

# The role of engram cells in the systems consolidation of memory

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**Abstract** | What happens to memories as days, weeks and years go by has long been a fundamental question in neuroscience and psychology. For decades, researchers have attempted to identify the brain regions in which memory is formed and to follow its changes across time. The theory of systems consolidation of memory (SCM) suggests that changes in circuitry and brain networks are required for the maintenance of a memory with time. Various mechanisms by which such changes may take place have been hypothesized. Recently, several studies have provided insight into the brain networks driving SCM through the characterization of memory engram cells, their biochemical and physiological changes and the circuits in which they operate. In this Review, we place these findings in the context of the field and describe how they have led to a revamped understanding of SCM in the brain.

## Episodic memory

The recollection of events with a specific spatial and temporal context, such as personal experiences. Often referred to as autobiographical memory.

Memory consolidation refers to the process by which a temporary, labile memory is transformed into a more stable and long-lasting state<sup>1,2</sup>. The representation of this more stable memory in the brain has been referred to as the memory trace<sup>3</sup> or memory engram<sup>4</sup>, and the quest to discover this neurological representation of memory has been at the forefront of neuroscience since its emergence as a major field of science.

During an experience, the complex system of memory in the brain combines massive amounts of sensory information and binds this together into a cohesive event, containing information about what occurred, where and when, in a form that is freely available to recall at a later time. It is this system that produces what is referred to as episodic memory. In the mammalian brain, the hippocampus serves as the key node of the episodic memory formation system. Here, an experience is encoded through plasticity (the formation of new synaptic connections and the reorganization of existing ones), as first proposed in Hebb's synaptic plasticity theory<sup>5</sup>. It is thought that this initial process lays down the circuitry required to retrieve this episode in the future. Indeed, recent technological advances have allowed researchers to label the hippocampal cells that are initially activated during an experience<sup>6,7</sup>. Combining this activity-dependent cell labelling with optogenetics led to the discovery of engram cells in the hippocampus; engram cells are defined as neurons that are activated during an experience, that have undergone enduring physical or chemical changes and that can subsequently be selectively reactivated to produce the retrieval of that experience or inhibited to prevent its retrieval<sup>7,8</sup>. This discovery produced the first

concrete evidence of an engram<sup>4</sup> for a specific memory in the brain.

However, the long-term storage of memory does not end there. Over the days, months or even years that follow an experience, it has been proposed that another type of consolidation takes place that strengthens and reorganizes the brain's networks into a long-term, more stable state at the systems level. This latter type of consolidation is known as systems consolidation of memory (SCM)<sup>9</sup>. Although the mechanisms of SCM and the networks involved in this process have been studied for decades, there are still many unknowns. In part, this lack of knowledge has arisen owing to the conflict between different theories that have been advanced based primarily on loss-of-function studies. In the past few years, however, great strides in advancing our understanding of SCM have been made. These include the identification and characterization of the engrams, engram cells and circuits that are associated with specific memories at times that are recent or remote in relation to the original experience. These advances have been achieved through the development of new technologies with convergent approaches, including gain-of-function and loss-of-function studies and observational studies<sup>7,8</sup>.

In this Review, we highlight the most recent advances in our understanding of the nature and dynamics of neocortical and subcortical memory engram cells, their circuits and their contributions to SCM. In addition to the major advances in our understanding of how episodic memories and the networks supporting them change and develop across time, we focus on the circuits and physiology that seem to be driving this process and discuss their roles and functions. Finally,

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<https://doi.org/10.1038/s41583-018-0031-2>

**Optogenetics**

The use of genetically encoded light-activated proteins (for example, ion channels) to control functional parameters (for example, the membrane potential) of targeted neuronal populations.

we discuss some of the major questions that remain to be answered.

**The history of systems consolidation**

**Temporally graded retrograde amnesia.** The original idea of SCM dates back to early psychological studies, in which it was observed that more recently formed memories were more susceptible to disruption than memories formed more remotely in time. This is the basis of Ribot's law of retrograde amnesia, which states that, with time, memories become resistant to decay or, in other words, that they need time to consolidate<sup>10</sup>. Temporally graded amnesia was first observed in patients in whom damage to the medial temporal lobes, a region that includes the hippocampus, produced both episodic memory-specific anterograde amnesia and a form of retrograde amnesia that appeared to be restricted to more recently formed memories while sparing much older memories<sup>11,12</sup>. Further investigations with patients in whom the damage was restricted to the hippocampus<sup>13,14</sup>, as well as experimental work with primates<sup>15,16</sup>, revealed that damage to the hippocampus was the key to the observed amnesia. This led to the idea that the hippocampus was essential for the formation and early retrieval of episodic memories but that processes taking place after learning permitted the role of the hippocampus in retrieval to be time limited<sup>17</sup>.

**Standard model and multiple trace theory.** Systems consolidation has always been a contentious area in the field and remains so, with several theories (outlined below) purporting different roles of the hippocampus in long-term memory, and some suggesting that systems consolidation does not occur at all<sup>18</sup>. At their core, however, the most popular of these theories appear to agree on a widely accepted view of hippocampal encoding, known as indexing theory, which states that the hippocampus forms an index of the cortical activity that was present during the actual experiencing of an event<sup>19</sup>.

but that the contents of component memories are actually stored in the distributed cortical networks in which this activity occurred<sup>20</sup>. According to this concept, the memory trace in the hippocampus is a representation of the patterns of neocortical activity that encode the content of an experience. Retrieval of the memory therefore involves the reactivation of the hippocampal cells that project to the neocortex to activate the neocortical pattern representing the entire experience. However, theories differ as to whether the hippocampus is always required to fully reactivate these distributed cortical networks to allow memory retrieval.

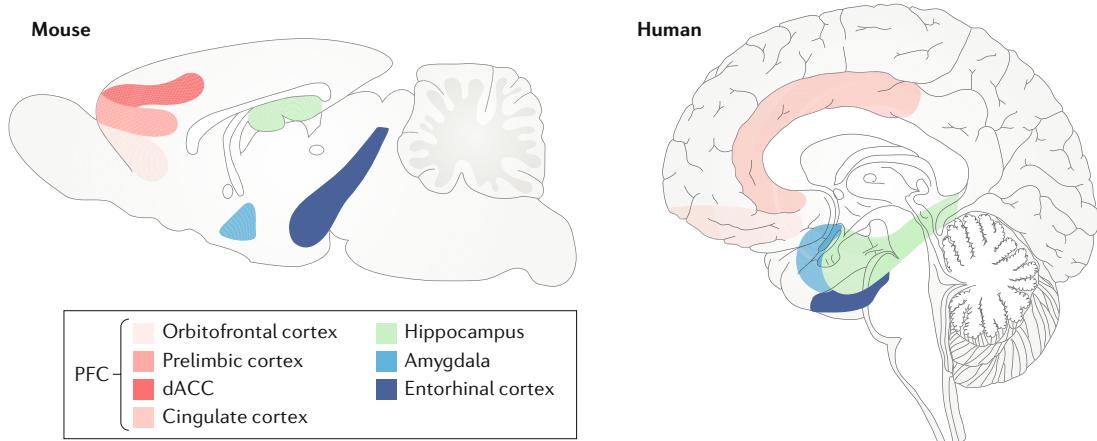
Early findings of temporally graded amnesia in humans, non-human primates and rodents led to several theories on the formation of memory in the brain<sup>9,21</sup>. The most commonly accepted theory at the time proposed that memories undergo a process of consolidation, wherein connections between the cortical regions in which the set of component memories is presumed to reside are strengthened with time, such that the requirement to initiate the retrieval of the entire memory by the hippocampus decreases<sup>22,23</sup>. This is known as the standard model of consolidation<sup>9</sup>.

