Finding the engram

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Abstract | Many attempts have been made to localize the physical trace of a memory, or engram, in the brain. However, until recently, engrams have remained largely elusive. In this Review, we develop four defining criteria that enable us to critically assess the recent progress that has been made towards finding the engram. Recent 'capture' studies use novel approaches to tag populations of neurons that are active during memory encoding, thereby allowing these engram-associated neurons to be manipulated at later times. We propose that findings from these capture studies represent considerable progress in allowing us to observe, erase and express the engram.

Neuronal ensembles
Collections of neurons that show coordinated firing activity, equivalent to the cell assembly defined by Hebb.

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Memories are thought to be encoded as enduring physical changes in the brain, or engrams[1,2]. Most neuroscientists agree that the formation of an engram involves strengthening of synaptic connections between populations of neurons (neuronal ensembles). However, characterizing the precise nature and location of engrams has been challenging. Lashley was among the first to attempt to localize engrams using an empirical approach[3,4]. Famously, his search proved unsuccessful, and his conclusion — that the engram is elusive — became widely influential[5–8]. Today, we appreciate that this elusivity was due, at least in part, to the sparse, widely distributed and dynamic nature of memory representations in the brain, making engrams challenging to identify using traditional scientific methods.

However, new tools have recently been developed that provide unprecedented opportunities to visualize and manipulate specific brain regions and cell populations. In particular, molecular and transgenic methods in rodents now allow neurons that were active at the time of learning (engram encoding) to be captured and tagged for later manipulation. In this Review, we develop four criteria for defining the engram and use them to evaluate whether recent studies have indeed uncovered the engram. We group the evidence on the basis of the type of experimental approach used, and discuss the advantages and limitations of each approach. We propose that findings from recent experiments have gone a considerable way towards satisfying these engram-defining criteria and, therefore, towards finding the engram.

Defining the engram

Memory is the capacity of an organism to acquire, store and recover information based on experience. The term engram was introduced by Semon more than 100 years ago[1,2] (BOX 1) and refers to the physical substrate of memory in the brain. Semon suggested that an engram has four defining characteristics[9] (FIG. 1). First, an engram is a persistent change in the brain that results from a specific experience or event. Second, an engram has the potential for ecphory; that is, an engram may be expressed behaviourally through interactions with retrieval cues, which could be sensory input, ongoing behaviour or voluntary goals. Third, the content of an engram reflects what transpired at encoding and predicts what can be recovered during subsequent retrieval. Fourth, an engram may exist in a dormant state between the two active processes of encoding and retrieval. That is, an engram exists beyond the operations and processes required to form and recover it. Therefore, an engram is not yet a memory but provides the necessary physical conditions for a memory to emerge[10]. Here, we use these four defining criteria — persistence, ecphory, content and dormancy — to evaluate whether recent studies have found the engram.

The prevailing view is that the formation of an engram involves strengthening of synaptic connections between populations of neurons that are active during encoding, leading to the formation of a neuronal ensemble[11–14]. This increase in synaptic strength between neurons increases the likelihood that the same spatiotemporal pattern of neural activity that occurred during encoding will be recreated at a later time (retrieval). Engrams need not be confined to a single brain region, but rather may be composed of widely distributed networks of neuronal ensembles. Nonetheless, the building blocks of the engram may be studied at smaller scales. For example, experience-induced epigenetic changes at the level of the nucleus, increases in synaptic strength and changes in neuronal excitability have been probed to investigate the cellular and subcellular components that may contribute to the formation and maintenance of an engram. Recent progress in finding the engram

Box 1 | **Origin of the term engram**

The term engram was introduced by Semon (1859–1918), a German scientist who wrote two books on human memory[9]. Semon made several important, albeit often overlooked, contributions to the understanding of how memories are formed, stored and retrieved. To avoid confusion with the vernacular, Semon called for precise scientific nomenclature in describing memory representations and processes. Specifically, he introduced two terms, engram and ecphory, that remain influential today. He defined an engram as "…the enduring though primarily latent modifications in the irritable substance produced by a stimulus…" (REF. 1). Ecphory was defined by Semon as the process which "…awakens the mnemic trace or engram out of its latent state into one of manifested activity…" (REF. 1). As such, the engram can be considered similar to a memory trace (and engraphy is the process used to form the engram), whereas ecphory is similar to memory retrieval. Although originally introduced and defined by Semon, it is Lashley (1890–1959) who popularized the term engram. In a series of related experiments that spanned over three decades, Lashley attempted to "find the locus of specific memory traces" (REF. 4). Lashley cited Semon on two occasions[143,144] but failed to credit Semon for introducing the term engram in his famous treatise *The Search for the Engram*.

has been fuelled in particular by advances in experimental techniques that allow investigation at the level of neuronal ensembles.

It is also important to note that the engram is not static. Following encoding, consolidation processes may alter the physical and chemical organization of engrams, which may alter an engram in terms of strength and quality[7]. Although consolidation implies a process of fixation or stabilization, engrams can be dynamic[15]. For instance, memory retrieval may transiently destabilize a previously consolidated engram and initiate a new consolidation cycle (that is, reconsolidation; see BOX 2) that can lead to further changes in the engram[16,17] (FIG. 1). Although the engram is a moving target over time, this characteristic does not preclude tractability and success in capturing the engram at any given moment in time.

Finally, different types of memory may be supported by engrams in distinct collections of brain regions. Here, we consider studies examining a range of memory types, in multiple species, but emphasize studies in rodents with a primary focus on memory functions that are mediated by the hippocampus and amygdala (that is, the medial temporal lobe). This is not to suggest that these are the only studies that have sought the engram. For an overview of other engram literature, see BOX 3.

Observing the engram

Observational studies have been designed to examine experience-induced changes in neural substrates (at the level of molecules, synapses, neurons, neuronal ensembles, and/or brain circuits and networks) that may reveal the location of the engram. Ramón y Cajal was among the first to articulate specific ideas about how and where experience-induced changes might be observed at the level of the neuron. Although he believed that the number of neurons was fixed after development, he maintained that the connections between neurons were modifiable by experience, a process he termed 'cerebral gymnastics' (REF. 18). Furthermore, Ramón y Cajal correctly hypothesized that the protrusions he observed in neurons (dendritic spines) represented the connection points with axon terminals and proposed that experience-induced modifications would occur at these points, which were later identified as synapses[19]. Evidence for the idea that experience changes neuronal morphology followed swiftly, as studies reported changes in dendritic spine number and shape following several interventions, including electrical brain stimulation[20,21]. Ramón y Cajal's influential ideas that experience sculpts the brain by modifying connections between neurons were subsequently championed by the Canadian psychologist Hebb[12,22]. Hebb proposed that learning strengthened the synaptic connections between neurons and thereby facilitated the formation of neuronal ensembles (or, as Hebb called them, 'cell assemblies'). These neuronal ensembles are thought to comprise collections of neurons that fire together at the time of learning and again at the time of memory retrieval. As such, they were proposed as a neural substrate for the engram.

Decades later, empirical studies established that environmental enrichment[23], learning[24] and induction of long-term potentiation[25,26] alter brain structure at the level of spine morphology. Beyond changes in dendritic spines, other persistent learning-induced changes in neurons have been characterized. These include changes in DNA structure (histone modifications and DNA methylation[27]), post-translational modification of kinases (for example, protein kinase Mζ (PKMζ)[28] and α-calcium/calmodulin-dependent kinase II (αCaMKII)[29]), activation of transcription machinery[30,31], induction of immediate-early genes (IEGs) such as *Fos* and activity-regulated cytoskeleton-associated protein (*Arc*)[32–34], phosphorylation and trafficking of receptors[35,36], alterations in synaptic strength[35,37,38] and changes in neuronal excitability[39] (FIG. 2).

