Engrams: Memory Consolidation and Retrieval

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ENGRAMS

Memory can be defined as the process by which organisms encode, consolidate and retrieve information. The concept of memory being stored as modifications in neuronal connections was first hypothesized by Ramón y Cajal [1]. This notion was furthered by the works of Donald Hebb [2], who proposed that the connections between co-active neurons are strengthened during learning[1].

Engrams are the physical substrates of memories. The neurons that display enhanced synaptic connections and are activated during both encoding and retrieval are proposed as the neural basis of engrams. The term 'engram' was coined by Richard Semon in two of his pioneering books [3][4]. The neurons which are active during both memory encoding & retrieval have been hypothesized to form the cellular and neuronal basis of engrams. He proposed that every engram possesses four defining characteristics. First, engrams are created by enduring physical changes in the brain. Second, an engram has the ability to be ecphoric (i.e.) memory stored in the engram can be retrieved upon the presentation of natural cues or by direct activation. Third, the contents of an engram can be used to both derive the information they encoded and predict the information they would retrieve upon activation. Finally, an engram must persist and should be able to exist in a dormant state between two processes, such as consolidation and retrieval or encoding and retrieval or encoding and consolidation.

Effectively, an engram must be created by persistent activity, possess ecphory, store content, and remain dormant for an extended duration. Over the years, this characterization of engrams has been used to identify them. Studies showed that engram cells are formed when synaptic connections are strengthened between neurons that are active during learning [5],[6]. Engram cells can be distinguished from non-engram cells based on distinctive features such as enhanced dendritic spine density, excitatory postsynaptic current amplitude, whole-cell intrinsic cell capacitance, AMPA to NMDA receptor density ratio, and selective activation of gene transcription. The above characteristics of engrams enable studies at different scales spanning from the molecular level to the network level.

This review will discuss some of the advances made in the study of engrams, their identification, activation, and their role in memory retrieval. Following this, the research findings of the physiological changes that differentiate engram neurons from non-engram neurons and the effects of these physiological changes on memory retrieval are described. Finally, some of the molecular underpinnings that result in these physiological changes and the role of these molecular mechanisms on memory retrieval are analyzed. It also provides possible future courses of research in this field.

Engram identification and activation

With the possession of this information about engrams, Karl Lashley undertook initial studies to identify them. Restricted by the unavailability of advanced experimental tools, Lashley [7] proposed

[1] Here, the term co-active refers to neurons that display temporal precedence in firing as opposed to simultaneous firing. Synaptic strengthening occurs only when a neuron's persistent firing induces action potentials in another neuron. The temporal gradation in firing and the persistence in firing corresponds to the causality and consistency components proposed by Hebb.

the equipotentiality (i.e.) all parts of the brain contribute equally to learning and the principle of "mass action", which states that the cortex works as a whole and the performance on a task improves when more of the cortex is involved. Lashley studied learning by training the rats on maze navigation. He induced lesions of varying degrees and found a positive correlation between the magnitude of the lesion and the induced learning deficit. This prompted him to reject the possibility of localized learning. In [7], he proposed equipotentiality and mass action as the main approaches by which learning is mediated.

By the late 20th century, immediate early genes (IEG) such as *c-fos, Zif*, and *Arc* were established as markers for neuronal activity [8]. Reijmers et al. [9] leveraged this knowledge and utilized it to generate the TetTag transgenic mice line for fear engram identification. TetTag mice comprise two transgene sub-systems as shown in Figure 1. The first sub-system is a tTA (Tetracycline-controlled transcriptional activation) gene which is present downstream of a c-fos promoter. tTAs are inducible gene expression systems, which can be reversibly activated by tetracycline or its derivatives such as doxycycline (DOX). Hence, in the absence of DOX, the gene is not transcribed. The second subsystem comprises a bi-cistronic [2] tetO promoter that drives a tau-LacZ marker and a mutated tTA gene (tTA^{H100Y}). The replacement of the histidine in the 100th position by a tyrosine (H100Y) in the tTA gene makes tTA DOX resistant. The second system, once activated, persists due to the feedback loop between the tetO promoter and tTA^{H100Y}. This enables tagging of cells that were active during the DOX-off to persist even during the subsequent DOX-on regime.

