The role of engram cells in the systems consolidation of memory

Title

The Engram Maturation Model of Systems Consolidation of Memory

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Abstract

The question of what happens to memories as days, weeks, or even years go by has long been one of the fundamental questions in neuroscience and psychology. For decades, researchers have attempted to identify brain regions where memory is initially formed and follow its potential changes across time, at the circuit, neuronal, molecular, and genetic levels. Various hypotheses have been generated for the phenomenon termed “systems consolidation of memory (SCM),” which describes changes in the circuitry and brain networks required for the maintenance of a memory with time. These models have largely been based on the analysis of amnesia patients, imaging of human and animal brains, and lesion, pharmacological, electrophysiological, and some gene manipulation studies in animal models. Recently, several important studies have accumulated evidence for the brain networks driving this phenomenon from the aspect of memory engrams cells, their biochemical and physiological changes, and their circuits. In this review, we will highlight these findings and place them among key findings in the field that lead to a revamped model of systems consolidation of memory in the brain, which we call the Engram Maturation Model. Furthermore, we will also discuss the possible mechanisms of how

the process of systems consolidation may change an initially episodic memory to become a part of a framework of knowledge, or schema, represented in a semantic-like manner.

1) Introduction

Memory consolidation refers to the process by which a temporary, labile memory is transformed into a more stable and long-lasting state[1,2]. The representation of this more stable memory in the brain has been referred to as the "memory trace"[3], or "memory engram"[4], 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 where, what, and when something took place, 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, encoding an experience through plasticity (the formation of new synaptic connections and the reorganization of existing ones), as proposed in Donald Hebb's[5] *synaptic plasticity theory*. This initial process lays down the circuitry required to retrieve this episode in the future. Recent technological advances have allowed researchers to label hippocampal cells that are initially activated during experience[6,7]. Combining this activity dependent cell labeling with optogenetics led to the discovery of engram cells in the hippocampus: neurons that are activated during an experience, undergo enduring physical or chemical changes, and can subsequently be selectively reactivated to produce the retrieval of that experience or inhibited to prevent retrieval[7,8]. This discovery produced the first concrete evidence of a memory engram in the brain, a concept originally described by Richard Semon in his *engram theory*[4].

However, the long-term storage of memory does not end there. Over the days, months, or even years following an experience, another type of consolidation takes place, strengthening and reorganizing 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)[9]. Although the mechanisms and networks of SCM have been studied for decades, there are still many unknowns, due in part to the conflict between theories that have been based primarily on loss of function studies. In the last several years, great strides have been made in advancing our understanding of this process, including the identification and characterization of the engrams,

engram cells and circuits associated with specific memories at recent or remote times, which has been achieved through the development of new technologies with convergent approaches, including gain and loss of function studies and observational studies[7,8].

In this review, we will highlight these most recent findings about the nature and dynamics of neocortical and subcortical memory engram cells, and their circuits, for SCM. Added to many major advances in our understanding of how episodic memories, and the networks supporting them, change and develop across time, we will focus on the circuits and physiology that seem to be driving this process and discuss their roles and functions. Finally, we will discuss some of the major questions that remain to be answered.

2) The history of systems consolidation

Temporally-graded retrograde amnesia

The original idea of SCM dates back to early psychological studies which 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, which states that with time, memories become resistant to decay, or in other words, that they need time to consolidate[10]. The first evidence for temporally graded amnesia was observed in patients, where lesions to the medial temporal lobes, including the hippocampus, produced both episodic memory-specific anterograde amnesia, and retrograde amnesia that appeared restricted to more recently formed memories, while sparing much older memories[11,12]. Further investigation with patients with damage more restricted to specifically the hippocampus[13,14], as well as experimental work with primates[15,16], 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, however SCM taking place after learning permitted the role of the hippocampus in retrieval to be time-limited[17].

Standard model of SCM and multiple trace theory

Systems consolidation has always been a contentious area in the field, and still remains so, with several theories (outlined below) purporting differing roles of the hippocampus in long-term memory. At their core, the most popular of these theories appear to agree on the most widely accepted view of hippocampal encoding, *indexing theory*, which states that the hippocampus forms an *index* of the cortical activity that was present during the actual experiencing of an

event[18] and that the *contents* of component memories are actually stored in the distributed cortical networks in which this activity took place[19]. In this concept, the 'memory trace' in the hippocampus is a representation of the patterns of neocortical activity that encode the content of an experience, and therefore retrieval of the memory involves the reactivation of these hippocampal engram cells projecting to the neocortical component engram cells 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, rodents etc., led to several theories on the formation of memory in the brain[9,20]. The most commonly accepted at the time proposed that memories undergo a process of consolidation, wherein connections between 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[21,22]. This is known as the standard model of consolidation[9].