Two themes emerge from these findings. First, learning-induced brain changes vary in their persistence. Although some changes are fleeting (for example, phosphorylation of αCaMKII), others persist for longer periods (for example, learning-induced alterations in synaptic strength). At the extreme, experience-induced changes in DNA structure may even be transmitted to future generations[40,41]. Second, many of the learning-induced changes described above are inter-related. For example, phosphorylation of αCaMKII enhances the function of AMPA and NMDA receptors, which in turn leads to a persistent increase in synaptic strength[42,43]. This increase in synaptic strength between cells underlies the formation of neuronal ensembles[44]. Therefore, although none of these changes alone constitutes the engram, they may be necessary for engram formation and, as such, can be used to point to the location of an engram at any given time.

However, in the absence of experimental intervention, it is unclear whether any observed brain changes following learning constitute an essential component of the engram. Some post-learning changes in the brain may reflect incidental aspects of the training experience or ongoing cellular housekeeping processes that are unrelated to memory formation or consolidation[45]. Moreover, although observed experience-induced changes in the brain may be linked to encoding, they do not necessarily predict subsequent retrieval success. For instance, the induction of IEGs is tied to encoding

Consolidation
The transformation of engrams from an initially labile state (in which they are vulnerable to disruption) to a more permanent state (in which they are resistant to disruption).

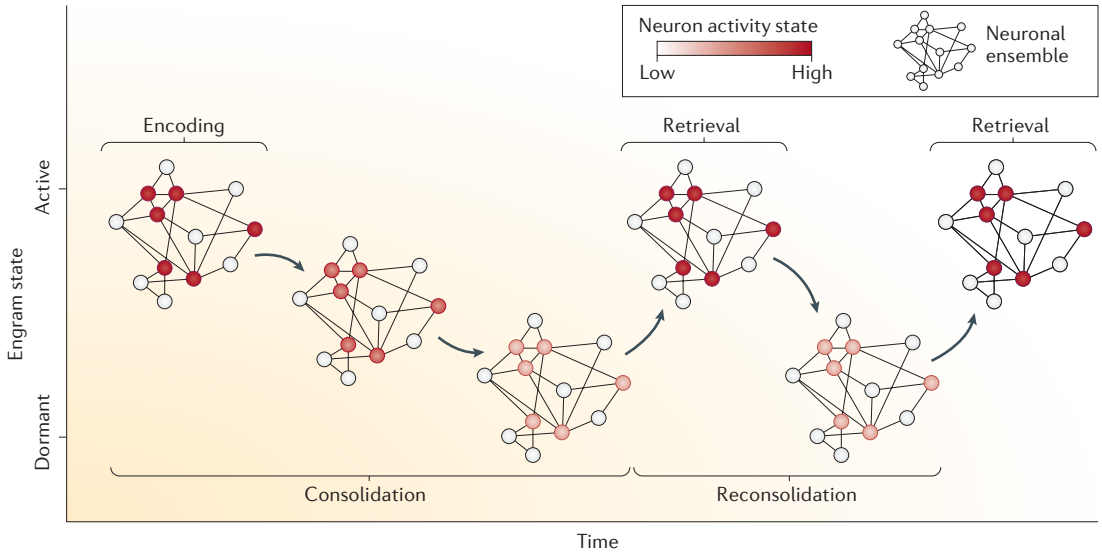


Figure 1 | **The lifetime of an engram.** The formation of an engram (encoding) involves strengthening of connections between collections of neurons (neuronal ensemble) that are active (red) during an event. Consolidation further strengthens the connections between these neurons, which increases the likelihood that the same activity pattern can be recreated at a later time, allowing for successful memory retrieval. During consolidation, the engram enters a mainly dormant state. Memory retrieval returns the engram back to an active state and transiently destabilizes this pattern of connections. The engram may be restabilized through a process of reconsolidation and re-enter a more dormant state. Therefore, an engram may exist in a dormant state between the active processes of encoding and retrieval required to form and recover the memory. In this way, an engram is not yet a memory, but provides the necessary conditions for a memory to emerge.

but has not been related directly to subsequent retrieval success. Nevertheless, a subset of observational studies convincingly show that some learning-induced brain changes reflect what transpired during encoding and also predict what will transpire during retrieval. Such observational studies can provide particularly compelling evidence with respect to the content criterion for engram identification.

Rodent multi-unit recordings and human fMRI studies. Observational approaches that address the content criterion are perhaps best exemplified by activity replay studies, which show that neuronal activity patterns that occur during a particular experience reoccur at later times[46]. Rodent studies suggest that spontaneous reactivation of event-specific neural activity occurs during brief high-frequency bursts in the hippocampus known as sharp wave–ripple events[47–49]. Activity replay may be observed while rats are actively engaged in a task, during rest periods after an experience[50] or even when the animal is sleeping and deprived of sensory input[51,52]. The strength of reactivation correlates with subsequent memory expression[53,54], and preventing replay by electrically disrupting sharp wave–ripples following learning impairs subsequent expression of that memory[55–57]. These findings indicate that replay is essential for consolidation of the engram. Further evidence suggests that replayed sequences contain information about specific prior experiences[58–62] and, moreover, that this firing sequence also predicts future behaviour[53]. For instance, in a spatial alternation task in which rodents were trained to remember past locations to guide future behaviour (that is, selection of a correct rewarded choice), sharp wave–ripple activity at the choice point was shown to predict future choices[62]. Finally, sensory cues related to the training experience that are present in the environment at later time points may act as a retrieval cue and induce replay[63–66].

Functional MRI (fMRI) studies in humans also show that neural activity patterns produced by an experience may reoccur during either subsequent sleep[67,68] or wakeful[69] periods. Regions of the hippocampus that are active during route learning have been found to be reactivated during subsequent slow-wave sleep, and the amount of replay activity has been shown to be positively correlated to successful retrieval of these routes the following day[67]. Importantly, event-specific activity patterns can be observed at times that do not overlap with encoding or retrieval and are therefore independent of external cues, active memorization or retrieval attempts. Moreover, activity patterns can be specific for discrete events. For example, in a paired-associate learning task, in which specific pairings produced unique, identifiable activity patterns, accuracy of recall for each paired-associate was predicted by its spontaneous reactivation frequency in the post-learning resting state[70] (see also REF. 71). Furthermore, patterns of replay alter functional connectivity between the medial temporal lobes and cortical structures that are involved in pertinent perceptual analyses during encoding and retrieval[72], and this connectivity is beginning to be understood at the level of neural oscillations. Neural oscillations occur simultaneously at multiple frequencies, and emerging evidence from intracranial and electroencephalogram scalp recordings suggests that

Replay
Recapitulation of experience-induced patterns of neuronal activity that occur during sleep or awake rest periods following an experience.

Sharp wave–ripple
High-frequency neural oscillations that occur in the hippocampus during periods of slow-wave sleep and behavioural immobility.

Functional connectivity
Task-specific coordination of activity between different elements (for example, neuronal ensembles) within neural systems.

Cued fear conditioning
A form of Pavlovian conditioning in which an initially neutral conditioned stimulus is paired with an aversive unconditioned stimulus. Subsequent presentation of the conditioned stimulus alone induces a conditioned fear response.