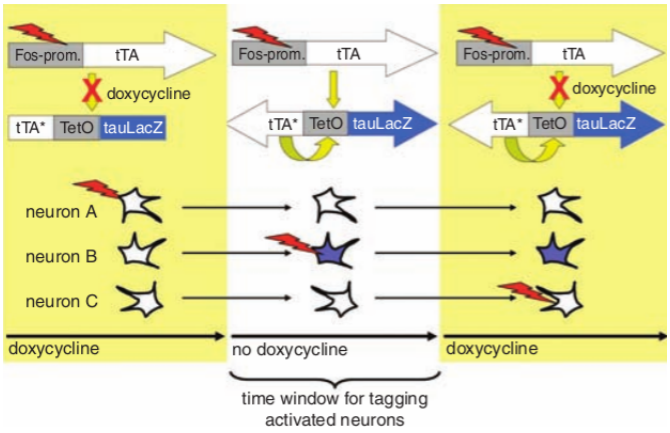

Figure 1: TetTag system used for engram identification. [9]

The mice were trained on classical fear conditioning involving foot shocks with a tone cue. The behavioral manifestation studied was the percentage freezing observed during the testing regime when compared to the training regime. In order to verify whether the neurons that were active during learning were also the ones that were activated during retrieval, Reijmers et al. used *Zif*. As *Zif* is also an IEG, its expression peaks around 1-2 hours after the neurons are activated. By analyzing the overlap between cells that are simultaneously tagged by Lac and express *Zif*, they showed that a subset of neurons were activated during both learning and retrieval. Additionally, they also showed that the number of reactivated neurons during memory retrieval positively correlated with the percentage freezing observed.

Subsequently, Liu et al. [10] built on this *c-fos*-tTA transgenic line and introduced an Adeno-Associated Virus (AAV) vector similar to the second system in [9]. This vector comprises a Tetracycline response

[2] Two Cistrons/genes get simultaneously transcribed.

element (TRE; 7 repeats of tetO), Channelrhodopsin (ChR2), and Enhanced Yellow Fluorescent Protein (EYFP). While the presence of EYFP ensures the specificity of tagging, ChR2 enables optogenetic activation of the tagged neurons. Hence, once the neurons were tagged in the absence of DOX, they can be optogenetically activated.

Engrams are sufficient for memory retrieval.

In addition to introducing the method of optogenetic stimulation of neurons described above, Lui et al. sought to understand the behavioral manifestation of the engram cell activations. They showed that activating the neurons in the dentate gyrus (DG) that were tagged during fear conditioning is sufficient to reproduce the freezing behavior in the absence of external cues. This is depicted pictorially in Figure 2. In their control experiments, they showed that activating engrams tagged in non-fear-conditioned animals and animals that are exposed only to the tone and not the shock, did not result in the freezing behavior. Lui et al. also showed that bi-lateral optogenetic stimulation of the tagged neurons elicits the same extent of behavioral response as the contextual cues.

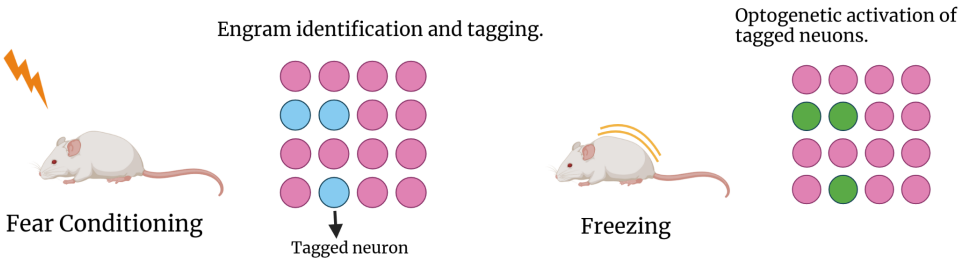

Figure 2: Engram cell activation is sufficient for memory retrieval [10].