However, patient and animal studies have sometimes produced conflicting reports on the sparing or loss of remote memories following damage to the medial temporal lobes and hippocampus<sup>17,24,25</sup> (TABLE 1). Such apparent conflicts inspired an alternative explanation of the findings: the multiple trace theory proposed that the hippocampus would always be required for the retrieval of an episodic memory that required the hippocampus for its formation<sup>24</sup>. According to this theory, each reactivation of a memory involves a re-experiencing of the original episode and creates additional traces in the hippocampus. The more traces of a particular memory within the hippocampus, the greater the probability that a trace of this memory will survive partial hippocampal disruption (such as that observed in many patients with retrograde amnesia and animal models)<sup>24</sup>. These multiple

Table 1 | Examples of the effects of prior hippocampal disruption on memory retrieval in rodents

Task	Effect on recent memory retrieval	Effect on remote memory retrieval	Refs
Contextual fear conditioning	Impaired	Intact	105–107
Contextual fear conditioning	Impaired (by acute optogenetic inhibition)	Impaired (by acute optogenetic inhibition)	28
	Impaired (by prolonged optogenetic inhibition)	Intact (following prolonged optogenetic inhibition)	
Contextual fear conditioning	Impaired	Impaired	108
Context discrimination	Impaired	Intact	98
Spatial five-arm maze	Impaired	Intact	40
Trace eyeblink conditioning	Impaired	Intact	38,109
Inhibitory avoidance	Impaired	Intact	110
Trace fear conditioning	Impaired	Intact	44
Socially acquired food preference	Impaired	Intact	111
Paired-associate memory	Impaired	Intact	112
Morris water maze	Impaired	Impaired	103,113–116

The table is intended to provide a general overview of examples of experimental findings in systems consolidation and does not serve as an exhaustive list of results.



**Fig. 1 | Brain regions and circuits implicated in the systems consolidation of contextual fear memory.** Brain regions shown to be involved in systems consolidation, indicated on a schematic illustration of the mouse brain, and their counterparts in the human brain. These include the orbitofrontal cortex, prelimbic cortex, dorsal anterior cingulate cortex (dACC), cingulate cortex, hippocampus, amygdala and entorhinal cortex. The prefrontal cortex (PFC) has several major subdivisions, of which the two important for systems consolidation are the medial PFC (mPFC, usually said to encompass the most anterior aspects of the cingulate cortex, containing the dACC, prelimbic and infralimbic cortices) and the orbitofrontal cortex (which contains medial, lateral and ventral components).

traces provide contextual information for an episode, promoting the neocortical extraction of the abstract or overlapping features of these episodes in a manner that is independent of context<sup>26</sup>. Therefore, according to this theory, memories of abstract and semantic information that are initially acquired in the context of a particular episode are separated from and stored independently of that context. This permits their retrieval without the aid of the hippocampus, whereas discrete, contextually rich or autobiographical information was proposed to always require the hippocampus for successful retrieval<sup>24</sup>.

The multiple trace theory was further advanced as the transformation theory, which hypothesized that the cortical gist-like, or abstract, memory and the hippocampal detailed contextual memory dynamically interact and that, depending on the memory strength and retrieval circumstance, the dominance of one over the other can change<sup>26,27</sup>. One study showed that the acute inhibition of the hippocampal CA1 by optogenetics timed to the onset of retrieval impairs retrieval in both recent and remote memory tests, whereas acute pharmacological hippocampal inhibition (taking effect 30 min after infusion) or prolonged optogenetic inhibition before and during memory retrieval impaired only recent memory recall<sup>28</sup>. These results could be explained if one assumes that memory traces coexist in both the hippocampus and neocortex and that either trace can be used for memory retrieval depending on the animal's situation and condition during recall.

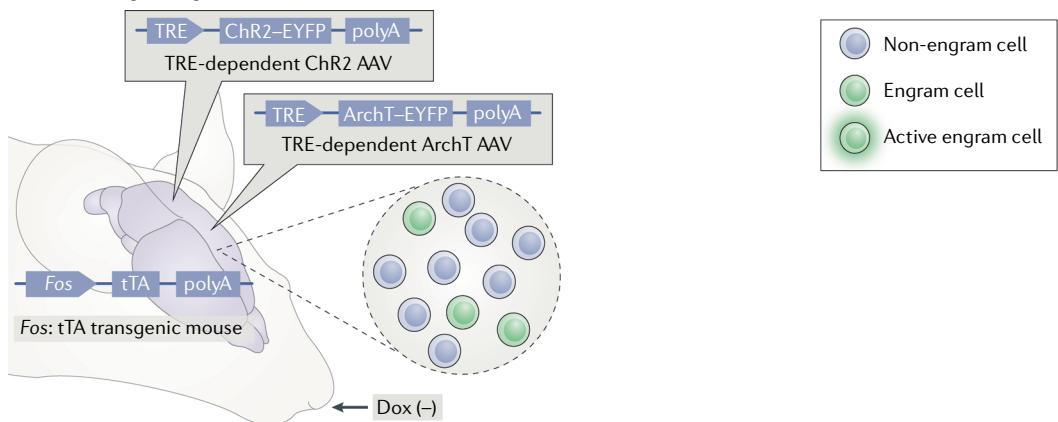
**Trace eyeblink conditioning**  
A form of classical conditioning extensively used to study neural structures and mechanisms that underlie learning and memory. It is based on a relatively simple procedure that often consists of pairing an auditory (or visual) stimulus with an eyeblink-eliciting unconditioned stimulus (such as a mild puff of air to the cornea or a mild shock), with the two stimuli being separated by a stimulus-free trace interval.

the consolidation of these memories requires both time and neocortical plasticity<sup>29</sup>. Regardless of the nature of the long-term memory, both the standard model and multiple trace theories suggest that cortical restructuring supports SCM and that it is these changes that permit the retrieval of the memory without input from the hippocampus<sup>22–24,26</sup>. Indeed, cortical synaptic plasticity over the period presumed to encompass these changes has been shown to be necessary for the successful retrieval of remote memory without affecting retrieval of recent memory<sup>30,31</sup>.

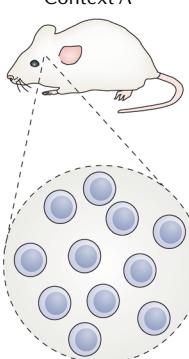
The idea that a specific brain region might acquire a crucial role in the retrieval of older memories was not predicted by any of the major theories of SCM<sup>22–24</sup>. However, a systematic mapping of the brain regions involved in the retrieval of recent or remote memory in mice by using [<sup>14</sup>C]2-deoxyglucose to measure regional levels of glucose metabolism revealed that specific remote memory centres may in fact exist<sup>32</sup>. This study identified several regions (the frontal cortex, the temporal cortex and the anterior cingulate cortex (ACC)) that unexpectedly showed greater activity during retrieval 25 days after learning than during retrieval 5 days after learning. The increased recruitment of frontal regions during the retrieval of older memories has also been reported in human memory experiments, particularly the medial prefrontal cortex (mPFC)<sup>33–35</sup> (FIG. 1).

Early discussions of the relationship between the mPFC and memory had typically focused on its role in working memory maintenance and in the formation of memory<sup>36,37</sup>. In one of the first experiments to assess the necessity of the mPFC (consisting of the dorsal anterior cingulate, prelimbic and infralimbic cortices) for memory retrieval at different time points, researchers revealed little effect of mPFC lesions shortly after memory acquisition in trace eyeblink conditioning in rats; however, severe memory impairments were observed when the mPFC was lesioned several weeks

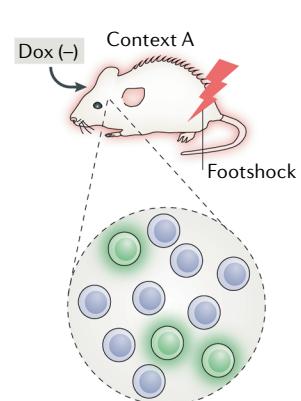
**The medial prefrontal cortex in remote memory retrieval.** The extent to which the role of the hippocampus in memory retrieval is time-limited and the nature of what memories are like without a hippocampus (whether episodic or semantic) are still fairly uncertain. However, what does seem clear is that, with time, certain memories can be retrieved without the hippocampus that was once essential for their formation and that