Contextual fear conditioning
A one-trial learning paradigm that is hippocampus and amygdala dependent, in which animals are placed in a specific context and administered one or a series of footshocks.

neural oscillations use a frequency- and phase-dependent coding scheme that may represent information at a high level of specificity[73].

To a large degree, multi-unit recording studies in rodents and resting-state fMRI studies in humans succeed in linking what transpired at encoding to what will transpire at retrieval; as such, they provide evidence that specifically addresses the content criterion. Moreover, as activity pattern replay occurs at times remote from the initial experience (and after other experiences have intervened), they also address the persistence criterion. However, many, if not all, of the learning-induced changes identified in these observational studies reflect different stages of ongoing consolidation of the engram. Therefore, in most observational studies (ranging from molecular to functional imaging studies), the engram is being observed in what could still be considered an active, rather than dormant, state (epigenetic changes may be an exception to this, in that they may be observed while the engram is likely to be in a dormant state[40,41]).

Observing a dormant engram presents a particular challenge. Processing of the engram — for example, during consolidation — allows for its detection by the observational methods described above. However, as the frequency of consolidation-related processes declines with time, so too does the possibility of engram detection. Indeed, replay events in rodents may be detected up to 24 hours following an experience using neurophysiological recordings[47,63,74] but, at least with current techniques, fall below detectable levels in the days after that experience. This limits opportunities for observing the engram at time points long after encoding.

Engram capture strategies. One solution to bridge this temporal divide is to permanently mark those neurons that are active during encoding, thus allowing the visualization of these neurons at time points after initial consolidation, when the engram is more likely to be in a dormant state. This temporal bridging has been achieved using neuronal capture strategies, which take advantage of the observation that IEGs (such as *Fos*, transcription factor zinc finger 268 (*Zif268*; also known as *Egr1*) and *Arc*) are induced by neural activity[75–79]. Indeed, analysis of IEG expression has been widely used to identify populations of active neurons during memory encoding[33,80]. However, IEG mRNA and protein levels return to baseline within minutes and hours, respectively, following induction.

To address this temporal limitation, transgenic mice have been engineered that, rather than using expression levels of the endogenous IEG itself to label neurons, use the IEG promoter to drive transcription of a genetically encoded label such as green fluorescent protein (GFP) or LacZ. This enables neuronal ensembles that were once active to be captured and permanently tagged[81–83] (FIG. 3). Various strategies have been used to restrict activity tagging to a particular time window. In the 'TetTag' method, tetracycline controls the capture of activated neurons (using a self-activating tTA–TetO system), and the window of activity tagging is opened by withdrawing mice from a diet containing the tetracycline derivative, doxycycline[81]. Another strategy (targeted recombination in active populations (TRAP)) uses a tamoxifen-inducible Cre recombinase ($CreER^{T2}$) system, and the window of activity tagging is opened by systemically injecting mice with tamoxifen[82,83]. These capture studies have shown that the populations of neurons that are active during training in memory tasks are also active during a recall test, suggesting that these neurons are part of the engram supporting this memory. For instance, TetTag mice were removed from a doxycycline diet (to open the window of activity tagging) before auditory cued fear conditioning such that active neurons were tagged with LacZ. Several days later, mice were tested for memory recall. Neurons active during this recall test were assessed using immunohistochemistry for endogenously expressed ZIF268. The degree of overlap observed between basolateral amygdala neuronal populations that were active during training (tagged with LacZ) and recall testing (immunostained with ZIF268) was above chance levels[81]. Similarly, subsets of neurons in the hippocampus[82,84,85] and cortex[84] that were activity-tagged during contextual fear conditioning were reactivated at above chance levels when that contextual fear memory was later recalled.

As with many fMRI studies in humans[86,87], these engram tagging studies in rodents find correspondence between neural activity during encoding and during retrieval. Whereas in fMRI studies this correspondence is observed at the level of brain regions or networks, in rodent tagging studies correspondence can be detected at the level of the neuronal ensemble. Furthermore, because tagged neurons may be observed throughout the brain in the weeks following training, these studies may also help to reveal the distributed and dynamic nature of the engram[88]. Indeed, patterns of neuronal reactivation that are induced by retrieval change over time, with reduced reactivation of tagged neurons at more remote time points in some hippocampal subfields (for examples, see REFS 82,84).

Two caveats are worth noting with respect to the rodent capture-and-tag studies. First, although the proportion of neurons that were tagged at encoding and reactivated by retrieval exceeded chance, it was nonetheless

Box 2 | **Reconsolidation and the engram**

Retrieving a memory transiently destabilizes the engram supporting that memory. Although originally described in the 1960s[145], interest in this process of reconsolidation was revived in 2000 (REF. 138). In the latter study, rats underwent auditory fear-conditioning training in which a footshock was paired with a tone. Twenty-four hours later, rats were presented with the tone in the absence of a footshock in an alternative context to retrieve the fear memory. Immediately after retrieval, rats received intra-amygdala microinjections of the protein synthesis inhibitor anisomycin. Upon subsequent presentation of the tone, the animals showed impaired auditory fear memory, suggesting that memory retrieval destabilized the engram and that protein synthesis is necessary for engram restabilization (or reconsolidation). Only the memories that are directly reactivated by this cue are sensitive to the amnestic effects of protein synthesis inhibition[146,147], and therefore memory impairments induced by blocking reconsolidation show high content specificity. However, the interventions used to disrupt reconsolidation involve systemic or brain-region-wide interventions (typically drug injections) that do not specifically target putative engram neurons. Therefore, at present, this approach lacks neural specificity and does not pinpoint the location of an engram beyond broad brain structures.

Box 3 | **Previous attempts to find the engram**

From a historical perspective, there have been many other examples of attempts to localize to engrams in addition to those described in this Review. These attempts have used several different approaches, searched in many parts of the brain and focused on many different types of behaviour. Notable, though definitely not exhaustive, examples include O'Keefe and Dostrovsky[96] who used electrophysiological recordings to identify hippocampal cells that stably respond to specific environmental locations and therefore provide the basis not only for navigating, but also for recognizing and remembering, previously encountered environments[148]. Using similar techniques, other researchers studied how neuronal activity changes during Pavlovian conditioning, in which initially neutral discrete stimuli (for example, a tone; the conditioned stimuli) acquire motivational salience by virtue of being paired with appetitive or aversive stimuli (the unconditioned stimuli) (for example, see REFS 13,149–151). For example, Thompson and colleagues[152] identified conditioned stimulus-related neuronal activity in a cerebellar circuit mediating the classical conditioning of an eyeblink response. Lesioning this same locus disrupted memory expression[6]. In fact, lesioning has been a particularly influential technique used to localize brain structures that contain the engram for many different types of memory, across many species (for example, see REFS 97,153–155). Neurophysiological approaches have also included probing reduced or increased responsiveness to a learned stimulus (including repetition suppression[156]), persistent firing during a memory delay[157] and cued activation of cells[158,159]. In humans, attempts to find the engram historically took advantage of neurophysiological recordings[73,160] and lesion studies[94,161,162], but perhaps the most prevalent technique used today to localize the engram in humans is functional neuroimaging[163].

surprisingly low (<10% of tagged neurons were reactivated)[81,82,84,85]. Previous studies using alternative labelling strategies (for example, cellular compartment analysis of temporal activity by fluorescence *in situ* hybridization (catFISH)) reported reactivation rates as high as ~40% in the CA1 region of the hippocampus[80]. The low reactivation rate observed in tagging studies might reflect engram contraction (that is, a reduction in the size of neuronal population in the engram following encoding)[89,90]. However, because the duration of the tagging window (hours to days) greatly exceeds the duration of training experience (typically minutes), it is more likely that these protocols overestimate the size of the encoding population for the experience of interest ('over-tagging') because neurons active in the hours or days before and/or after training might be unintentionally tagged. Second, as is true for all of these observational studies, the evidence is correlational. Thus, tagging alone does not indicate whether identified neurons are necessary and/or sufficient for memory expression and therefore does not address the criterion of ecphory. However, ecphory may be assessed by selectively manipulating these tagged neuronal populations.