However, patient and animal studies have often produced conflicting reports on the sparing of remote memories following damage to the medial temporal lobes and hippocampus[17,23,24] (see Table 1 for some examples). 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 episodic memory that required the hippocampus for its formation[23]. According to this theory, each reactivation of a memory which represents re-experiencing of the original episode creates additional traces in the hippocampus. Therefore, the more traces 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[23]. These multiple traces provide contextual information for an episode, promoting the neocortical extraction of the abstract or overlapping features of these episodes independent of context.[25] Therefore, according to this theory, memories of abstract and semantic information, initially acquired in the context of a particular episode, are separated and stored independently of it, permitting 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[23]. The 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 depending on the memory strength and retrieval

circumstance, the dominance of one over the other can change[25,26]. One study[27] showed that immediate inhibition of hippocampal CA1 by optogenetics impairs retrieval in both recent and remote memory tests, whereas acute pharmacological hippocampal inhibition (30 min after infusion) or prolonged optogenetic inhibition prior to memory retrieval impaired only recent memory recall. These results could be explained if one assumes that memory traces co-exist in both the hippocampus and neocortex and either trace can be used for memory retrieval depending on the animal's situation and condition during recall.

mPFC as center of remote memory retrieval

The nature of the time-limited role of the hippocampus and the nature of what memories are like without a hippocampus (episodic or semantic) is still fairly uncertain. However, what does seem clear, is that with time, certain memories can be retrieved independent of the hippocampus that was once essential for their formation and that the consolidation of these memories requires both time and neocortical plasticity[28]. Regardless of the nature of the long-term memory, both the standard model and multiple trace theories suggest that cortical restructuring supports SCM and it is these changes that permit the retrieval of the memory without input from the hippocampus[21–23,25]. 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[29,30].

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[21–23]. However, a systematic mapping of the brain regions involved in the retrieval of recent or remote memory in mice by using (14C)2-deoxyglucose to measure regional levels of glucose metabolism revealed that specific remote-memory centers may in fact exist[31]. This study identified several regions (the frontal cortex, the temporal cortex, and the ACC) that unexpectedly showed greater activity during retrieval 25-days after learning compared to 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)[32–34] (Figure 1).

Early discussions of the relationship between mPFC and memory had been typically linked to its role in working memory maintenance and also in the formation of memory[35,36]. 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, but severe memory impairments were observed when the mPFC was lesioned several weeks after learning in rats[37]. Studies to follow strengthened this notion, revealing greater immediate early gene activity within the mPFC during remote memory retrieval than during recent memory retrieval[38,39]. Furthermore, targeted reversible inactivation of mPFC sub-regions revealed the necessity of these regions in the retrieval of remote but not recent memory[38–40] in rodents in contextual fear conditioning[27,38], the Morris Water Maze[41,42], trace fear conditioning[43], trace eyeblink conditioning[40] and paired-associate memory[44]. While many of these studies revealed the importance of the mPFC in remote memory recall, other cortical areas like the orbitofrontal, auditory and retrosplenial cortex, are also important for other types of remote memories. Furthermore, it was not clear 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.

New approach to find memory engrams for systems consolidation

In 1904, the German Scientist Richard Semon, proposed the physical theory of human memory. He coined the term ‘‘engram’’ for the physical substrate of memory, which he defined as ‘‘the enduring though primarily latent modification in the irritable substance produced by a stimulus’’[4]. The term engram is roughly equivalent to the commonly used *memory trace*, and can be defined as the enduring physical/chemical changes that occur in the neural network (criterion 1) as a result of activation of neuronal subpopulations by episodic stimuli (criterion 2) and can be subsequently re-activated by stimuli that were part of the original set of encoded stimuli, resulting in the recall of the original memory (criterion 3)[3,7,8]. Recent technological advances have made it possible to identity engram cells for a specific memory and examine the effect of their activation or inactivation on mnemonic behaviors[45–51] (Figure 2). These technologies include the utilization of immediate early genes (IEGs), cell-type restricted transgenic mice, optogenetics, pharmacogenetics, electrophysiological recording and optical imaging[52]. Based on the above three sets of criteria, engram cells have been shown to be present in the hippocampus and in many other brain areas[52].

Previous findings and insights into the mechanisms of SCM were generated primarily 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 inform the correlation of activity to behavior. But by the definition, the latter type of studies falls short of identifying a causal link of the observed activity to behavior. In this review, we will discuss current understanding of the neural mechanisms for SCM from the points of view of the specific engrams of episodic memory cells and their circuits that play an obligatory role in SCM. These findings form the basis of an Engram Maturation Model of systems consolidation.

3) Neocortical memory generation

Early tagging hypothesis

While several studies had suggested a role of the mPFC in remote memory retrieval (see above), it was uncertain whether the mPFC or other frontal cortical areas encode an episode rapidly during learning, as the hippocampus does. The prevalent models of SCM posit that episodic memories are initially formed within the medial temporal lobes with rapid synaptic plasticity in these areas, and the functional retrieval of the memory slowly shifts to neocortical networks during the post-encoding period. 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[53]. Injecting a competitive AMPA/kinase receptor antagonist (CNQX) or an NMDA receptor antagonist (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 7 days after[53]. These results indicate that activation and plasticity in the neocortex during learning is necessary for remote memory recall, but not for recent memory recall. Could an involvement of putative neocortical engrams in recent memory recall simply be masked by the active hippocampal engram at this time, or, although formed during learning are they in an inactive state and require conversion to an active form for remote memory recall? The authors of REF[53] hypothesized the latter possibility, and introduced the concept that some OFC cells may be ‘tagged’ during training and become part of the future engram.