**a Generating transgenic mice****b Labelling engrams**

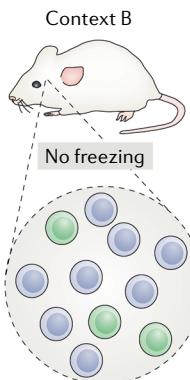
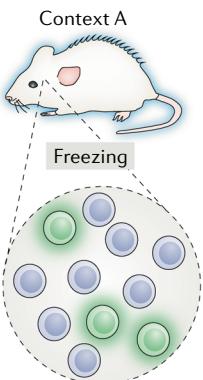
## Context A



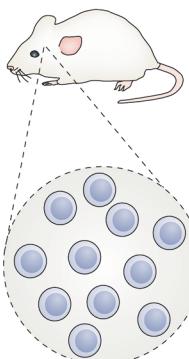
## Memory formation



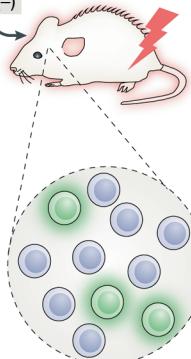
## Natural recall

**c Activating engrams**

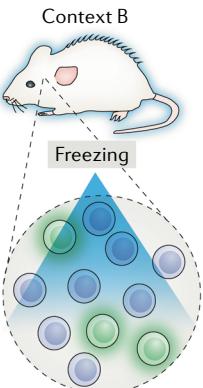
## Context A



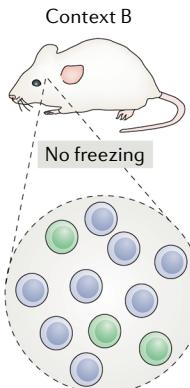
## Context A



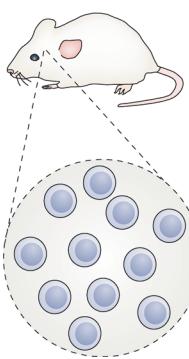
## ChR2 + blue laser



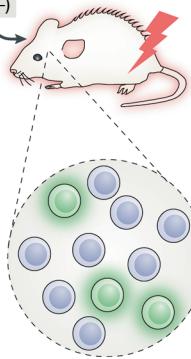
## ChR2, no laser

**d Inhibiting engrams**

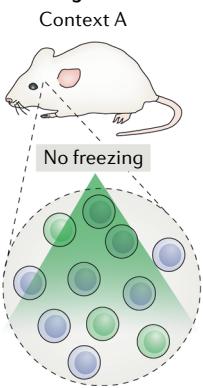
## Context A



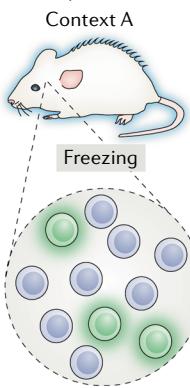
## Context A



## ArchT + green laser



## ArchT, no laser



◀ Fig. 2 | Tools to identify memory engrams in the brain. **a** | Activity-dependent cell labelling. In this approach, a transgenic mouse that expresses the tetracycline transactivator protein (tTA) under the control of the promoter of the immediate early gene *Fos*<sup>6</sup> is injected with an adeno-associated virus (AAV) expressing the light-activated cation channel rhodopsin 2 (ChR2) fused to an enhanced yellow fluorescent protein fluorophore (EYFP) under the control of a tetracycline-responsive element (TRE) or with a virus expressing the light-activated proton pump archaerhodopsin (ArchT) fused to EYFP under the control of a TRE<sup>48</sup>. Providing the mice with a diet containing doxycycline (Dox) inhibits the binding of tTA to its target TRE site, which in turn prevents it from driving ChR2-EYFP or ArchT-EYFP expression. Mice are implanted with an optical fibre targeting the hippocampus. When Dox is removed from the animal's diet, neuronal activity and the resultant *Fos* activation induce the expression of tTA, which binds to TRE and drives the expression of ChR2-EYFP (or ArchT-EYFP), labelling a subpopulation of activated cells in the hippocampus. **b** | During training (memory formation) in a contextual fear conditioning paradigm, a mouse is placed in context A and given a footshock. In the absence of Dox, the activated neuronal ensembles in the hippocampus are tagged with ChR2-EYFP. When returned to context A during the natural recall phase, the mouse displays a conditioned fear response (freezing), and the tagged engram cells are reactivated. When placed in a neutral context (context B), however, the mouse does not display freezing behaviour, and the tagged engram cells are not activated<sup>48</sup>. **c** | Artificially activating hippocampal context A engram cells that have been tagged with ChR2 with a blue laser light in the safe context B will result in the mouse freezing. **d** | Conversely, inhibiting context A engram cells tagged with ArchT with green laser light during the recall phase in context A produces deficits in memory retrieval, evidenced by a lack of freezing behaviour.

**Immediate early gene**  
A gene that encodes a transcription factor that is induced within minutes of raised neuronal activity without requiring a protein signal.

**Immediate early gene**  
activation is, therefore, used as an indirect marker of neuronal activation.

**Contextual fear conditioning (CFC)**. A behavioural test in which an aversive stimulus is given to an animal in a conditioning chamber, such that the fear response can subsequently be elicited in the conditioning chamber in the absence of the aversive stimulus.

**Morris water maze**  
A hippocampus-dependent spatial learning and memory task in which a rodent learns the position of an escape platform placed beneath the surface of a pool of opaque water using a set of distal extra-maze visual cues.

**Trace fear conditioning**  
An associative memory task in which a stimulus (the conditioned stimulus, such as a tone) predicts an aversive stimulus (the unconditioned stimulus, such as a footshock), with the two stimuli being separated by a stimulus-free trace interval. Subsequent presentation of the conditioned stimulus alone in a neutral context can elicit a fear response.

after learning<sup>38</sup>. Subsequent studies strengthened this notion, revealing greater immediate early gene activity within the mPFC during remote memory retrieval than during recent memory retrieval<sup>39,40</sup>. Furthermore, targeted reversible inactivation of mPFC subregions revealed the necessity of these regions in the retrieval of remote but not recent memory<sup>39–41</sup> in rodents in contextual fear conditioning (CFC)<sup>28,39</sup>, the Morris water maze<sup>42,43</sup>, trace fear conditioning<sup>44</sup>, trace eyeblink conditioning<sup>41</sup> and paired-associate memory<sup>45</sup>. Although many of these studies revealed the importance of the mPFC in remote memory recall, it is important to note that other cortical areas, including the orbitofrontal, auditory and retrosplenial cortices, are also important for other types of remote memories. Furthermore, these findings did not clarify whether the specific role of the mPFC is to provide a remote memory engram or to regulate the retrieval of remote memories stored in other cortical areas.

**A new approach to find memory engrams.** In 1904, Richard Semon proposed the physical theory of human memory. He coined the term *engram* to describe the physical substrate of memory, which he defined as “the enduring though primarily latent modification in the irritable substance produced by a stimulus” (REF. 4). The term *engram* is roughly equivalent to another commonly used term, *memory trace*, and can be defined by three criteria: an engram is the enduring physical and/or chemical change that occurs in the neural network (criterion 1) as a result of activation of neuronal subpopulations by episodic stimuli (criterion 2) and can be subsequently reactivated by stimuli that were part of the original set of encoded stimuli, resulting in the recall of the original memory (criterion 3)<sup>3,7,8</sup>. Recent technological advances have made it possible to identify engram cells for a specific memory and to examine the effect of their activation or inactivation on mnemonic

behaviours<sup>46–52</sup> (FIG. 2). These technologies include the utilization of immediate early genes (to drive gene expression in activated cells), transgenic mice exhibiting cell type-restricted patterns of gene expression, optogenetics, pharmacogenetics, electrophysiological recording and optical imaging<sup>53</sup>. In particular, the engram identified in the hippocampal dentate gyrus granule cells for CFC memory met all three of Semon's criteria<sup>48,49,51,52,54</sup>.