Extending this study, Ramirez et al. [11] found that engrams can be manipulated to create false memories. They created these false memories by associating the engrams activated during one context with fear conditioning performed in another context. In their experiments, as shown in Figure 3, using mice as the animal model, they tagged the DG neurons pertaining to the exploration of context A and optically stimulated them during fear conditioning in context B. These animals, upon being placed back in context A, exhibit freezing. Ramirez et al. in addition to showing the sufficiency of engram activation for memory retrieval, also showed for the first time, the context specificity of the engrams. Besides the classical fear conditioning, they also trained the mice on the conditional place avoidance (CPA) paradigm. The CPA comprises two chambers (chambers A and B), and the behavioral outcome studied is the percentage of time the animals spend in each of the chambers (i.e.) preference. The DG neurons corresponding to the exploration of chamber A were tagged and stimulated during fear conditioning in a different context. Upon the animal being placed back into the CPA setup, the animals showed an aversion to chamber A. The control animals had no preference for either of the chambers.

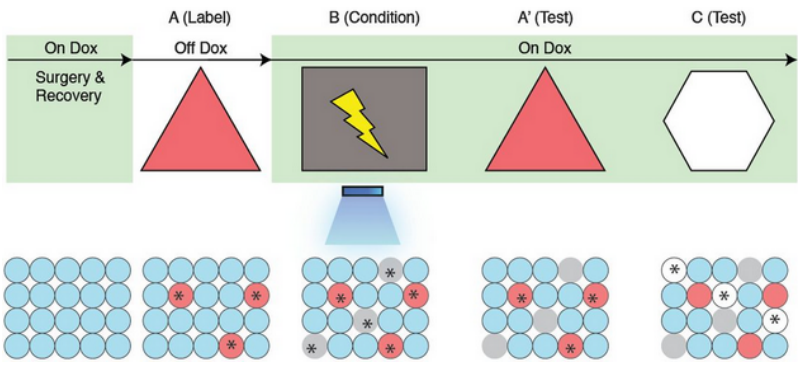

Figure 3: Manipulation of engrams results in generation of false memories [11]. When the animals are reintroduced into the context A (i.e.) A', they exhibit freezing.

Engrams are necessary for memory retrieval.

Subsequently, Tanaka et al. [12] showed that engram cells are necessary for memory retrieval. Using the *c-fos*-tTA/tetO-Cre/tetO-H2B-GFP mice as the animal models and AAV-FLEX-Archaerhodopsin (ArchT) vectors, they were able to inhibit neurons that were active during learning. H2B-GFP is a longer-lasting version of the standard GFP and contains the human histone protein H2B. FLEX (flip-excision) is a loxP-based gene switch that enables conditional gene expression in the presence of Cre. ArchT is an optogenetically activated outward proton pump that hyperpolarizes cells, thereby inhibiting them. Hence, when tTA is transcribed, both Cre and H2B-GFP are expressed. The presence of Cre activates the FLEX system, resulting in the flipping of the inverted ArchT gene and its subsequent expression.

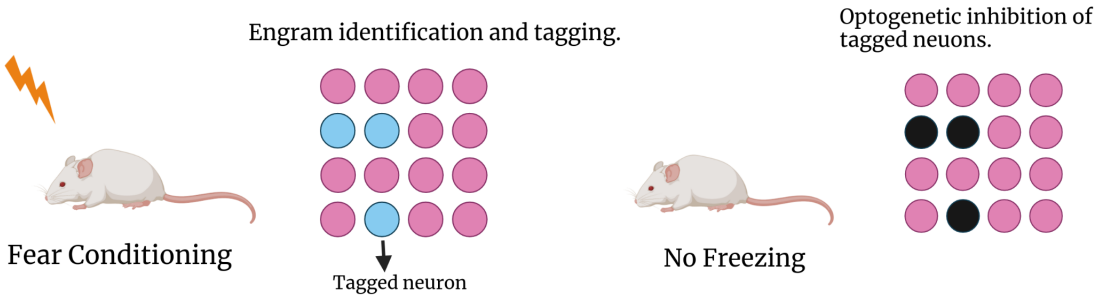

Figure 4: Engram cell activation is necessary for memory retrieval [12].