Inputs to mPFC Crucial During Learning for Future Formation of Remote Memory

Recently[54], the tagging phenomenon in SCM was investigated by combining neural circuit mapping with the retrograde tracer CTB, and axonal projection-specific optogenetic manipulations. 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 HPC (dHPC) cells,

into several neocortical areas, including the mPFC and the basolateral amygdala (BLA) were identified[54,55]. When the input from MEC-Va to the mPFC was optogenetically inhibited specifically during contextual fear conditioning (CFC), a selective impairment in remote memory retrieval (post-training test days 15 and 22) was observed without deficits in recent memory retrieval (post-training test days 2 and 8). In contrast, optogenetic inhibition of the axonal projections to other cortical areas, like the caudal anterior cingulate cortex (cACC), and retrosplenial cortex (RSC), specifically during CFC training impaired neither remote nor recent recall. Furthermore, optogenetic inhibition of MEC-Va axonal projections in these cortical areas, including the mPFC, specifically during recent or remote 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 also selectively impaired the retrieval of the remote fear memory, but not the retrieval of the recent memory. Using a gain of function experiment, another study[56] generated an artificial contextual fear remote memory in mice by the simultaneous optogenetic stimulation of blue light-sensitive channel rhodopsin (ChR2) expressing memory engram cells in both the dHPC and BLA. 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 (Figure 2).

Generation of "Silent" Engram Cells in the mPFC During Learning

These findings suggested that engram cells are already 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[57,58]. The circuit study described above was extended[54] to address this issue further by applying engram identification and manipulation technologies to characterize mPFC engrams. The authors of REF[54] and another group[59] both examined immediate early gene expression in the neocortex during CFC and found that there was a subset of mPFC neurons that strongly expressed the c-Fos protein during learning. 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 c-Fos expression within the mPFC (Figure 2).

To functionally characterize the c-Fos-expressing neurons that form the memory engram in the mPFC, Kitamura et al.[54] expressed channelrhodopsin (ChR2)[60] specifically in these cells and examined the effect of activating this sub-population of mPFC cells in a neutral context. Blue light-pulsed stimulation of ChR2-expressing cells in the mPFC induced freezing in a neutral context at both recent and remote times (Figure 2). Thus, it appeared mPFC engram cells are in

fact generated during initial training, as their optogenetic reactivation could induce memory retrieval one-day after training until at least two-weeks after learning. Further evidence that these cells met the defined criteria of engram cells was provided by the confirmation that the c-Fos expressed cells in the mPFC 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[54]. However, these mPFC engram cells were not reactivated by natural recall cues (as assessed by the cue-induced expression of endogenous c-Fos) one 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 by optogenetic stimulation, but not by natural recall cues one day after training. The engrams in this state were referred to as “silent” engrams. The silent nature of the engram may provide the cellular basis for the tagging phenomenon, as tagging in this case refers to the creation of a population of neurons as the source of functionally mature engram cells. Engram cells in a silent state were previously observed in a mouse model of retrograde amnesia[51], in models of early Alzheimer’s disease[61], and in social memory[62]. Thus, the silent state of engrams is not just a phenomenon unique to early mPFC engrams, rather a more general phenomenon of memory engram cells. Furthermore, silent engrams cannot be merely equated to "tagging", because the conversion between silent and mature engram cells is bidirectional, whereas tagging refers only to unidirectional relationships from silent to mature 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 to the ‘active engram’ counterparts. In the case of silent engram cells present in retrograde amnesia, it has been shown that these cells have weaker synaptic connections with downstream engram cells compared to the active engram cells in non-amnesic mice[51]. From the evidence, we hypothesize that silent engram cells may have weaker synaptic connections between memory engrams that are activated during learning, and are therefore more difficult to reactivate.

We expect that there are a diverse set of underlying molecular and cellular features that define silent and active memory engram cells. One common difference that is shared amongst the aforementioned cases is a paucity of dendritic spines in the silent engram cells compared to their active counterparts[51,54,61]. 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 to active state, and vice versa. Specific signaling cascades involved in the regulation of chromatin remodeling have been

observed to occur within the neocortex during learning[53]. For example, increases in histone H3 acetylation in the OFC was observed after learning, and interference with this cascade before learning impaired remote memory retrieval[53]. DNA methylation, a transcriptional repression mechanism, has also been shown to be a crucial step in remote cortical memory formation: it was revealed that persistent, gene-specific cortical hypermethylation was induced in the mPFC following CFC that persisted for at least 30 days following learning[63]. Pharmacologic inhibition of methylation in the mPFC at this remote time-point also disrupted memory retrieval, suggesting that DNA methylation that occurs during learning may serve to preserve long-term memories[63]. 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[64]. 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[64]. An examination of such changes between engram and non-engram cell populations, and between silent and active engram cells, would greatly inform these mechanisms.