Many of the findings described above and the insights into the mechanisms of SCM that they provided were generated from the experimental results of loss-of-function studies, which do not generally provide highly restricted interventions. Evidence obtained by these studies has been combined with observational studies, which can enable the correlation of activity to behaviour. However, by definition, the latter type of studies falls short of identifying a causal link between the observed activity and behaviour. In more recent years, and as described below, a series of studies have provided evidence for such a causal link between the specific engrams of episodic memory cells, their circuits and SCM.

## Neocortical memory generation

Our understanding of the neocortical activity, mechanisms and circuits supporting long-term memory formation have been greatly advanced over the past decade. Converging evidence from multiple lines of study suggests that neocortical activity, particularly within the mPFC, during learning and the gradual strengthening of neocortical connections over time support long-lasting memory and the process of systems consolidation.

**Early tagging hypothesis.** Although several studies had suggested a role of the mPFC in remote memory retrieval (see above), it was unclear whether the mPFC and/or other frontal cortical areas encode an episode rapidly during learning, as the hippocampus does. The prevalent models of SCM posited that episodic memories are initially formed within the medial temporal lobes through rapid synaptic plasticity in these areas and that the site of memory storage slowly shifts from these lobes to neocortical networks during the post-encoding period<sup>22</sup>. One of the first hints that a frontal cortical area may be in some way involved in the rapid formation of a memory trace on the day of training came from a study using the social transmission of food preference paradigm<sup>55</sup>. Injecting a competitive AMPA and/or kainate receptor antagonist (6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX)) or an NMDA receptor antagonist ((2R)-amino-5-phosphonovaleric acid (AP-5)) into the orbitofrontal cortex (OFC) to block synaptic activity specifically during the training period impaired remote retrieval of this memory 30 days after training but not retrieval 7 days after training<sup>55</sup>. These results suggest that activation and plasticity in the neocortex during learning are necessary for remote memory recall but not for recent memory recall. This led researchers to question whether an involvement of putative neocortical engrams in recent memory recall could be masked by the contribution of the active hippocampal engram

**Paired-associate memory**

A memory task in which arbitrary paired associations are learned and recalled, for example, certain locations in a space may be paired with a particular object or flavour of food reward.

**Social transmission of food preference paradigm**

A memory paradigm in rodents that takes advantage of the animals' natural food neophobia. If a naïve subject rat interacts with a demonstrator rat that has recently sampled a particular novel food substance, the naïve animal acquires a preference for that food that can persist for many days.

at this time or whether, although formed in the OFC during learning, these engrams are in an inactive state and require conversion into an active form for remote memory recall. The authors of REF.<sup>55</sup> hypothesized the latter possibility and introduced the concept that some OFC cells may be tagged during training and become part of the future engram. However, the nature of this tagging remains undefined.

**Medial prefrontal cortex inputs and the formation of remote memories.** The tagging phenomenon in SCM has since been investigated by combining neural circuit mapping using the retrograde tracer cholera toxin subunit B with axonal projection-specific optogenetic manipulations<sup>56</sup>. First, the specific and direct projections of layer Va cells in the medial entorhinal cortex (MEC-Va), which are one of the immediate output targets of dorsal hippocampus cells, were identified. These projections were shown to target several neocortical areas, including the mPFC and the basolateral amygdala (BLA)<sup>56,57</sup>. When the input from MEC-Va to the mPFC was optogenetically inhibited during CFC training, a selective impairment in remote memory retrieval (on post-training test days 15 and 22) was observed, but there were no deficits in recent memory retrieval (on post-training test days 2 and 8). By contrast, optogenetic inhibition of the axonal projections of MEC-Va to other cortical areas, including the caudal ACC and the retrosplenial cortex, during CFC training impaired neither remote nor recent recall. Furthermore, optogenetic inhibition of MEC-Va axonal projections to any of these cortical areas, including the mPFC, during recall periods did not impair recall.

Another major input to mPFC cells originates in the BLA, and optogenetic inhibition of these BLA projections during CFC training also selectively impaired the retrieval of the remote fear memory but not the retrieval of the recent memory<sup>56</sup>. Using a gain-of-function experiment, another study generated an artificial remote contextual fear memory in mice through the simultaneous optogenetic stimulation of blue light-sensitive channel rhodopsin 2(ChR2)-expressing memory engram cells in the dorsal hippocampus and BLA<sup>58</sup>. These results indicated that inputs to the mPFC from MEC-Va and BLA during learning are crucial and sufficient for the formation of remote memory in the mPFC.

**Generation of silent engram cells in the medial prefrontal cortex.** The findings described above suggested that engram cells are formed in the mPFC during training. Previously, plasticity within the mPFC has been shown to be important both during and shortly after learning for contextual fear memory<sup>59,60</sup>. The circuit study described above was therefore extended to address this issue by applying engram identification and manipulation technologies to characterize mPFC engrams. Two groups examined immediate early gene expression in the neocortex during CFC and found that there was a subset of mPFC neurons that strongly expressed the FOS protein during learning<sup>56,61</sup>. In addition, both hippocampal and BLA inputs into the mPFC, which are crucial for the formation of remote contextual fear memory, were

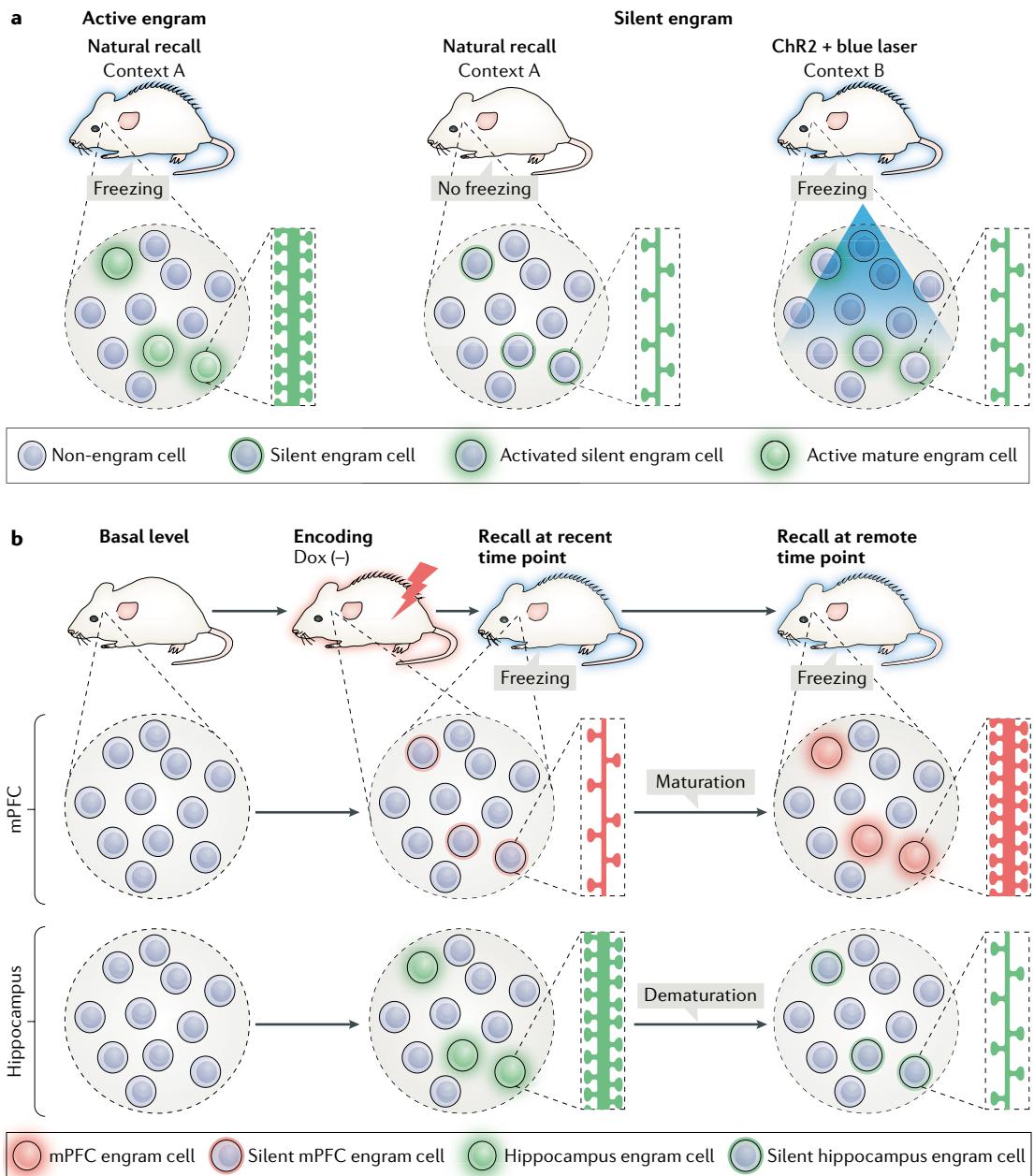
required for the observed induction of FOS expression within the mPFC.