Tanaka et al. showed that when the engram neurons are optogenetically inhibited, the animal's fear memory retrieval is significantly impaired. This is depicted graphically in Figure 4. They analyzed the behavioral manifestation of engram inhibition and verified that inhibition of non-engram hippocampal neurons did not affect memory retrieval. They also showed that memory retrieval in the hippocampal dorsal CA1 (dCA1) results in cortical activation in downstream areas and provide the first evidence of orthogonality between the computations performed in the ventral and dorsal streams of CA1.

Physiological changes observed in engrams.

While engrams have been proven to be both necessary and sufficient for memory retrieval, the physiological characteristics of the engram neurons were not explored in the studies discussed above. The work of Ryan et al. [5] bridged this gap. In their paper, Ryan et al. proposed and analyzed several physiological metrics of both engram and non-engram cells. The animal model they used is the same as described in [10]. They tagged the DG neurons that were active during learning with an AAV-TRE-mCherry construct and tagged the upstream entorhinal cortex (EC) neurons with an AAV-Calcium/calmodulin-dependent protein kinase II(CaMKIIα) promoter-ChR2-EYFP construct. CaMKII is a gene that is expressed in excitatory neurons in the EC. Hence, the construct injected into the EC tagged only the excitatory neurons.

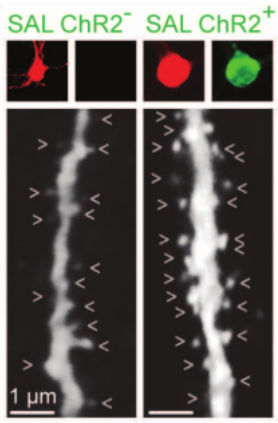

Figure 5: Dendritic spine density variation observed between engram and non-engram neurons.

In order to compare the electrophysiological responses between the engram and non-engram cells in the DG, paired recordings were obtained from engram and non-engram cells when the EC cells were optogenetically activated. The excitatory postsynaptic current (EPSC) amplitude was used as an estimate of the synaptic strength of the neurons. They found that engram cells exhibited significantly greater synaptic strength than non-engram cells. They also found that engram cells have greater α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) to N-methyl-D-aspartate (NMDA) receptor current ratios. Previous works such as [13], [14] have established the AMPA/NMDA receptor current ratio as a measure of postsynaptic strength. These studies showed that synaptic strength could be varied as a function of the type and density of the neurotransmitter receptors. Long-term potentiation (LTP) usually results in a higher AMPA/NMDA receptor current ratio, while long-term depression (LTD) results in a lower AMPA/NMDA receptor current ratio [15]. Ryan et al. also showed that engram cells have a higher spontaneous EPSC amplitude and higher dendritic spine density (Figure 5).

Persistence of a Memory, its Retrieval and Forgetting

Memory retrieval is an act of recalling prior information initiated by the presence of a cue. While memory retrieval is usually studied as the end consequence in most studies, it is preceded by memory encoding and memory consolidation. Memory encoding, as the name suggests, is the process of efficiently converting external high-dimensional information into a functional, low-dimensional information space. Memory encoding in both biological systems and artificial systems is a function of

input statistics. The dependence of encoding on the input statistics is the basis of the entire subfield of efficient coding in neuroscience. Consolidation can be defined as the process by which a learned/encoded memory is stored for a longer duration (i.e.) the conversion of a short-term memory into a long-term memory. The key feature of consolidation is that the memories are extremely fragile and susceptible to external disturbances during this time frame. Protein synthesis inhibition is one such external perturbation that inhibits memory consolidation, resulting in retrograde amnesia. Once a memory is encoded and converted from short-term memory into long-term memory, cue-based activation of the engram cells results in memory retrieval.

Memories stored in engrams persist through induced retrograde amnesia

All the studies described in the previous section disrupt memory retrieval by activating or inhibiting the engram cells and thereby directly targeting the final stage of the process. But can memory retrieval be disturbed by perturbing any of the upstream processes, such as memory encoding and consolidation? And if a memory is disrupted, can it ever be retrieved? These were two of the main questions addressed by Ryan et al. [5]. In addition to studying the various physiological changes that occur in engram cells, Ryan et al. also showed that memory retrieval could be disrupted by inducing perturbations to the system during the consolidation stage. In order to hinder memory consolidation, anisomycin (ANI), a protein synthesis inhibitor, is injected into the mice after contextual fear conditioning (CFC). In the control group, saline was injected into mice at the same time at which ANI was injected into the experimental group. The experimental protocol is depicted graphically in Figure 6.