4) Slow maturation and de-maturation of memory engrams during SCM

Physiology of mPFC Engram Cell Maturation

Discovery of silent engram cells in the mPFC that are essential for remote memory recall suggests the gradual maturation of these silent engram cells. Before silent engram cells were identified in mPFC, a study[65] 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 two paired stimuli (Figure 3a-b). These new patterns developed over a period of several weeks after learning, with and without continued conditioning trials: this is 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 (Figure 3a,b)[65]. 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[54] examined single cell activity longitudinally by monitoring transient calcium (Ca2+) events in the same putative mPFC engram cells in vivo during and after CFC over a two week period. Using a miniaturized, head-mounted fluorescence microscope via a micro–gradient-index lens implantation[66] into the mPFC, it was possible to investigate changes in neural activity within individual cells across time that may represent the process of memory engram cell maturation in the mPFC. On day 1, mice were first exposed to context B, followed by CFC in context A. Mice were then re-exposed to both contexts in the same order 1 and 14 days later. mPFC cells did not appear to discriminate between the two contexts on day 1 before footshock presentation. However, after footshock presentation, about 11% of cells showed a significant increase in Ca2+ transients [shock-responding (SR) cells]. The remaining ~89% of PFC cells did not respond to the shocks [shock non-responding (SNR) cells]. During recall, the transient Ca2+ activity of SR cells in context A was significantly higher compared with that in context B on day 15, but not on days 1 or 2 (Figure 3c-d). SR cells are likely to be the mPFC memory engram cells, given that the generation of mPFC engram cells requires both context exposure and foot-shocks during learning. Furthermore, SR cells were silent in response to the conditioned stimuli during recent recall and active during remote recall (Figure 3c, d). However, it would be desirable to ascertain with engram labelling technology that SR cells are indeed mPFC engram cells.

Crucial role of Hippocampal Engram Cells for the Maturation of mPFC Engrams

In most models of SCM, the functional retrieval of episodic memory can be gradually transferred from the hippocampus to the neocortex through the strengthening of cortio-cortical connections. This postulated transfer has now been shown to be due to the slow functional maturation of mPFC engram cells formed rapidly in a silent state during learning[54]. In addition, it has been shown that this maturation of the mPFC engram cells requires post-learning input from the hippocampal engram cells; this suggests that the need of an intact hippocampus for remote memory is for the maturation of the mPFC's silent engram cells. The need of an intact hippocampus for remote memory at the level of circuits and oscillatory activity was investigated in an earlier study[67] in which tetanus toxin (TeTX) was expressed in hippocampal CA3 cells using a triple transgenic mouse line in which CA3 output was blocked only 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 to the extent that blocking the medial entorhinal cortex layer III to CA1 circuit

had no effect on ripples during sleep, or on remote memory[68,69]. It has been shown that sharp-wave ripple-induced replay of place cell activity in CA1 contributes to the consolidation of spatial memory within the HPC[70,71]. A similar mechanism that operates repeatedly over a longer distance from the HPC to mPFC, taking days or weeks, may promote the slow maturation of the silent mPFC engram cells. For example, the coupling of cortical spindles to hippocampal sharp-wave activity has been shown to be important for memory consolidation[72,73].

To examine the contribution of hippocampal engram cells to the maturation of mPFC engram cells, a more recent study[54] investigated the effect of chronic inhibition of the output of hippocampal dentate gyrus (DG) engram cells using selective TeTX expression in these cells starting one day after training[54]. TeTX expression in DG engram cells inhibited the reactivation of mPFC engram cells during exposure to the conditioned context 12 days after CFC, a cellular denominator 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 calcium imaging revealed that TeTX expression in HPC engram cells after CFC blocked the time-dependent increase in the context-specific $Ca2^{+}$ transients observed in SR cells in the mPFC. These results together show that hippocampal activity, and specifically the activity of hippocampal memory engram cells, following the learning period (SCM period), is necessary for the gradual maturation of mPFC engram cells. There are also other network changes taking place in the brain over this consolidation process[74], and we do not rule out the possibility that parallel changes in other brain networks (e.g. thalamic networks) may also support this cortical maturation process.

De-maturation (or Silencing) of Hippocampal Engram Cells with Time

As we described above, it is still debated whether the hippocampus would always be required for the retrieval of a memory[28] based on lesion studies in rodents and human patients. However, loss of function experiments cannot provide information about the existence of a memory engram, so it is important to also analyze the effects of a gain of function by stimulating the hippocampal memory engram at the remote time point. Examining the post-consolidation fate of HPC engram cells, it was found that HPC dentate gyrus engram cells were not reactivated by natural cues during retrieval on day 15 (remote)[54,75] and their spine density was significantly reduced compared to day 5 (recent)[54], however their optogenetic activation was still able to induce freezing behavior[54]. Thus, at 2 weeks after learning, hippocampal engram cells persist in a silent state. Although it was not determined how long after encoding these silent hippocampal

engram cells last beyond 15 days, we speculate that the hippocampal engram eventually may lose the original memory information[9,21,22]. It is also possible these hippocampal engrams remain accessible long-term. It has been demonstrated that the presentation of a reminder cue, for example briefly re-exposing the animal to the conditioned context, one-day before the retrieval test, can reinstate hippocampal dependency at remote time-points[76,77]. This reminder also has the effect of regaining the memories context specificity, something that is typically lost in very remote contextual memories[77–79]. Therefore, 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 the transformation theory[25]. This would need to be demonstrated experimentally and can be tested with available tools. The issue of how long silent hippocampal engram cells last would be addressed with long-lasting labeling of hippocampal engram cells for months[49,50,80]

We currently do not know how hippocampal engram cells become silent. A possible mechanism for de-maturation/silencing of hippocampal engram cells could be circuit reorganization based on the erasure of old connections and the creation of new connections by the addition of newborn neurons[81–83] in the perforant path-dentate gyrus pathway and dentate gyrus-CA3 pathway[84]. This integration of newborn neurons into the hippocampal circuits from entorhinal to dentate gyrus-CA3 would disrupt existing synaptic connections, creating competition during their development and the process of new wiring, and eventually might cause the state changes from active to silent engrams due to decreased synaptic connections between memory engram cells. Another circuit mechanism of de-maturation of hippocampal engram cells could be top-down PFC control of hippocampal function to inhibit hippocampal activity[28].