In one study, the FOS-expressing neurons that form the memory engram in the mPFC were characterized through the specific expression of ChR2 (REF.<sup>52</sup>) in these cells and examination of the effect of activating this subpopulation of mPFC cells in a neutral context<sup>56</sup>. Blue light-pulsed stimulation of ChR2-expressing cells in the mPFC induced freezing in a neutral context at both recent and remote times. Thus, it appeared that mPFC engram cells are generated during initial training, as their optogenetic reactivation could induce memory retrieval as early as 1 day after training and until at least 2 weeks after learning.

Evidence that these cells met the defined criteria of engram cells was provided by the finding that the mPFC cells that expressed FOS during learning were necessary for the recall of remote memory and that these cells were reactivated by natural recall cues during remote memory recall in a context-specific manner<sup>56</sup>. However, these mPFC engram cells were not reactivated by natural recall cues 1 day after training, and their inhibition had no effect on retrieval of the memory at this recent time point. Thus, mPFC engram cells are generated quickly on the day of training, and the memory is retrievable from these cells through optogenetic stimulation, but not by natural recall cues, 1 day after training. The engrams in this state were referred to as silent engrams.

Engram cells in a silent state were previously observed in a mouse model of retrograde amnesia<sup>52,54</sup>, in models of early Alzheimer disease<sup>63</sup> and in social memory<sup>64</sup>. Thus, the silent state of engrams is not unique to early mPFC engrams but is a more general phenomenon of memory engram cells. Current understanding of silent engrams is based on several lines of evidence that these cells are not reactivated by natural cues but can be reactivated artificially to elicit their encoded memory. In all cases, silent engram cells display relatively low spine density compared with their active engram counterparts<sup>52,54,56</sup> (FIG. 3). Furthermore, in the case of the silent engram cells demonstrated to be present in a retrograde amnesia model<sup>52,54</sup>, it has been shown that, on average, these cells exhibit reduced potentiation at the synapses that connect them to upstream engram cells compared with those of active engram cells in non-amnesic mice<sup>52</sup>. We hypothesize that a similar reduced synaptic potentiation is present in the early mPFC engram cells, and therefore memory is not retrievable from these cells by natural cues, although it is through optogenetic stimulation, which bypasses synapses.

The silent nature of the neocortical engram may provide a cellular-level mechanism for the tagging phenomenon, with tagging in this case referring to the creation of a population of neurons that are the source of functionally mature engram cells. However, silent engrams cannot be merely equated to tagging because the relationship between silent and mature engram cells is bidirectional (that is, silent engram cells can be converted into active engram cells and, as described below, active engram cells can become silent), whereas the tagging phenomenon described previously implied a



**Fig. 3 | Silent and active memory engrams.** **a** | Active engram cells typically show dense spines and are reactivated by natural cues. For example, in the left panel, the engram cells are reactivated by re-exposure to a context (context A) previously associated with an aversive stimulus, triggering activation of these cells and an appropriate behavioural response (freezing). Silent engram cells, such as those shown in the right two panels, contain more sparse spine density and are not reactivated by natural cues; however, if tagged with the light-activated channel rhodopsin 2 (ChR2), they can be artificially reactivated with blue laser light and can produce memory retrieval even in the absence of contextual cues<sup>52,56,63,64</sup>. **b** | In one study, medial prefrontal cortex (mPFC) engram cells were rapidly formed during contextual fear conditioning on day 1 and labelled with doxycycline (Dox) removed from the animal's diet. However, they were not reactivated with natural recall cues and displayed low spine density at recent time points. The immature mPFC engram cells functionally, structurally and physiologically matured during the subsequent few weeks and were active during retrieval at a remote time point, and displayed increased spine density. Conversely, hippocampal engram cells were rapidly formed during day 1 of training, at which point they were also functionally, structurally and physiologically mature. They gradually became silent with time, accompanied by a reduction in the density of dendritic spines<sup>56</sup>.

unidirectional relationship between tagged, but inactive, and active cells.

We expect that there is a diverse set of underlying molecular and cellular features that define silent and active memory engram cells. One common difference

between these cells that has been noted in all the aforementioned studies is a paucity of dendritic spines in the silent engram cells compared with their active counterparts<sup>52,56,63</sup>. There are also a number of genetic changes that take place during learning that are essential for

the formation of long-term cortical memory, any of which may be involved in the conversion of engram cells from a silent into an active state and vice versa. For example, specific signalling cascades involved in the regulation of chromatin remodelling have been observed to occur within the neocortex during learning<sup>55</sup>. Increases in histone H3 acetylation in the OFC were observed after learning, and interference with this cascade before learning impaired remote memory retrieval<sup>55</sup>. DNA methylation, a transcriptional repression mechanism, has also been shown to be a crucial step in remote cortical memory formation: persistent, gene-specific cortical hypermethylation was induced in the mPFC following CFC and persisted for at least 30 days following learning<sup>65</sup>. Pharmacological inhibition of methylation in the mPFC at this remote time point also disrupted memory retrieval, suggesting that DNA methylation that occurs during learning serves to preserve long-term memories<sup>65</sup>.

Epigenetic changes during learning have also been shown to be important for the formation of recent and remote memory. Histone variant exchange, in which canonical histones are replaced with their variant counterparts, occurs during learning, and histone H2A.Z, a variant of histone H2A, is actively exchanged in response to fear conditioning in the hippocampus and the mPFC<sup>66</sup>. H2A.Z was shown to mediate the expression of hundreds of genes in the hippocampus and mPFC, and its regulation in these brain regions was shown to restrain the formation of recent and remote memory, respectively<sup>66</sup>. An examination of the differences between these epigenetic changes in engram and non-engram cell populations, and between silent and active engram cells, would provide us with important information about what defines these cells and the mechanisms by which they are formed.

#### **Engram maturation and de-maturation**

We now have strong evidence that the nature of engram cells is quite dynamic. Engram cells can be generated in a silent form and, with maturation, become active; likewise, engram cells can be generated in an active form and, with time or amnesia, become silent.