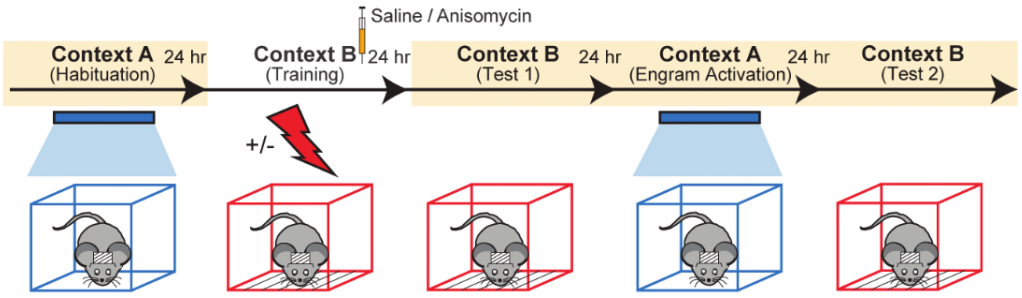

Figure 6: Amnesia induction and reversal of physiological changes [5].

Several physiological properties of engrams observed in the control group were absent in the ANI group. There was no significant difference between the engram and non-engram neurons in the ANI group in terms of synaptic strength, AMPA/NMDA receptor current ratio, and dendritic spine density. Once trained on CFC, the mice were tested by placing them back in the training context, inducing natural recall. The control mice displayed significantly higher freezing than the ANI mice group, thus proving that memory retrieval has been hampered. Next, Ryan et al. checked if the memory can be retrieved by activating the engram cells. Optogenetic activation of the neurons that were active during the training phase resulted in the same level of freezing in the ANI group mice as in the control group. This highlighted that memory stored in engrams persists even under retrograde amnesia and reversal of physiological property alterations. Ryan et al. showed that while memory consolidation is disrupted under the application of a protein synthesis inhibitor, the underlying memory stored in the engram itself is not disrupted and surprisingly, the memory can be retrieved by directly optogenetically stimulating the engram cells.

The causal link between LTP and memory retrieval

While the physiological changes manifested in engrams such as synaptic strengthening, increase in AMPA/NMDA receptor current ratio and increase in dendritic spine density correlates with the physiological changes associated with LTP, a causal link between LTP and memory storage in engrams has not been established. Nabavi et al. [16] used optogenetically induced LTP and LTD to analyze this causality. Using rats as model organisms, they injected an AAV vector containing oChIEF (a modified ChR opsin) [3]. The vector is injected into the auditory nuclei input to the amygdala. The animals were fear-conditioned with a tone as the conditioned stimuli.

All the animals used in the experiments were previously trained on a tone-induced lever press task and the normalized number of lever presses was used as a metric of memory storage. The lower the normalized lever presses, the better the storage of fear-conditioned memory. The rats were tested for the ability to retrieve the fear memory after they were trained. The AMPA/NMDA receptor current ratio was used to determine if LTP or LTD occurred. In addition to using tone as the cue, Nabavi et al. also used direct optogenetic stimulation of the auditory nuclei as the conditioned stimuli for another set of animals. When either tone/optogenetic activation was used, fear conditioning resulted in a higher AMPA/NMDA receptor current ratio and hence, LTP. The trained animals also demonstrated low normalized lever presses upon application of the conditional stimuli.

Following this, Nabavi et al. induced LTD in trained mice by using prolonged, low-frequency optogenetic stimulation. When these animals were tested one day after the application of LTD, they showed no conditioned response to the stimuli of tone or direct optogenetic activation. This indicated that the animals were no longer able to retrieve the memory associated with fear conditioning. Nabavi et al. also showed that LTP-LTD mediated memory retrieval is extremely plastic by applying repeated rounds of LTD and LTP in the animals. A model of the synaptic plasticity is depicted in Figure 7.