Erasing the engram

Nonspecific lesion studies. Erasure studies are designed to chronically remove or to acutely inhibit a necessary component of the engram to prevent its reactivation and subsequent memory expression. Early erasure experiments attempted to find the engram by nonspecifically lesioning various brain regions in rodents. In a seminal study, Lashley and Franz[3] used this strategy but failed to find the engram. Rats were trained to navigate a maze to obtain food, and different parts of the cortex were subsequently ablated using heat (thermocautery) or knife cuts. Animals showed memory impairment only after large cortical lesions irrespective of location. This led to the conclusion that the engram is not localized in a discrete cortical region but instead is widely distributed. This concept continues to resonate today, particularly in studies focused on understanding the organization of memory in the brain at the network level (for example, see REF. 91).

However, these pioneering studies had limitations. In most of these experiments, memory for a spatial task was assessed in rats that were trained extensively, and recent findings indicate that memories produced by over-training are less prone to disruption by brain lesioning than memories produced by moderate training[92,93]. Furthermore, although it was not known at the time, it is now appreciated that there are multiple memory systems[94,95] and that the hippocampus, in particular, has an essential role in the formation and maintenance of spatial memory[96,97]. In the rat brain, the cortex lies just dorsal to the hippocampus, and a re-examination of Lashley's data has led to the suggestion that the degree of memory impairment observed in his classic experiments may correspond to (unintended) hippocampal damage produced only by large cortical lesions[98].

Although modern techniques produce more localized, neuron-specific lesions, such approaches do not target specific neuronal ensembles (that is, the collection of neurons that might correspond to the engram). Instead, an optimal strategy would be to lesion only those neurons involved in a given engram, leaving other neurons unaffected (BOX 4). By identifying neurons that were active during encoding of a given experience, current capture strategies allow this to be achieved. Two primary capture strategies that allow for specific manipulation (ablation, silencing or activation) of engram neurons at later time points ('allocate-and-manipulate' and 'tag-and-manipulate') have been developed. Crucially, both are based on the premise that neurons that are active during memory encoding are likely to become part of the engram supporting that memory.

Allocate-and-erase strategies. Previous findings have shown that, during a training experience in mice, individual neurons with relatively high expression levels of the transcription factor cyclic AMP-responsive element-binding protein (CREB) are selectively recruited, or allocated, into a resulting engram[99,100] (FIG. 4). In these initial studies, CREB was virally overexpressed before training in a small, random subset (~15%) of pyramidal neurons in the lateral amygdala (LA), a structure known to be important in auditory fear conditioning[13,101–103]. Using *Arc* mRNA expression as a marker of a recently active neuron[80], it was found that neurons with higher CREB activity at the time of training were more likely, than their neighbours with lower CREB activity, to be active during memory retrieval, suggesting that higher levels of CREB activity mediate allocation to the engram[8,104]. Although this effect was initially described in the LA for auditory fear memory, it has also been observed in the LA during encoding of conditioned taste aversion[105] and encoding of cocaine-conditioned place preference[106] memories, in the insular cortex during

Cyclic AMP-responsive element-binding protein (CREB). A transcription factor that, when activated, results in the expression of downstream proteins thought to be important for long-term memory.

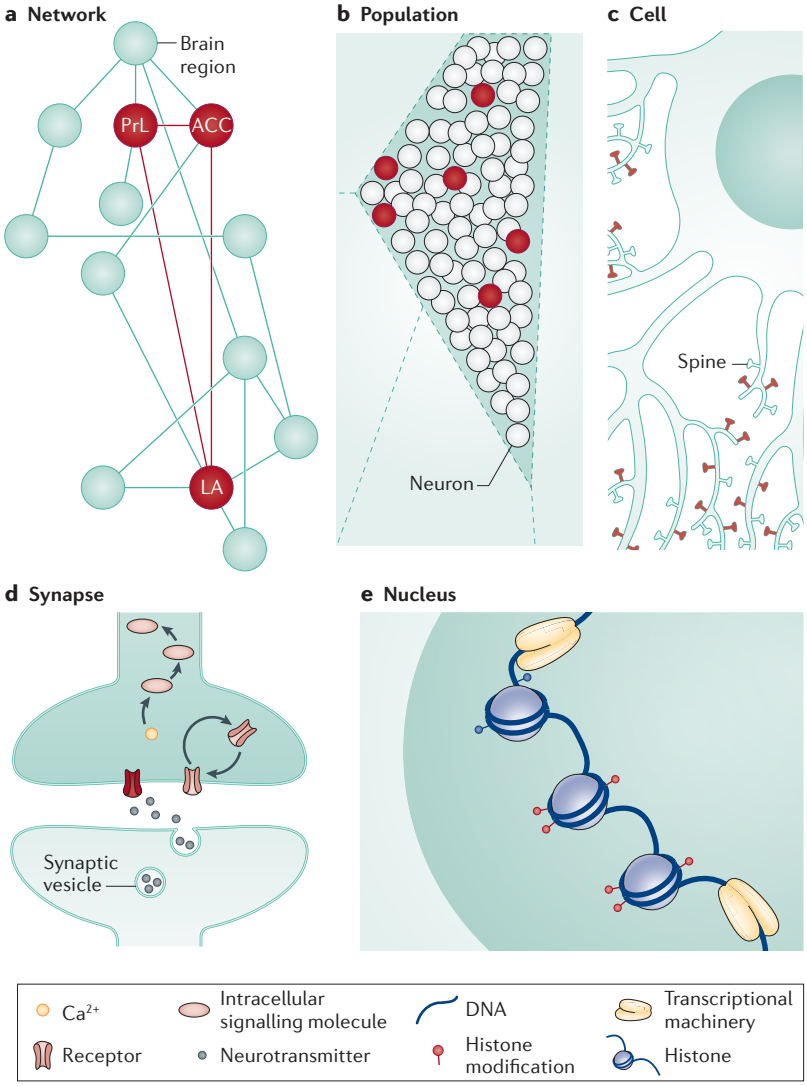


Figure 2 | **Multiple levels of analysis of an engram.** Although engrams are thought to involve strengthening of connections between neurons (neuronal ensembles) widely distributed throughout the brain, the engram can be probed at different scales and levels of analysis. This schematic depicts the components of a hypothetical fear engram (shown in red) at different levels of analysis, from a brain network to a neuronal nucleus. **a** | At the brain network level, a subset of brain regions may be involved in this engram. Red lines depict functional connections between these engram brain regions. Cyan lines depict underlying anatomical connections between brain regions. **b** | At the neuronal population level, subsets of neurons within a brain region may be involved in this engram. **c** | With the formation of each engram, changes occur at the level of individual neurons (for example, changes in the pattern of connectivity). **d** | Changes can also occur at subsets of synapses (for example, synaptic strengthening). **e** | At the nuclear level, the engram can be reflected in transcriptional and epigenetic changes. ACC, anterior cingulate cortex; LA, lateral amygdala; PrL, prelimbic cortex.

encoding of a conditioned taste aversion memory[107] and in the dentate gyrus (DG) during encoding of a contextual fear memory[108]. Subsequent experiments showed that neurons with higher CREB activity were preferentially allocated to the engram because they were more excitable than their neighbours[105,108,109]. Indeed, increasing the excitability in populations of LA neurons in the minutes before fear conditioning using genetically encoded modulators of neuronal activity — for example, by pharmacological activation of excitatory designer receptors exclusively activated by designer drugs (DREADDs)[110] or light-induced activation of opsins[111] — also resulted in preferential allocation of these more active neurons to the engram[109]. Experimentally increasing excitability in a subset of neurons at the time of training may mimic and amplify endogenous processes that occur during normal memory encoding. Thus, during natural engram formation, neurons that happen to be more excitable at the time of training are preferentially allocated to the resulting engram[109,112], an effect predicted by *in silico* modelling data[113].