**Evidence for medial prefrontal cortex engram cell maturation.** The discovery of silent engram cells in the mPFC that are essential for remote memory recall suggests that these silent engram cells gradually mature in the period after learning. Indeed, an older study<sup>67</sup> examined the time course over which neural activity in the mPFC becomes selective for an acquired memory during this cortical memory maturation period. After acquisition of conditional memory associations in trace eyeblink conditioning, subpopulations of neurons in the mPFC of rats began to exhibit sustained activity during the interval between the presentation of two paired stimuli (FIG. 4a,b). These new patterns developed over a period of several weeks after learning: the same time period in which it has been previously shown that the mPFC becomes important for retrieval and in which plasticity mechanisms appear to be crucial<sup>67</sup>. Although this study did not longitudinally monitor engram cells,

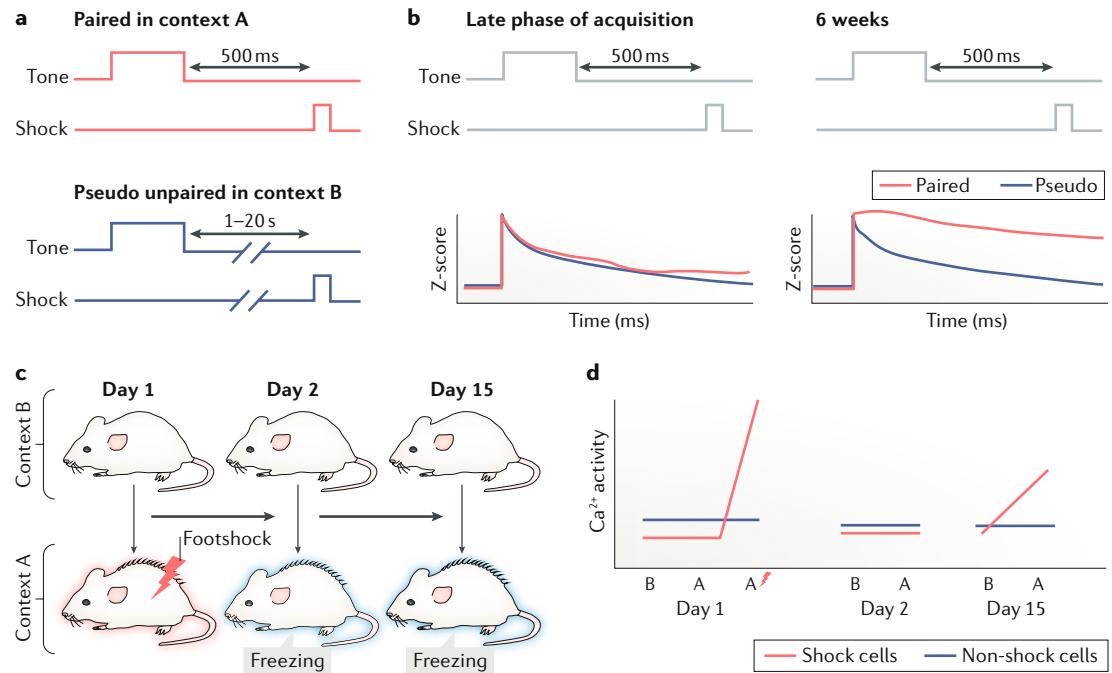
the results are consistent with the notion that mPFC engram cells mature as the memory ages.

A more recent study<sup>56</sup> examined single cell activity longitudinally by monitoring transient calcium ( $\text{Ca}^{2+}$ ) events, tracking intracellular  $\text{Ca}^{2+}$  spikes with a genetically encoded  $\text{Ca}^{2+}$  indicator (GcaMP) in putative mPFC engram cells *in vivo* during and after CFC training. Using a miniaturized, head-mounted fluorescence microscope<sup>68</sup> implanted into the mPFC of mice, it was possible to investigate changes in neural activity within individual cells across time. On day 1, the mice were exposed to a neutral context (context B), followed by CFC in context A. The mice were then re-exposed to both contexts in the same order 1 and 14 days later. The activity of mPFC cells did not appear to discriminate between the two contexts at baseline (before footshock presentation on day 1). However, after footshock presentation, about 11% of cells (termed shock cells) showed a significant increase in  $\text{Ca}^{2+}$  transients. During recall on day 15, the transient  $\text{Ca}^{2+}$  activity of shock cells in context A was significantly higher than it was in context B; however, this was not the case on days 1 or 2 (FIG. 4c,d). It appears likely that shock cells are mPFC memory engram cells, given that the generation of mPFC engram cells requires both context exposure and footshocks during learning. Furthermore, shock cells were silent in response to the conditioned stimuli during recent recall and active during remote recall. Thus, these findings would show the clear maturation of the physiological activity of mPFC memory engram cells with time. However, it would be desirable to ascertain with engram labelling technology that shock cells are indeed mPFC engram cells.

**Role of hippocampal engram cells in neocortical engram maturation.** According to most models of SCM, the memory storage site shifts from the hippocampus (or medial temporal lobes) to the neocortex through the strengthening of cortico-cortical connections. However, as described above, this postulated shift has now been shown to also involve the slow functional maturation of mPFC engram cells formed rapidly in a silent state during learning<sup>56</sup>. In addition, it has been demonstrated that the maturation of the mPFC engram cells requires post-learning input from the hippocampal engram cells<sup>56</sup>; this suggests that the requirement for an intact hippocampus during the systems consolidation period for the formation of remote memory is a consequence of its role in the maturation of the silent engram cells of the mPFC.

The mechanisms by which the hippocampus contributes to remote memory were investigated in an earlier study<sup>69</sup> in which tetanus toxin (TeTX) was expressed in hippocampal CA3 cells in mice in the post-training period that followed CFC. This revealed that the blockade of CA3 output during the SCM period reduced the intrinsic frequency of high-frequency oscillatory activity (sharp-wave ripples) and place cell replay in CA1, and impaired remote CFC memory. This effect was specific to the CA3–CA1 circuit because blocking the medial entorhinal cortex layer III–CA1 circuit had no effect on the frequency of sharp-wave ripples during sleep or on remote memory<sup>70,71</sup>. It has been shown that sharp-wave ripple-induced replay of place cell activity in CA1

Sharp-wave ripples  
Brief (approximately 100 ms)  
episodes of high-frequency  
(>100 Hz) population activity.



**Fig. 4 | Physiological maturation of neocortical representations.** **a** | Context-dependent acquisition and maturation of memory associations with time in a trace eyelink conditioning paradigm. In this study, rats were exposed to an environment in which an auditory conditioned stimulus (CS, a tone) was presented within 500 ms of an unconditioned eyelid stimulation (US, labelled shock), resulting in pairing of the two stimuli, and they were also exposed to a separate environment in which the CS and US were separated by 1–20 seconds and thus pseudo unpaired. Rats learned to associate the CS with the US in the paired context but not the pseudo unpaired context. **b** | Traces showing activity (standardized firing rates (Z-score)) of medial prefrontal cortex (mPFC) cells during these exposures. Over the 6 weeks following training, the cells began to exhibit sustained activity during the interval between the presentation of the two paired stimuli (CS and US)<sup>67</sup>. **c** | Longitudinal calcium imaging of the mouse mPFC was measured during the systems consolidation of a contextual fear memory over 2 weeks. On day 1, mice were exposed to context B, followed by contextual fear conditioning (forming an association between context and footshock) in context A. The mice were re-exposed to both contexts in the same order on days 2 and 15 and exhibited a conditioned fear response (freezing) in context A. **d** | Calcium ( $\text{Ca}^{2+}$ ) activity recorded in mPFC cells in mice expressing the calcium sensor GCaMP6 during these exposures. About 11% of mPFC cells (known as shock cells) showed a significant increase in  $\text{Ca}^{2+}$  activity during conditioning. The remaining ~89% of mPFC cells (non-shock cells) did not respond to the shocks. During recall, the transient  $\text{Ca}^{2+}$  activity of the shock cells in context A was significantly higher than that in context B on day 15 but not on days 1 or 2, whereas the frequency of  $\text{Ca}^{2+}$  transient events in non-shock cells remained constant, irrespective of contexts and days<sup>56</sup>.

contributes to the consolidation of spatial memory within the hippocampus<sup>72,73</sup>. A similar mechanism that operates repeatedly over a longer distance (from the hippocampus to the mPFC), taking days or weeks, could be hypothesized to promote the slow maturation of the silent mPFC engram cells. For example, the coupling of cortical spinles to hippocampal sharp-wave activity has been shown to be important for memory consolidation<sup>74,75</sup>.

To examine the contribution of hippocampal engram cells to the maturation of mPFC engram cells, a more recent study<sup>56</sup> investigated the effect of chronic inhibition of the output of hippocampal dentate gyrus (DG) engram cells through selective TeTX expression in these cells starting 1 day after training<sup>56</sup>. This inhibited the reactivation of mPFC engram cells during exposure to the conditioned context 12 days after CFC (an indicator of engram cell maturation). TeTX expression also blocked the increase in the dendritic spine density of mPFC engram cells that was observed in the control group. *In vivo*  $\text{Ca}^{2+}$  imaging revealed that TeTX expression in hippocampal

engram cells after CFC also blocked the time-dependent increase in the context-specific  $\text{Ca}^{2+}$  transients observed in shock cells in the mPFC. These results together show that hippocampal activity, and specifically the activity of hippocampal memory engram cells, following the learning period (that is, during the SCM period) is necessary for the gradual maturation of mPFC engram cells.