Maintenance of Active BLA CFC Engram Cells Throughout SCM

The intact BLA has been shown to be necessary for the retrieval of both recent and remote fear memory[85], and the existence of active BLA memory engrams has been demonstrated in the recent time points after learning[6,86]. One recent study[54] mapped the neuronal circuits that are necessary for the formation and retrieval of BLA engram-mediated CFC memory. Input from dHPC engram cells delivered to the BLA via MEC-Va is required for the formation of BLA engrams during conditioning, and for fear memory retrieval during recent times. In contrast, at remote time points, input from mPFC engram cells to BLA engram cells is essential for fear memory retrieval. Thus, there exists a circuit switch during SCM in the route through which the retrieval input is delivered to the BLA for recent versus remote fear memory retrieval. 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 significant overlap between the BLA engram cells activated during recent and remote recall[54]. Thus, unlike the PFC and HPC engrams which undergo silent to active, and active to silent conversions, respectively, BLA engram cells seem to stay persistently active throughout SCM, although the route of input to activate them switches (Figure 5). BLA activity is essential for the valence aspect of the fear memory, and likewise we would expect a similar circuit for the consolidation of a rewarding memory given the known role of the BLA in positive valence behaviors[87]. However, for episodic memory lacking such a strong valence component, we predict a similar consolidation dependent network switch to drive the activity of whatever necessary downstream structure ultimately leads to the behavior.

(5) Is the mPFC in Remote Episodic Memory What the HPC is for Recent Episodic Memory?

Many neocortical areas are activated during episodic memory formation and retrieval. These include the caudal anterior cingulate cortex (cACC)[38,39], entorhinal cortex[75,88], perirhinal and postrhinal cortices [89] and retrosplenial cortex[39,90]. Furthermore, formation of memory engrams has been reported in several higher order structures of the sensory and association cortices[91–93]. 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[18]. The role of the mPFC in remote episodic memories may be equivalent to that of the HPC for recent memory[28]. Indeed, it has been shown[54] that, whereas inhibition of hippocampal output to mPFC (via MEC-Va terminals) during learning resulted in an impairment of remote memory retrieval, the same treatment of MEC-Va terminals in other cortical areas, such as caudal ACC and retrosplenial cortex, during learning had no effect on remote memory retrieval. Thus, among neocortical regions, the mPFC seems to have a special function which 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[74]. The similar role of the hippocampus and mPFC in recent and remote memory, respectively, can also be seen in the way the emotion-invoking component of an episode is handled. For this purpose, 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).

(6) 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, primarily from the aspect of their morphology, physiology, and function. We have placed these engram studies among previous key findings and theories in the field. This led to the proposal of The Engram Maturation Model, a revamped model of SCM in the brain.

Previously, behavioral loss of function studies of rodents in which hippocampal function is blocked by lesion and pharmacological manipulations have suggested the slow gradual formation of neocortical memory in the process of SCM[22,94]. However, an earlier study indicated that neocortical activity during learning is crucial for the formation of remote memory[53]. The nature of this requirement was revealed by recent gain-of-function studies which identified the rapid generation of 'silent' memory engram cells in the mPFC during learning[54]. Similar inactive engrams have previously been identified in the hippocampus of mice in retrograde amnesia[51], of mice models of early Alzheimer's disease[61], and of mice in which social memory could no longer be retrieved[62]. One common feature of these silent engram cells generated under various conditions is their abnormally low spine density. The silent mPFC engram cells gradually mature into active engram cells (activatable by natural recall cues) during the weeks after learning and this maturation process requires post-training input from the hippocampal engram cells[54]. Thus, we propose that SCM occurs in two major steps: the rapid generation of a silent engram in the mPFC during learning and the slow functional maturation of these engrams aided by input from hippocampal engram cells during the post-training period lasting a few weeks in rodents. This maturation process includes augmentation of spine density in the mPFC engram cells, which also requires input from the hippocampal engram cells. We assume these dynamics of memory engrams in the PFC and the HPC during systems consolidation would be also observed in other types of episodic-like memory (e.g. social transmission memory, trace eye blink conditioning).

There are many questions associated with the Engram Maturation Model of SCM. Perhaps the most burning question is how the input from hippocampal engram cells converts mPFC engrams from the silent to active state with a concomitant increase in spine density in the engram-holding cells. 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[70,71] by presumably disrupting consolidation within the hippocampus-entorhinal cortex, but its role in systems consolidation has not been tested. In the case of the

early Alzheimer disease mouse models, it has been shown that silent dentate gyrus engram cells can be converted to active ones by repeated optogenetic activation of the upstream entorhinal cortex engram cells at a high frequency (100Hz)[61]. Similarly, in retrograde amnesia mice, augmented expression of the PAK-1 kinase in CA1 engram cells restores spine density of the engram cells and converts them from a silent to an active state[95]. 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 where unused engrams see progressive loss of active synapses, or an active process in order to ensure 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[96,97].