Using these techniques to allocate and capture engram neurons, the effects of post-training ablation of captured neurons on subsequent memory retrieval have been examined. In one study, mice in which a subpopulation of LA neurons overexpressed CREB underwent auditory fear conditioning. When initially tested, mice showed high levels of freezing to the auditory tone (indicating intact tone-fear memory). However, selectively ablating these engram neurons (via a diphtheria toxin system) after training decreased subsequent expression of that fear memory[114]. After these engram neurons were ablated, mice were able to re-learn this task normally, indicating that the decrease in freezing did not reflect a simple performance deficit. In addition, ablating a similar number of non-allocated (that is, non-engram) neurons had no effect on subsequent memory expression[114]. Moreover, reversibly silencing (rather than permanently ablating) engram neurons in the minutes before a memory test using an allatostatin receptor–ligand system also impaired memory expression and, crucially, mice showed normal tone-fear memory when these neurons were no longer silenced[105]. This allocate-and-erase strategy has also been used to disrupt the expression of conditioned taste aversion[105,107] and cocaine-induced conditioned place preference[106] memories. Therefore, using different methods to silence allocated neurons, in various tasks, these allocate-and-erase experiments show that it is possible to disrupt the expression of a specific engram.

Tag-and-erase strategies. The tag-and-erase approach takes advantage of activity-dependent neuronal tagging strategies described above[81–83,85] not only to tag active neurons but also to express genetically encoded inhibitors of neural activity (such as inhibitory DREADDs or opsins). As with the allocate-and-erase techniques, this allows captured engram neurons to be inhibited at later time points. This strategy has been used to tag populations of neurons in the DG[82], CA3 (REF. 82) and CA1 (REF. 115) regions of the hippocampus that were active during contextual fear training in mice. Subsequent silencing of these tagged engram neurons decreased the expression of the corresponding contextual fear memory. Memory deficits were observed days[115] or weeks[82] after training, indicating that these tagged neurons remained essential for memory expression at these time points (FIG. 3). In addition to studies of conditioned fear, a similar strategy has been used to disrupt the expression of memories of contexts associated with access to rewarding drugs[116,117].

Designer receptors exclusively activated by designer drugs (DREADDs). Engineered G protein-coupled receptors that are no longer activated by the endogenous ligand but are instead activated by otherwise inert drug-like small molecules, used to control G protein signalling *in vivo*.