Importantly, there are also other network changes taking place in the brain during this consolidation period<sup>76</sup>, and we do not rule out the possibility that parallel changes in other brain networks (such as thalamic networks) may also support this cortical maturation process.

**De-maturation (silencing) of hippocampal engram cells.** As described above, whether the hippocampus is always required for the retrieval of a memory<sup>29</sup> continues to be debated, with arguments on both sides being primarily based on lesion studies in rodents and human patients (TABLE 1). However, loss-of-function experiments

cannot rule out the existence of a memory engram, and it is therefore important to also analyse the effects of a gain of function by stimulating the hippocampal memory engram at the remote time point. By examining the post-consolidation fate of hippocampal engram cells, researchers found that hippocampal DG engram cells were not reactivated by natural cues during retrieval on day 15 after CFC<sup>56,77</sup>, and their spine density was significantly reduced at this stage in comparison to day 5 (REF.<sup>56</sup>); however, their optogenetic activation was still able to induce freezing behaviour<sup>56</sup>. Thus, 2 weeks after learning, hippocampal engram cells appear to persist in a silent state (FIG. 3). Although it was not determined how long after encoding these silent hippocampal engram cells last, we speculate that the hippocampal engram may eventually lose the original memory information<sup>9,22,23</sup>. Nevertheless, it is also possible these hippocampal engrams remain accessible long term.

It has been demonstrated that the presentation of a reminder cue, such as a brief re-exposure to the conditioned context, 1 day before the retrieval test, can re-instate hippocampal dependency at remote time points<sup>78,79</sup>. This reminder also has the effect of regaining the context specificity of the memory, something that is typically lost in very remote contextual memories<sup>79–81</sup>. It is possible that this reminder effect is the result of the reactivation of the original silent hippocampal engram, a process that would be similar to that proposed by transformation theory<sup>26</sup>. However, this would need to be demonstrated experimentally. This issue could be tested with available tools: for example, how long silent hippocampal engram cells last could be addressed through long-lasting labelling of hippocampal engram cells over a period of months<sup>50,51,82</sup>. Furthermore, one can easily test whether various reminders can convert the hippocampal engram cells from the silent state into the active state.

It is currently unknown how hippocampal engram cells become silent. A possible mechanism for the de-maturation (silencing) of hippocampal engram cells could be circuit reorganization driven by the erasure of old connections and the creation of new connections through the addition of newborn neurons<sup>83–85</sup> in the perforant path–DG pathway and the DG–CA3 pathway<sup>86</sup>. The integration of newborn neurons into the hippocampal circuits from the entorhinal cortex to the DG–CA3 pathway would disrupt existing synaptic connections and eventually might cause the state change from active to silent engrams owing to decreased synaptic connections between memory engram cells. Another circuit mechanism of de-maturation of hippocampal engram cells could involve top-down PFC-mediated control of hippocampal function to inhibit hippocampal activity<sup>29</sup>.

**Maintenance of active engram cells in the basolateral amygdala.** The intact BLA has been shown to be necessary for the retrieval of both recent and remote fear memory<sup>87</sup>, and the existence of active BLA memory engrams has been demonstrated at recent time points after learning<sup>6,88</sup>. One recent study<sup>56</sup> mapped the neuronal circuits that are necessary for the formation and retrieval of BLA engram-mediated CFC memory. This showed that input from dorsal hippocampal

engram cells, delivered to the BLA via MEC-Va, is required for the formation of BLA engrams during conditioning and for fear memory retrieval at recent time points. By contrast, at remote time points, input from mPFC engram cells to BLA engram cells is essential for fear memory retrieval. Thus, during SCM, there is a switch in the route through which the retrieval input is delivered to the BLA. The same BLA fear memory engram cells that are generated by CFC and used for retrieval of recent memory persist during SCM, demonstrated by a substantial overlap between the BLA engram cells activated during recent and remote recall<sup>56</sup>. Thus, unlike the mPFC and hippocampal engrams, which are converted from silent into active engrams and active into silent engrams, respectively, BLA engram cells seem to stay persistently active throughout SCM (although the route of input to activate them switches) (FIG. 5).

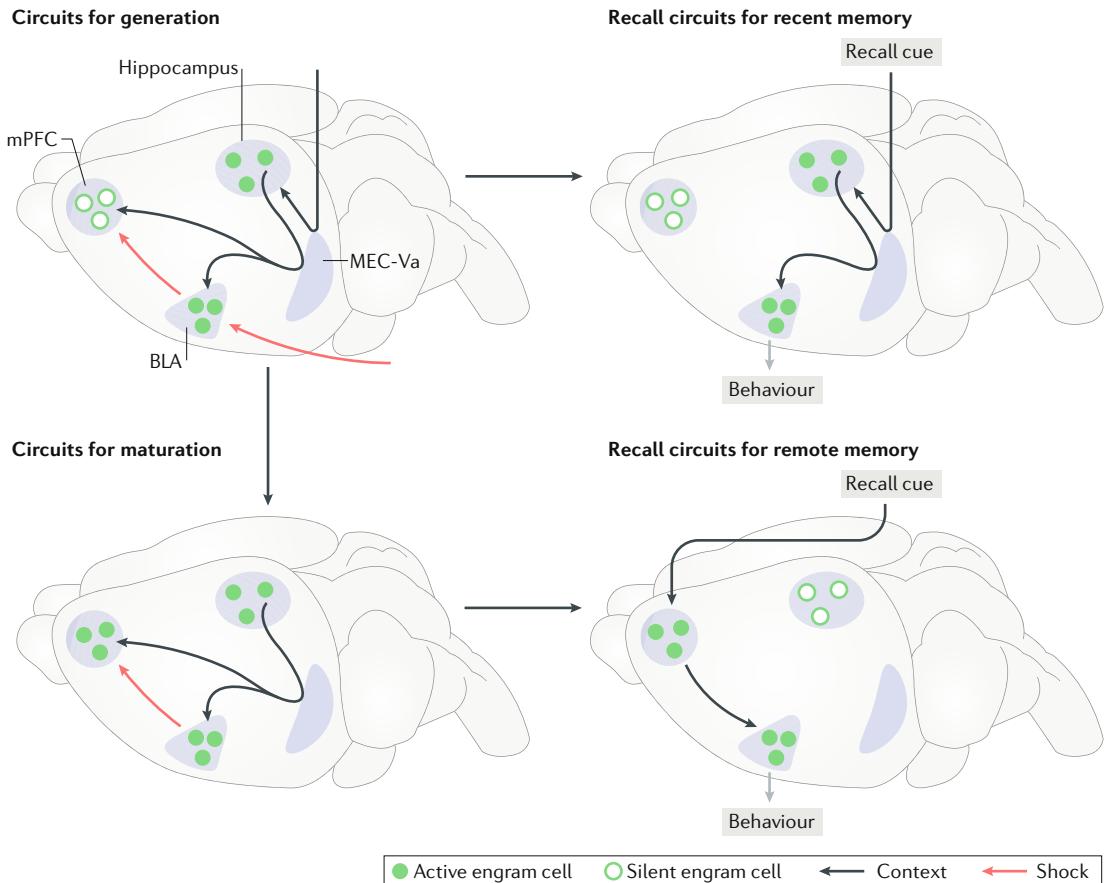
BLA activity is essential for the valence aspect of fear memory, and we would expect a similar circuit to exist for the consolidation of a rewarding memory, given the known role of the BLA in positive valence behaviours<sup>89</sup>. Furthermore, for episodic memories that lack such a strong valence component, we predict that there may be a similar consolidation-dependent network switch in the circuits that drive the activity of whatever necessary downstream structure ultimately leads to the behaviour.