What is the relationship between mPFC engrams for remote episodic memories and semantic memories? Early studies showed that 30 day-old cortical CFC memory loses context specificity, which the few days-old hippocampus memory retains[78,98,99]. 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[100,101], and also in the formation of schematic frameworks for episodic memories[44,102,103], which are all more semantic than episodic. However, at least 2 weeks after learning in mice, CFC memory in the mPFC is clearly as context-specific as 2 day old hippocampal CFC memory[54,78]. These results suggest the mPFC engram itself can provide episodic information[24]. Furthermore, 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 HPC to the mPFC via entorhinal cortex does not disrupt the retrieval of CFC memory from the mPFC[56]. These results challenge the concept that the hippocampus is *always* required for successful retrieval of an episodic memory, including at remote times[24]. 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 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 them for the formation of a semantic memory engram in the PFC, independent of the hippocampal engrams.

Long-term recordings have revealed that over a one-month period the activity of neurons in the mPFC gradually becomes more sensitive to the latent, relational features of a memory task, and while over a slightly different time-course information about the perceptual/physical

features of the environment is significantly weakened, it still remains[103]. Another possibility that cannot be excluded is that hippocampal structures downstream of the dentate gyrus (DG), (i.e. CA3-CA1-Subiculum) may remain involved in retrieval longer than the DG[27], and such contextual information could be provided to the mPFC at these remote time-points from the hippocampus[104], despite the DG engram being silent.

One important question associated with cortical memory would be to address whether there are distinct populations of mPFC cells for remote episodic and semantic memory and whether these cells interact, or if an episodic to semantic conversion takes place within single 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.

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Highlighted References

6. Reijmers, L. G., Perkins, B. L., Matsuo, N. & Mayford, M. Localization of a Stable Neural Correlate of Associative Memory. Science 317, 1230–1233 (2007). **This study developed the cfos-tta transgenic mouse line allowing for the long-term tagging of neurons active during experience, setting up for the ability to manipulate these neurons in the future.**

18. Teyler, T. J. & DiScenna, P. The hippocampal memory indexing theory. Behav. Neurosci. 100, 147–154 (1986). **This paper proposed that the role of the hippocampus in learning and memory is to act as an index of the cortical activity present during experience.**

28. Frankland, P. W. & Bontempi, B. The organization of recent and remote memories. Nat. Rev. Neurosci. 6, 119–130 (2005). **This excellent review on systems consolidation written and published during the pre-engram discovery era highlighted the importance of the prefrontal cortex for remote memory and outlines several hypotheses still to be tested, for example top-down inhibition of the hippocampus by the mPFC during remote memory.**

44. Wang, S.-H., Tse, D. & Morris, R. G. M. Anterior cingulate cortex in schema assimilation and expression. Learn. Mem. 19, 315–318 (2012). **This study built off a previous paper from the same lab that created a task to study schema memory and identified a time-limited role of the hippocampus in schema learning. Here they identified the prefrontal cortex as an important node in schema memory and schema learning.**

45. Han, J.-H. *et al.* Selective erasure of a fear memory. *Science* **323,** 1492–1496 (2009). **Using a loss-of-function approach, this study demonstrated the existence of engram cells; genetic ablation of amygdala cells with high excitability resulted in the specific loss of a fear memory.**

47. Liu, X. et al. Optogenetic stimulation of a hippocampal engram activates fear memory recall. Nature 484, 381–385 (2012). **This seminal study was the first to identify engram cells for a specific memory using a gain-of-function approach; optogenetic re-activation of hippocampal cells activated by learning resulted in recall of the specific memory.**

51. Ryan, T. J., Roy, D. S., Pignatelli, M., Arons, A. & Tonegawa, S. Engram cells retain memory under retrograde amnesia. Science 348, 1007–1013 (2015). **This study demonstrated enduring physical changes specifically in engram cells following learning, and was the first to demonstrate that post-encoding protein synthesis was dispensable for memory storage. This paper also provided the first evidence of the existence silent engram cells.**

53. Lesburgueres, E. et al. Early Tagging of Cortical Networks Is Required for the Formation of Enduring Associative Memory. Science 331, 924–928 (2011). **This study inhibited plasticity in the prefrontal cortex during learning that prevented the formation of remote memory, showing that prefrontal cortex activity during learning is crucial for remote memory.**

54. Kitamura, T. et al. Engrams and circuits crucial for systems consolidation of a memory. Science 356, 73–78 (2017). **This study discovered that silent engrams are formed in the prefrontal cortex during learning and mature during systems consolidation with aid from hippocampal engram cells; while active engram cells formed during training de-mature to silent engram cells during the systems consolidation.**

64. Zovkic, I. B., Paulukaitis, B. S., Day, J. J., Etikala, D. M. & Sweatt, J. D. Histone H2A.Z subunit exchange controls consolidation of recent and remote memory. Nature 515, 582–586 (2014). **This study discovered epigenetic changes during learning in the hippocampus and prefrontal cortex important for the formation of recent and remote memory.**

65. Takehara-Nishiuchi, K. & McNaughton, B. L. Spontaneous Changes of Neocortical Code for Associative Memory During Consolidation. Science 322, 960–963 (2008). **This study recorded from prefrontal cortex neurons across learning and consolidation and uncovered physiological correlates of these neurons during systems consolidation.**

Key Points

- Over the days, months, or even years following an experience, the brain's networks strengthen and reorganize into a long-term, more stable state at the systems level, referred to as systems consolidation of memory (SCM). The mechanisms and networks of SCM have been studied for decades, however in the last several years great strides have been made in advancing our understanding of this process, most recently aided by identifying and characterizing the engrams and engram cells for a specific memory.