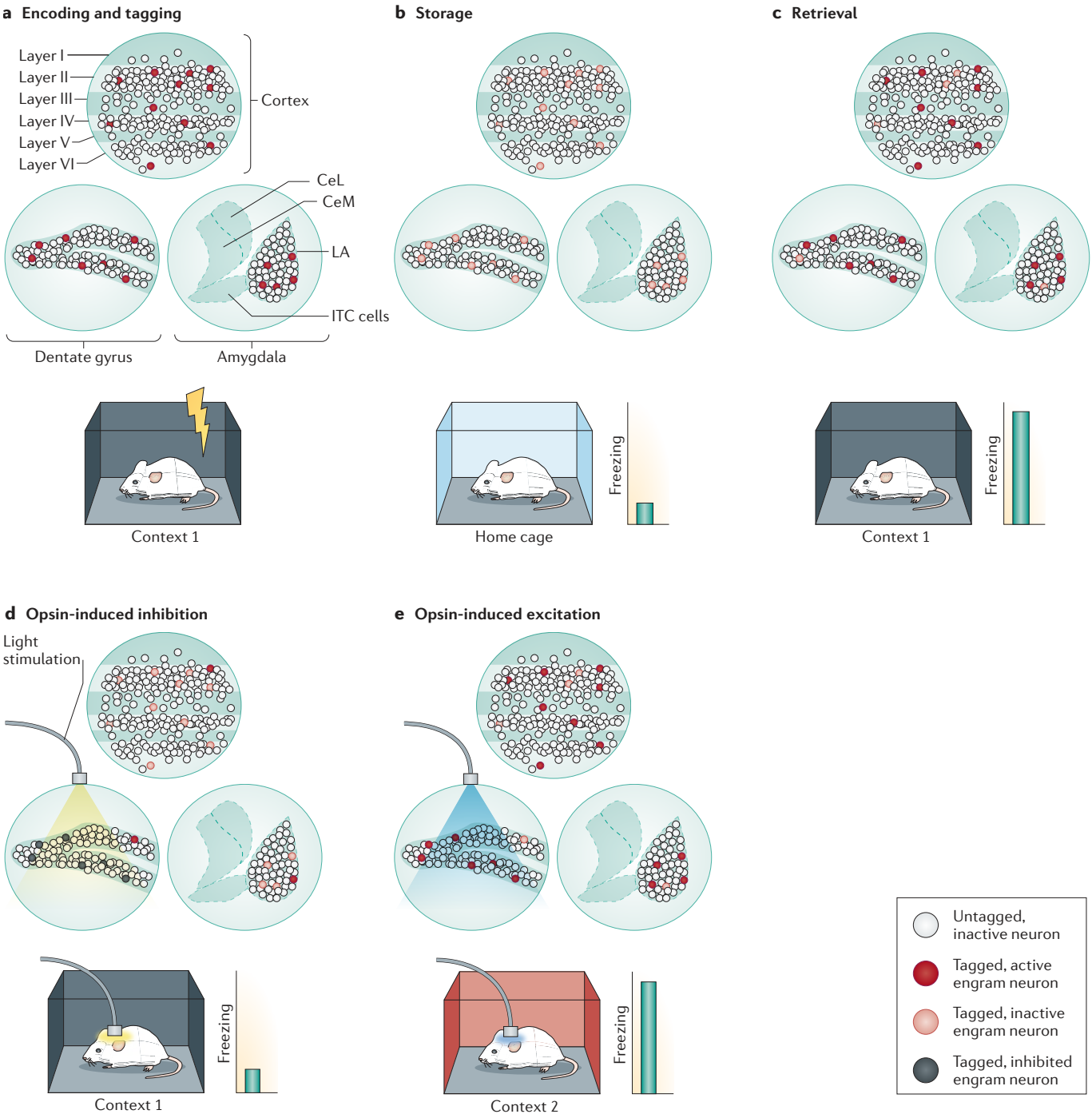


Figure 3 | **The tag-and-manipulate approach to finding the engram.** In this approach, transgenic mice are generated in which neurons that are active during a memory-encoding event are captured and tagged. Through the use of immediate-early gene promoters, these tagged neurons express genetically encoded modulators of neuronal activity (for example, inhibitory or excitatory opsins), allowing them to be silenced or activated at later times. **a** | During training for contextual fear conditioning, a mouse is placed in context 1 and given a footshock. This activates widely distributed neuronal ensembles in the dentate gyrus, cortex and lateral amygdala (LA) and these engram neurons are tagged. **b** | Following training, mice are returned to their home cage (where they do not freeze). The engram is consolidated, and tagged engram neurons become inactive. **c** | When returned to context 1, the mouse shows conditioned fear (freezing behaviour), showing successful retrieval. This successful retrieval is associated with above chance reactivation of engram neurons. **d** | If tagged neurons in the dentate gyrus are optogenetically silenced when the mouse is returned to context 1, then successful memory retrieval is blocked, and mice show reduced conditioned fear. Inhibiting engram neurons in the dentate gyrus is sufficient to decrease reactivation of tagged neurons in the cortex and amygdala. **e** | Conversely, artificially activating tagged engram neurons in the dentate gyrus alone is sufficient to act as a memory retrieval cue such that mice now freeze in a third, unique context (context 2). Activating dentate engram neurons is sufficient to induce reactivation of tagged neurons in the cortex and amygdala. CeL, central nucleus of the amygdala lateral division; CeM central nucleus of the amygdala, medial division; ITC cells, intercalated cells.

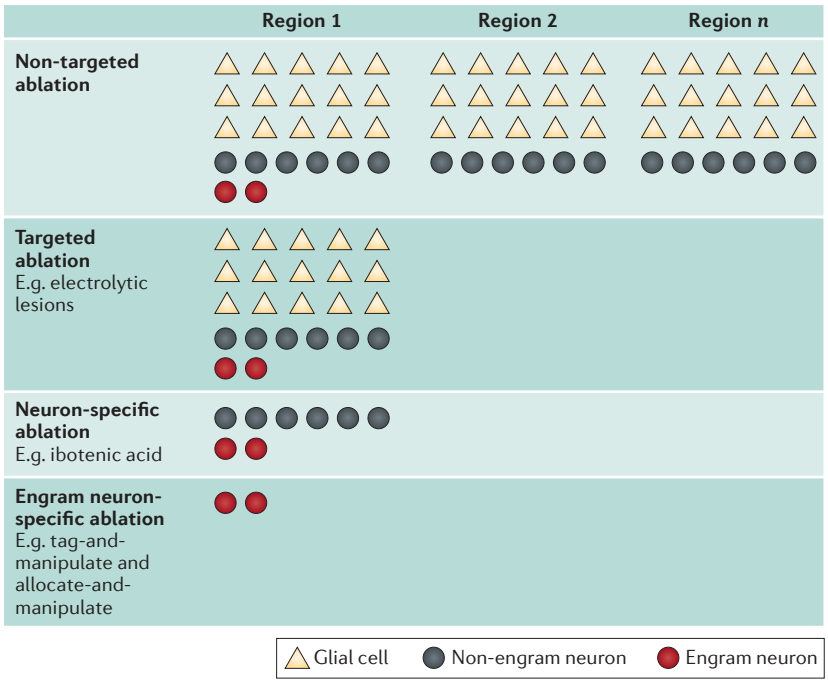
Whereas other conceptually related approaches have been used to disrupt memory expression (BOX 2), these capture-and-erase experiments have made notable progress in finding the engram. Because the erasure experiments reveal that preventing reactivation of populations of engram neurons interferes with subsequent memory expression, they begin to address the ecphory criterion. Moreover, as memory deficits were observed at time points remote from initial encoding, these studies address the persistence and dormancy criteria. Indeed, activation of the same population of neurons is necessary for successful memory retrieval weeks after encoding[82], at a time when, presumably, the engram is in a dormant state.

Limitations of capture-and-erase experiments. Although these capture-and-erase strategies offer improvements over nonspecific lesion methods to localize and erase the engram, they remain imperfect. For instance, many studies capture and erase engram neurons in only one brain region (such as the LA or a specific subfield of the hippocampus), even though an engram may span multiple brain regions[115], in line with Lashley's idea that the engram is widely distributed[4]. Nonetheless, silencing only a subpopulation of these putative engram cells in the LA[105–107,114] or hippocampal subfields (in CA1 (REF. 115), DG or CA3 (REF. 82)) was sufficient to impair subsequent memory expression. These findings indicate that, within what is probably a broad engram network, particular neuronal populations within certain brain regions have more critical roles in memory expression. Indeed, the LA has been shown to be essential in motivationally relevant memories, and the hippocampus has long been hypothesized to have a crucial role in retrieving contextual and spatial memory by reinstating training-like patterns of neural activity across the cortex. Consistent with the idea that neuronal ensembles in the hippocampus coordinate retrieval of memories stored in the cortex[118–120], silencing tagged neurons in the CA1 both impaired memory retrieval and reduced the reactivation of tagged cortical neurons during retrieval attempts[115]. Therefore, although engrams may involve interactions between neuronal populations across multiple brain regions, these results indicate that there is a degree of specialization within these larger networks. Graph theory analyses suggest that particular regions in the brain that interact extensively with other regions within the overall engram network (that is, hub-like regions) may have more prominent roles in memory expression[121,122].

In addition, the duration of capturing time windows in both the allocate-and-erase and tag-and-erase studies greatly exceeds the duration of training, leaving open the possibility that both strategies 'over-tag' by capturing neurons that are not part of that engram. Might the observed memory deficits arise from ablating or silencing these non-engram neurons? Thoughtful control studies suggest not. In one example, researchers[115] tagged CA1 neurons when mice were exposed to one environment and then subsequently silenced these tagged neurons

Box 4 | **The evolution of lesion techniques in the search for the engram**

Progress in finding the engram is directly linked to tool evolution. Lashley and Franz[3,4] first began their engram search using non-targeted lesions (thermocautery) that affected several brain regions and destroyed astrocytes, oligodendrocytes and non-engram neurons as well as the intended target, engram neurons (see the figure). Because these lesions did not offer regional or cell-type specificity, there was substantial collateral damage. Ablation techniques that targeted more discrete brain regions became available in the 1970s and 1980s, and were widely used in the search for the engram. These included electrolytic lesions that targeted one brain region rather than many[6,97]. However, these types of lesions damaged all cell types (including glia) and fibres of passage. Neuron-specific lesion approaches (for example, ibotenic acid-induced lesions) emerged next but, again, both engram and non-engram neurons were targeted. The engram capture approach allowed engram-specific neurons to be ablated and was first successfully applied in the search for the engram in 2009 (REF. 114). In this way, the ability to find the engram was guided by the specificity of the tools available.



Opsins
Light-sensitive proteins that change their conformation from a resting state to a signalling state upon light absorption, used to excite or inhibit neuronal populations using light (that is, optogenetics).

Graph theory
Graph theory is a branch of mathematics used to compare both global (for example, 'small worldness') and local ('hubs') properties of networks.