### The mPFC in remote episodic memory

Many neocortical areas are activated during episodic memory formation and retrieval. These include the caudal ACC<sup>39,40</sup>, entorhinal cortex<sup>77,90</sup>, perirhinal and postrhinal cortices<sup>91</sup> and the retrosplenial cortex<sup>40,92</sup>. Furthermore, formation of memory engrams has been reported in several higher order structures of the sensory and association cortices<sup>93–95</sup>. Outputs from some of these engrams are likely to be integrated by the hippocampus during the formation of recent episodic memory, as suggested by indexing theory<sup>19</sup>.

It has been suggested that the role of the mPFC in remote episodic memories is equivalent to that of the hippocampus for recent memory<sup>29</sup>. Indeed, it has been shown that, whereas inhibition of hippocampal output to mPFC (via MEC-Va terminals) during learning impairs remote memory retrieval, the same treatment of MEC-Va terminals in other cortical areas has no effect on remote memory retrieval<sup>56</sup>. Thus, the mPFC seems to have a special function that may include the integration of individual component engrams stored in various other cortical areas. This is further supported by the finding that the mPFC emerges as one of the key neocortical hubs in the long-term memory network, assessed by activation patterns and functional connectivity analyses<sup>76</sup>.

The similar role of the hippocampus and mPFC in recent and remote memory, respectively, can also be seen in the way that the emotion-invoking component of an episode is handled. For this purpose, the BLA engram cells that hold the emotional component of a recent memory largely overlap with those of the remote memory, and it is the hippocampus and mPFC engram cells that send stimuli to the BLA engram for its reactivation in recent and remote times, respectively (FIG. 5).



**Fig. 5 | Engrams and their circuits for systems consolidation of memory.** A new model to describe the mechanisms of systems consolidation of memory is shown<sup>56</sup>. In the medial prefrontal cortex (mPFC) memory, engram cells are rapidly formed during day 1 of a contextual fear conditioning paradigm by inputs from layer Va cells in the medial entorhinal cortex (MEC-Va) and the basolateral amygdala (BLA). However, these engram cells are not activated by natural recall cues when examined soon after training (day 2). These silent mPFC engram cells functionally, structurally and physiologically become active during the subsequent few weeks. This process requires inputs from hippocampal engram cells, which are thought to reach the mPFC via MEC-Va. By contrast, retrieval of the active mPFC engram at a remote time point does not require MEC-Va input. Functional hippocampal engram cells that are formed during training become silent with time; they are not retrieved at remote time points by natural recall cues but are still reactivatable optogenetically for recall (not shown). Conversely, BLA engrams formed during training are functionally maintained even after the consolidation-mediated switch in recall circuits. Adapted with permission from REF<sup>56</sup>, AAAS.

### Conclusion and perspectives

In this Review, we have summarized some of the most recent advances in our understanding of the nature and dynamics of neocortical and subcortical memory engrams for SCM and have placed these studies among previous key findings and theories in the field. On the basis of these recent findings, we have proposed that SCM occurs in two major steps: the rapid generation of silent engrams in the mPFC during learning and the slow functional maturation of these engrams, which is aided by input from hippocampal engram cells during the post-training period lasting a few weeks in rodents<sup>56</sup>. This maturation process includes augmentation of spine density in the mPFC engram cells, which also requires input from the hippocampal engram cells. We assume that the dynamics of memory engrams in the PFC and the hippocampus during SCM would be mirrored in other types of episodic-like memory (such as social transmission

memory or trace eye blink conditioning), but this remains to be tested.

There are many questions associated with this model of SCM. Perhaps the most burning question is how the input from hippocampal engram cells converts mPFC engram cells from a silent into an active state. One exciting and testable possibility is that repeated sharp-wave ripple-mediated replay of hippocampal CA1 engram cell activity during the animal's slow-wave sleep or quiet awake periods could boost the synaptic strength and spine density of mPFC engram cells. Disruption of sharp-wave ripple activity in the hippocampus has been demonstrated to impair spatial learning<sup>72,73</sup>, presumably by disrupting consolidation within the hippocampus-entorhinal cortex, but its role in SCM has not been tested. In early Alzheimer disease mouse models, it has been shown that silent DG engram cells can be converted into active engram cells by repeated optogenetic activation of the upstream entorhinal cortex engram

**Semantic memories**  
Recollections of factual information that are independent of the specific episodes in which that information was acquired.

cells at a high frequency (100 Hz)<sup>63</sup>. Similarly, in retrograde amnesia mice, augmented expression of the serine/threonine-protein kinase PAK1 in CA1 engram cells restores their spine density and converts them from a silent into an active state<sup>54</sup>.

Related to the mechanisms underlying the maturation of mPFC engrams is the mechanism for the de-maturation of hippocampal engrams. The question here is whether this is a passive process in which unused engrams undergo a progressive loss of active synapses or an active process that ensures turnover and reuse of hippocampal cells for new memories. The mature mPFC engram cells could have a role in this process through their back projections to the hippocampus<sup>96,97</sup>.

What is the relationship between mPFC engrams for remote episodic memories and those for semantic memories? Early studies showed that a 30-day-old cortical CFC memory loses its context specificity, while the few-days-old hippocampus memory retains<sup>80,98,99</sup>. This has been taken as evidence that remote cortical memory is more semantic. Indeed, the mPFC is known to play a role in rule and categorization memories<sup>100,101</sup> and also in the formation of schematic frameworks for episodic memories<sup>45,102,103</sup>, which are all more semantic than episodic. However, at 2 weeks after learning in mice, when CFC memory recall is dependent on mPFC activity, the memory and mPFC engrams are as context-specific as 2-day-old hippocampal CFC memory; although the context specificity of the memory declines at 4–6 weeks after learning, we do not know the state of the mPFC engram at this later time point<sup>56,80</sup>. These results suggest the mPFC engram itself can, at least at the 2-week time point, provide episodic information<sup>25</sup>. At this time point after learning, the hippocampal engram is silent and cannot provide contextually rich information to the mPFC engram for episodic recall. In addition, a blockade of the major projection from the hippocampus to the mPFC via entorhinal cortex does not disrupt the retrieval of CFC memory from

the mPFC<sup>56</sup>. These results challenge the concept that the hippocampus is always required for successful retrieval of an episodic memory, including at remote times<sup>24</sup>. An interesting possibility that emerged from these data is that remote episodic memory engrams and the related semantic memory engram can coexist in the mPFC and that the retrieval of neither type of remote memory requires the functional hippocampus. Perhaps experiencing multiple, related episodes results in the formation of multiple, remote episodic memories in the PFC, and a gist-like engram is extracted from these memories for the formation of a semantic memory engram in the PFC, independent of the hippocampal engrams.

Long-term recordings have revealed that over a 1 month period, the activity of neurons in the mPFC gradually becomes more sensitive to the latent, relational features of a memory task and that although information about the perceptual and/or physical features of the environment is considerably weakened (over a slightly different time course), it still remains<sup>103</sup>. Another possibility that cannot be excluded is that hippocampal structures downstream of the DG (that is, the CA3–CA1–subiculum) may remain involved in retrieval longer than the DG<sup>28</sup> and that such contextual information could be provided to the mPFC at these remote time points from the hippocampus<sup>104</sup>, despite the DG engram being silent.

It will be important in the future to address whether there are distinct populations of mPFC cells for remote episodic and semantic memory (and, if so, whether these cells interact) or whether an episodic-to-semantic conversion takes place within individual mPFC cells. At the moment, neither engram cells nor their associated circuits have been identified for a specific semantic memory, whether it is for a rule, category or schema. However, the good news is that such investigations seem to be within reach with the availability of new tools.

Published online 3 July 2018

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**Author contributions**

M.D.M. and T.K. researched data for the article. S.T., M.D.M. and T.K. made substantial contributions to discussions of the content, wrote the article and reviewed and/or edited the manuscript before submission.

**Competing interests**

The authors declare no competing interests.

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