- The term engram can be defined as (i) enduring physical/chemical changes that occur in the neural network as a result of (ii) activation of neuronal subpopulations by episodic stimuli, and (iii) a subsequent re-activation by stimuli that were part of the original set of encoded stimuli, resulting in the recall of the original memory. Recent technological advances (including the utilization of immediate early genes, cell-type restricted transgenic mice, optogenetics, pharmacogenetics, electrophysiological recording and optical imaging) have made it possible to identity and manipulate engram cells for a specific memory leading to the discovery of engram cells in the hippocampus (HPC), amygdala, prefrontal cortex (PFC), and many other brain areas.

- Many brain regions are activated during episodic memory formation and retrieval, yet among them, the hippocampus seems to have a special function which may include the integration of individual component engrams stored in various other cortical areas. With systems consolidation, this function appears to shift to the PFC, and this role of the PFC in remote episodic memories therefore may be equivalent to the role played by the HPC for recent memory.

- Interestingly, engram cells in the PFC are generated quickly on the day of training, and the memory is retrievable from these cells by optogenetic stimulation, but not by natural recall cues at recent time points after training. Thus, the engram exists in the PFC in a silent state, and like silent engrams observed in the hippocampus in retrograde amnesia, dendritic spine density is reduced in these engram cells compared to their active counterparts. Genetic changes that take place during learning that are essential for the formation of long-term cortical memory may be involved in the conversion of engram cells from a silent to active state.

- Hippocampal activity, specifically the activity of hippocampal memory engram cells, during the post-learning period (i.e. SCM period), is necessary for the gradual maturation of PFC engram cells and thereby for the formation of remote memory. In contrast, over this same period, hippocampal engram cells spine density decreases, and they are no longer activated by natural recall cues, but persist in a silent state retaining functional connectivity and memory information.

- The 2 week-old CFC memory stored in the PFC is context-specific, while at this time point, the HPC memory engram cells are silent and cannot provide contextually rich information to the PFC. Furthermore, the retrieval of episodic CFC memory from the PFC is independent of input from the HPC. These results suggest that the PFC engram itself can provide episodic information for the remote memory and challenges the theory that the HPC is necessary for retrieval of episodic memory at remote times.

- Unlike the PFC and HPC engrams which change from silent to active, and active to silent states, BLA engram cells are persistently active throughout SCM, although the route of input to activate them switches from the HPC to the mPFC.

Display Item Legends

Table 1. Examples of effect of hippocampal disruption prior to memory retrieval in different types of memories

Task	Recent Memory Retrieval	Remote Memory Retrieval	Studies
Contextual Fear Conditioning	Impaired	Intact	Kim & Fanselow, 1992[105]; Anagnostaras et al., 1999[106]; Winocur et al., 2013[107]
Contextual Fear Conditioning	Impaired (by acute opto) Impaired (prolonged opto)	Impaired (by acute opto) Intact (by prolonged opto)	Goshen et al., 2013[27]
Contextual Fear Conditioning	Impaired	Impaired	Sutherland et al., 2008[108]
Context Discrimination	Impaired	Intact	Wang et al., 2009[98]
Spatial 5-Arm Maze	Impaired	Intact	Maviel et al., 2004[39]
Trace Eyeblink Conditioning	Impaired	Intact	Kim et al., 1995[109]; Takehara et al., 2003[37]
Inhibitory Avoidance	Impaired	Intact	Quillfeldt et al., 1996[110]
Trace Fear Conditioning	Impaired	Intact	Quinn et al., 2008[43]
Socially Acquired Food Preference	Impaired	Intact	Winocur et al., 1990[111]
Paired-Associate Memory	Impaired	Intact	Tse et al., 2007[112]
Morris Water Maze	Impaired	Impaired	Broadbent et al., 2006[113]; Clark et al., 2005[114]; Mumby et al., 1999[115]; Sutherland et al., 2001[116]; Winocur et al., 2013[103]

Figure 1: Brain regions and circuits implicated for systems consolidation of contextual fear memory

a, Brain regions shown to be involved in systems consolidation, indicated on a schematic illustration of the mouse brain (right), and their counterparts in the human brain (left). These include the orbitofrontal cortex (OFC, blue), prelimbic cortex (PrL, Mouse-yellow), dorsal anterior cingulate cortex (Mouse-dACC, green), cingulate cortex (human-turquoise), hippocampus (HPC, purple), amygdala (red) and entorhinal cortex (EC, Mouse-brown). The PFC has several major subdivisions, of which the two important for systems consolidation are the medial PFC (mPFC, green and red) and OFC (blue). The mPFC most commonly refers to the most anterior aspects of the cingulate cortex, containing the dorsal anterior cingulate dACC, prelimbic (PrL) and infralimbic cortices. The OFC contains, medial, lateral, and ventral components.

