder. In addition, positive memory engram activation has recently been shown to alleviate stress-induced models of depression in mice [49<sup>\*</sup>]. Furthermore, tagging and interfering with engram cells for cocaine-related memories has been reported as possible treatment avenues of drug addiction [65]. Cases of pathological amnesia that are due to retrieval failures should be much more amenable to restorative interventions than instances

of *bona fide* memory loss. The particular approach to amnesia discussed in this review could be employed for investigating and potentially treating various types of clinical amnesia, such as Alzheimer’s disease.

### Evolutionary significance

From an evolutionary perspective, synaptic plasticity is a ubiquitous feature of neurons that seems to have arisen with the first nervous system in a common ancestor of cnidarians and bilaterians over a billion years ago [66]. On this basis, synaptic plasticity can be considered a fundamental neuronal property, the disruption of which in brain regions such as the hippocampus or amygdala will impair the encoding and retrieval of memory. On the other hand, engram cell connectivity is a substrate that naturally increases in complexity as brain anatomy evolves (Table 1). Therefore the more complex the brain, the greater the opportunity for the storage of detailed memories through hierarchical engram circuits distributed throughout brain regions. Connectivity patterns among engram cell assemblies are a potential mechanism of information storage that is in keeping with what Hebb originally envisioned [16]. Further research in this direction may provide significant new insights into the storage of memory.

### Conflict of interest statement

Nothing declared.

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