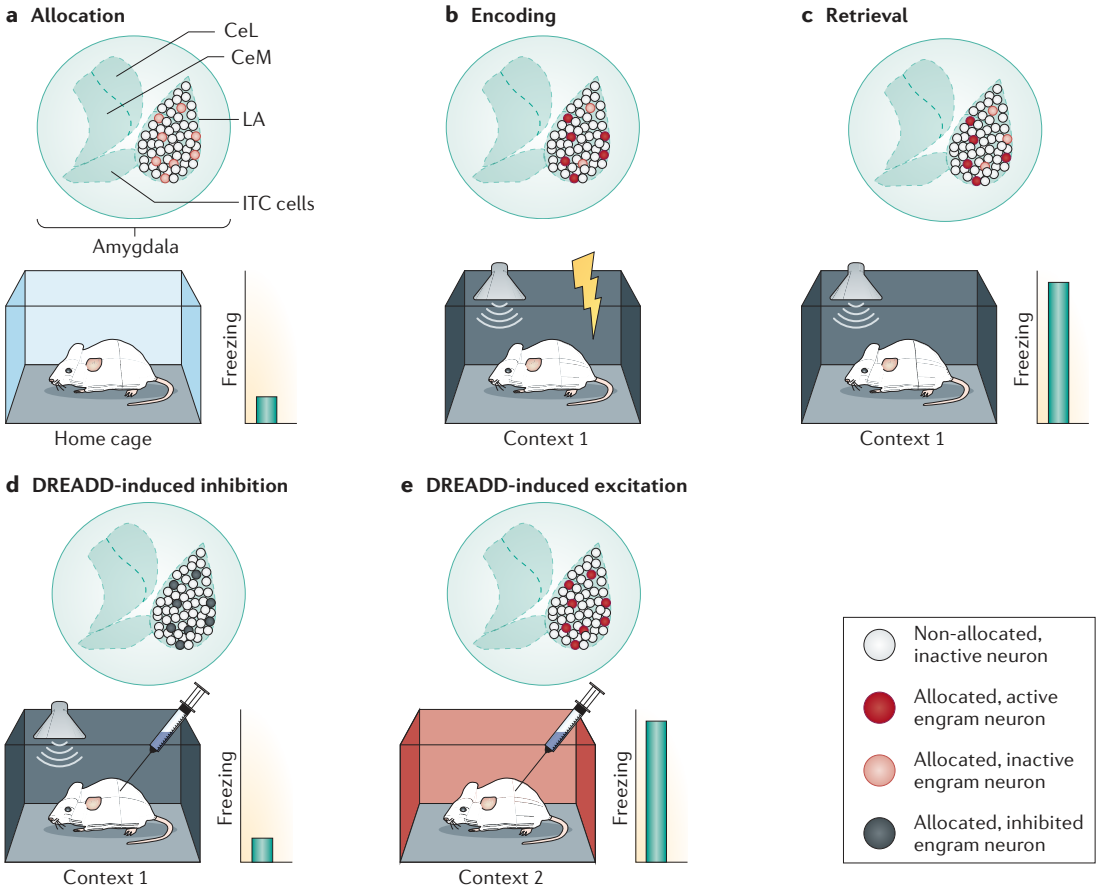


Figure 4 | **The allocate-and-manipulate approach to finding the engram.** In this approach, the activity of a small subset of neurons is increased at the time of memory encoding (through the use of viral expression of the transcription factor CREB (cyclic AMP-responsive element-binding protein), excitatory DREADDs (designer receptors exclusively activated by designer drugs) or optogenetic constructs), facilitating the allocation of these neurons to the resulting engram. At any time after training, the activity of these allocated engram neurons can be manipulated via the DREADD or optogenetic construct. **a** | During allocation, the activity of a subset of lateral amygdala (LA) neurons is increased just before training in an auditory fear-conditioning paradigm. The mouse is then placed in context 1 and presented with a tone that co-terminates with a footshock. Neurons made more excitable are shown in pink. **b** | Neurons that are active during training (tone–footshock pairing) are allocated to a fear memory engram in the LA (engram neurons, shown in red). **c** | Successful retrieval is associated with above chance reactivation of engram neurons, and the mouse shows conditioned fear (freezing behaviour when the tone is replayed). **d** | Memory retrieval is blocked by silencing tagged engram LA neurons, via inhibitory DREADD activation, and mice show reduced conditioned fear (freezing) when the tone is replayed. **e** | Conversely, memory retrieval can be artificially induced by activating tagged engram LA neurons. This activation is sufficient to act as a memory retrieval cue such that mice now freeze in a unique context (context 2) in the absence of the tone. CeL, central nucleus of the amygdala lateral division; CeM central nucleus of the amygdala, medial division; ITC cells, intercalated cells.

while the mice underwent fear conditioning in a second environment, thereby preventing the previously tagged neurons from becoming part of the contextual fear memory engram for the second context. Silencing the neurons tagged in the first environment had no effect on the subsequent expression of the contextual fear memory from the second environment. This indicates that silencing components of one hippocampal engram does not affect expression of another engram or, more generally, that silencing a subpopulation of neurons in the hippocampus does not broadly disrupt processes required for memory retrieval. Similarly, silencing neurons allocated to one LA engram does not disrupt expression of another[105]. Therefore, the use of capturing strategies allows the specific manipulation of one engram (which supports one memory) rather than all engrams (and all memories), and thereby begins to address the content criterion.

Artificially expressing the engram

Typically, a memory emerges when the latent engram is awakened by an external retrieval cue, the process of ecphory[1,2,10]. Studies in both humans and animals have revealed that engrams can also be awakened artificially by stimulating the neuronal components of the engram. During the surgical treatment of patients with epilepsy, Penfield found that focal electrical stimulation of the human brain could awaken latent engrams and artificially induce memory retrieval[123]. Although most

stimulation-induced experiential responses were vague, some were specific (for contemporary examples, see REFS 124,125). In this way, Penfield showed that it is possible to bring to mind past experiences by directly activating the engram in the absence of an external sensory retrieval cue or internal retrieval attempt.

At first glance, these early findings satisfy several of the criteria for engram identification. Stimulation led to memory expression, which addressed the ecphory criterion. Furthermore, in some instances, evoked memories corresponded to events from the distant past, thereby addressing the persistence and dormancy criteria. However, there was no a priori way of predicting which memory would be evoked at any given stimulation site, a limitation that remains relevant to contemporary human stimulation studies[126]. By using neuron capture strategies to restrict activation to those neurons that were active during encoding, present-day rodent studies build on this tradition and begin to address the content criterion.

Activating captured neurons. By combining allocation or tagging methods with techniques that allow selective activation of neurons, modern-day rodent studies can predict the content of a particular memory that will be retrieved when a particular engram is stimulated. In this way, a latent engram can be awakened by artificial stimulation of captured engram neurons using excitatory DREADDs or opsins in the absence of a retrieval cue. For example, using a tag-and-manipulate strategy, DG neurons that were activated by auditory fear conditioning were tagged with channelrhodopsin 2 (ChR2) in mice[85]. Over the next 5 days, the mice were placed in a familiar context that had not been paired with a footshock, and freezing behaviour was measured. Remarkably, when the DG neurons that were active during initial fear conditioning were transiently stimulated using light, mice froze in this familiar context that had never been paired with a footshock. Interestingly, this artificially awakened memory did not seem to undergo extinction over repeated test days. This approach has also been used to reawaken an engram for a rewarding event, in which male mice encountered a female mouse[127,128]. Therefore, these and other[129] tag-and-manipulate studies show that experimentally reactivating neurons that have been incorporated into an engram is sufficient to act as a retrieval cue, similar to the nonspecific neural stimulation used in Penfield's experiments.

Similar findings have been obtained from allocate-and-manipulate studies in which a memory emerged following artificial stimulation of allocated neurons[109]. To allocate LA neurons to a fear engram, researchers expressed an excitatory DREADD in a small random population of neurons and administered the DREADD ligand clozapine-*N*-oxide (CNO) to activate these neurons immediately before auditory fear conditioning. Artificially activating these allocated engram neurons by administering CNO in the days after training induced freezing in a novel context, whereas artificially activating a similarly sized population of non-engram neurons did not.