Figure 2: Tools to identify memory engrams in the brain

a, Activity-dependent cell labeling with the c-fos tTA system. In this approach, a c-fos-tTA transgenic mouse, which expresses the tetracycline transactivator (tTA) under the control of a promoter (the immediate early gene c-fos)[6], was injected with AAV-TRE (tetracycline-responsive element)-ChR2-eYFP (enhanced yellow fluorescent protein) or AAV-TRE-ArchT-eYFP[47]. The presence of doxycycline (DOX) inhibits c-fos-promoter driven tTA from binding to its target TRE site, which in turn prevents it from driving ChR2–eYFP expression. Mice are implanted with an optical fibre targeting the hippocampus. When DOX is removed from the animal's diet (DOX (-)), training and the resultant c-fos activation induces the expression of tTA, which binds to TRE and drives the expression of ChR2–eYFP (or ArchT-eYFP), labelling a subpopulation of activated cells (green, Fig 1c) in the hippocampus. **b**, During training for contextual fear conditioning, a mouse is placed in context A (Cxt A) and given a footshock. The activated neuronal ensembles in the hippocampus during conditioning, in the absence of Doxycycline, will express the genes of interest, for example ChR2 or ArchT, and express the accompanied fluorescent marker, e.g. eYFP (flashing green). When returned to context A, the mouse displays a conditioned fear response and the tagged engram cells are reactivated by natural recall cues. When places in a neutral context (Cxt B) the mouse does not display freezing behaviour and the tagged engram cells are not activated. **c**, Artificially activating hippocampal context A engram cells tagged with ChR2 with 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 in A produces deficits in memory retrieval evidenced by a lack of freezing behaviour.

Figure 3: Physiological maturation of neocortical representation

a, Context-dependent acquisition and maturation of memory associations with time in trace eyeblink conditioning. Rats were exposed to an environment in which an auditory conditioned stimulus (CS) was paired with an unconditioned eyelid stimulation (US, labelled 'shock')(Paired), and a separate environment in which the CS and US were unpaired (Pseudo). Rats learn to associate the CS with the US in the Paired, but not the Pseudo condition. **b**, mPFC cells began to exhibit sustained activity during the interval between the presentation of the two paired stimuli (CS and US) in the period up to 6weeks after learning, whereas mPFC cells did not in the period of learning shown with standardized firing rates (Z-score)[65]. **c**, Experiment for longitudinal calcium imaging from the mouse mPFC during systems consolidation of a contextual fear memory over 2 weeks. On day 1, mice were first exposed to context B, followed by contextual

fear conditioning (association between context and foot-shock) in context A. Mice were then re-exposed to both contexts in the same order on days 2 and 15. **d**, Longitudinal calcium imaging from mPFC cells with GCaMP6. About 11% of mPFC cells (known as 'shock cells', red) showed a significant increase in Ca2+ transients during conditioning. The remaining ~89% ('non-shock cells', black) of mPFC cells did not respond to the shocks. During recall, the transient Ca2+ activity of the shock cells in context A was significantly higher compared with that in context B on day 15, but not on days 1 or 2, whereas the frequency of Ca2+ transient events in non-shock cells remained constant, irrespective of contexts and days[54].

Figure 4: Silent and active memory engrams

a, mPFC engram cells (green) were rapidly formed during training on day 1. However, they were not retrievable with natural recall cues and displayed low spine density. The immature mPFC engram cells functionally, structurally, and physiologically matured during the subsequent few weeks and were active during retrieval and displayed increased spine density during the remote time-point. On the other hand, hippocampal engram cells (purple, bottom) were rapidly formed during day 1 training, at which point they were also functionally, structurally, and physiologically mature. They gradually become silent with time accompanied by a reduction of dendritic spines. **b**, Active engrams typically show dense spines and are reactivated by natural cues (left, yellow). Silent engrams on the other hand contain more sparse spine density and are not reactivated by natural cues, however, if tagged with ChR2, they can be artificially reactivated with blue laser light and can produce memory retrieval[51,54,61,62].

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Figure 5: Engrams and their circuits for systems consolidation of memory

New model for the mechanisms of systems consolidation of memory[54]. mPFC (green) memory engram cells for contextual fear conditioning are rapidly formed during Day-1 training by inputs from both MEC-Va and BLA, but they are not retrievable with natural recall cues (red open circles). The silent mPFC engram cells functionally, structurally, and physiologically become active during the subsequent few weeks (red opaque circle) and this process requires inputs from HPC engram cells presumably through MEC-Va. In contrast to the formation on Day-1, retrieval of the active mPFC engram (red filled circles) at a remote time does not require MEC-Va input. Functional HPC (purple) engram cells (red filled circle) formed during training become silent with time; they are not retrieved on Day-14 by natural recall cues but are still re-activatable optogenetically for recall (red open circles). On the other hand, fear memory BLA (red) engrams formed during training are functionally maintained even after the consolidation-mediated switch in recall circuits (red filled circles).