Context-associated fear memories are thought to depend on a distributed hippocampal–cortical memory trace[88]. Just as artificial stimulation of tagged DG neurons was sufficient to induce freezing[85], stimulation of tagged neurons in the retrosplenial cortex also resulted in fearful behaviour[130]. Presumably, this focal reactivation of engram neurons in either the DG or the retrosplenial cortex triggered a broader pattern of reactivation across the entire engram. Indeed, reactivating tagged retrosplenial cortex neurons activated other tagged neurons in the amygdala and entorhinal cortex, in a manner similar to the reactivation pattern produced by the natural external retrieval cue (the conditioning context)[130]. These findings are consistent with Penfield's assertion that focal stimulation of a particular brain site produced a broader reactivation of the engram in a process similar to pattern completion[131].

Criteria satisfied by artificial expression studies. Have these artificial expression studies successfully transformed latent engrams into expressed memories? We think that the evidence is compelling. Stimulation of engram neurons in the examples described above led to involuntary memory expression, addressing the ecphory criterion. Moreover, memories could be evoked at time points remote from encoding when the likelihood that these engrams are being actively processed is low, addressing the persistence and dormancy criteria. However, is the expressed memory 'real'? To what extent do these studies satisfy the content criterion? At first glance, artificial reactivation of captured engram neurons and physical presentation of an external retrieval cue seemed to be functionally equivalent, but in fact there were some differences. In particular, in most experiments, the neurally reinstated behavioural response was smaller in magnitude than the naturally reinstated response, which was evoked by presentation of the external sensory cue[85,109,127]. At the psychological level, memory retrieval is thought to be most successful when environmental and internal cues available at encoding are also present at the time of retrieval (that is, the principle of encoding specificity[132]). Similarly, at the neural level, artificial reactivation of an engram is likely to be most successful for behavioural memory expression when it faithfully recapitulates the pattern of neural activity present at encoding[133]. Of course, artificial reactivation is not likely to recapitulate the precise spatiotemporal activity pattern that accompanied encoding. The loss of this fidelity may occur in the spatial domain, temporal domain or both, leading to less effective reinstatement and subsequently weaker behavioural expression of the memory.

Another way to address this question is to ask whether 'artificial memories' behave similarly to 'real memories'. Classical psychological studies emphasize that memory is a constructive process[134], with engrams being reconstructed into memories in a use-dependent manner that is influenced by the amount of pertinent detail available and the individual's current goals, among other factors. Many types of memory errors described in the cognitive literature support this notion[135]. Reconstruction may, in turn, lead to changes in the corresponding engram (that is, the engram may be updated), including the formation of new associations with salient stimuli or events.

Pattern completion
The ability of a network to retrieve stored information on the basis of partial or degraded input patterns.

A recent study examined whether artificial activation of an engram could be associated with a new event and thereby create a false memory[136]. In this study, active neurons were captured while mice were exploring a novel context. These captured neurons were optogenetically reactivated while the mice underwent fear conditioning in a second context. Remarkably, mice did not learn to fear this second context but now froze when placed back in the original context that had never been paired with shock, indicating that the experimentally reinstated memory had been conditioned and a 'false' memory had been created. Taking this strategy one step further, another group of researchers successfully created a false association between two distinct engrams in mice[137]. Hippocampal CA1 neuronal ensembles that were active while the animal was in a neutral context (that is, a context not paired with shock) were labelled. Basolateral amygdala neuronal ensembles that were active during footshock in a different context were also labelled. Subsequent co-activation of both engrams produced an association between the two memories, such that freezing now occurred in the neutral context that had not previously been paired with footshock.

The process of memory retrieval and reconstruction is thought to involve destabilization of the engram, followed by a protein synthesis-dependent restabilization process (collectively termed reconsolidation) (BOX 2). For instance, in an auditory fear-conditioning experiment, presenting the external retrieval cue (tone) immediately before intra-LA administration of the protein synthesis inhibitor anisomycin disrupted reconsolidation, and the tone fear memory was no longer expressed[138]. Might a neurally reinstated memory also undergo reconsolidation? Intra-LA administration of anisomycin before artificial activation of engram neurons, in the absence of a retrieval cue, also disrupted subsequent memory expression[139]. This suggests that disrupting protein synthesis prevented the restabilization of a neurally reinstated engram, a finding similar to that observed following reconsolidation blockade in traditional studies of memory. This susceptibility to reconsolidation blockade provides evidence that the artificially expressed memory behaves like a real memory.

One cautionary note is that these artificial expression studies so far have been limited to fear- and reward-conditioning paradigms. In these tasks, the behavioural expression of memory is relatively impoverished (that is, limited to freezing or approach or avoidance behaviour), and so it is not obvious whether the artificially expressed memory captures the full details of the encoding experience (for example, beyond the footshock or the presence of a female mouse). As such, behavioural paradigms used in current research pose limits for addressing the content criterion. One promising avenue for future studies is to use more complex tasks in which the details of the encoding experience can be probed more extensively. A potentially suitable task, for example, is sequence learning in which correct recognition of particular temporal sequences of events (for example, ordered presentations of odours A, B, C and D) is reinforced[140]. As the temporal organization of events is a defining feature of episodic memory[141,142], such tasks might more closely probe 'remembering what transpired' than conditioning paradigms, and more thoroughly address the content criterion than the studies performed to date.

Conclusions

More than 60 years ago, Lashley conceded defeat in his search for the engram. Here, we applied four engram-defining criteria to evaluate recent experimental evidence from three types of studies that have attempted to find the engram. Observational studies have been the most successful in addressing the content criterion. In rodent multi-unit electrophysiological recording studies, patterns of neuronal activity that occurred during encoding are detected at later time points, and these replayed patterns predict future behaviour. Perhaps with even greater specificity at the content level, human fMRI studies have identified event-specific neural activity that occurs in the period between encoding and retrieval and, furthermore, reflects what transpired at encoding and predicts success in subsequent retrieval.

Contemporary techniques in rodents that capture neurons that are active at the time of encoding have provided a powerful new way of hunting for the engram and have been especially successful in addressing the other three criteria through the manipulation of specific 'engram neurons'. Erasure studies have shown that silencing engram neurons prevents memory expression, and thus establish that activation of these neurons is necessary for successful retrieval. Conversely stimulation of these engram neurons has been used effectively to induce artificial memory recovery, and thus establish that their activation is sufficient for retrieval. Therefore, these types of experiments may be the first line of evidence that convincingly satisfies the ecphory criterion. These studies show that it is possible to induce memory expression, even without interactions with any external retrieval cue or dedicated retrieval attempts, by directly activating engram neurons. That these manipulations are effective at time points remote from encoding, when the engram is likely to be dormant, additionally satisfies the persistence and dormancy criteria.

To date, these capture strategies have only been applied in fear- and reward-conditioning experiments. In these paradigms, the behavioural readout is limited in complexity, which makes it challenging to fully address the content criterion. Nonetheless, these studies reveal some content specificity by showing that capture strategies allow the erasure of a targeted engram, and not just any engram. Similarly, activating captured neurons leads to the artificial expression of the corresponding memory, and not just random memories. Moreover, these artificially expressed memories share properties with real memories in that they reconsolidate and can form *de novo* associations. In addressing each of the four engram-defining criteria that were derived from the definitions originally offered by Semon[1,2], not only can contemporary rodent studies claim to have found the engram, but also have identified means to control it.

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Competing interests statement
The authors declare no competing interests.