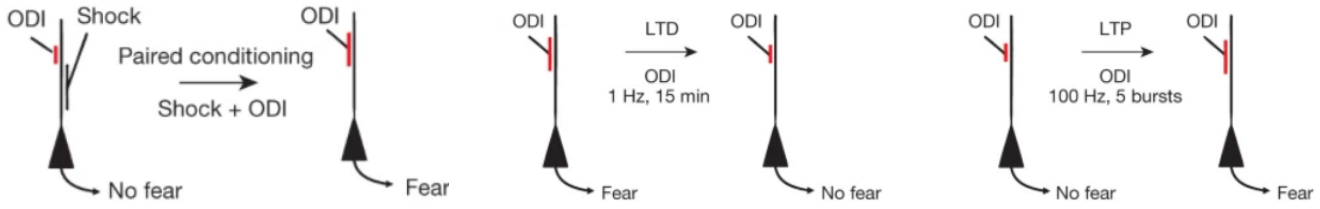

Figure 7: Model of synaptic plasticity backing the causal link between LTP/LTD and memory retrieval [16]. ODI stands for Optogenetically Driven Input.

As the vector induction in this study was not specific to the neurons activated during tone-induced fear conditioning, they used controls to verify that the activation of the auditory nuclei resulted in retrieval of previously learned fear-conditioning. They optogenetically activated the auditory nuclei input to the amygdala of animals that weren't previously fear-conditioned and showed that there was no change in the normalized lever presses. This proved that the fear-conditioned memory was retrieved during activation as opposed to the behavioral manifestation being caused by the general activation of the amygdala, the fear center of the brain.

As a second control, to verify the association learning between tone/optogenetic activation and shock,

[3] ChIEF is derived from ChEF by the addition of a point mutation of Ile^{170}. ChEF is derived from ChR and has a crossover site at loop E-F. oChIEF is an optimized version of ChIEF. [17]

Nabavi et al. performed stimulus extinction experiments. In stimulus extension experiments, the stimulus is repeatedly presented to the animals without the shock. Once trained, the animals no longer displayed fear-conditioned memory retrieval. The animals failed to retrieve the memory even under induced LTP, hence, verifying that tone and shock paired memory was only studied. Through these experiments, Nabavi et al., for the first time, provided insights into the causal link between LTP and memory retrieval.

Is memory retrieval a passive or dynamic process?

While Ryan et al. [5] showed that memory consolidation is a dynamic and protein synthesis-dependent process, memory retrieval was predominantly believed to be passive lookup of memories that were previously stored. However, studies that focused on understanding the role of AMPA receptors (AMPAR) in synaptic plasticity hinted at the possibility of memory retrieval being a dynamic, protein synthesis-dependent process [18], [19], [20], [21].

In order to test whether memory retrieval is dependent on protein synthesis, Lopez et al. [22] infused ANI, a protein synthesis inhibitor, minutes before memory retrieval in fear-conditioned mice. They showed that protein synthesis inhibition (PSI) hampered memory retrieval, as mice injected with ANI exhibited significantly reduced freezing when compared to the controls. They then checked if PSI hindered the memory itself by testing fear memory retrieval in the same mice after 3 hours, without ANI, and found that freezing behavior was restored to control levels. In order to rule out the off-target effects of ANI, they also used rapamycin (RPI), another protein synthesis inhibitor with significantly reduced off-target effects, and obtained the same results.

NMDAR-mediated memory retrieval

Lopez et al. then worked on identifying key proteins involved in this protein synthesis-mediated memory retrieval. They followed up on previous studies such as [23], which showed that NMDAR mediates the recycling of AMPAR at the postsynaptic side. They used ifenprodil, an NMDAR inhibitor, to test how the blockage of AMPAR recycling affects memory retrieval. A blockage in the recycling, while preventing the insertion of newly synthesized AMPARs at the cell membrane, maintains a constant density of AMPA receptors at the postsynaptic side. They found that animals treated with ifenprodil, when tested, exhibited no deterioration in memory retrieval. This indicated that for a single test involving memory retrieval, maintaining the density of AMPAR on the postsynaptic side was sufficient for memory retrieval. They also injected a mixture containing ifenprodil and RPI into the mice and found that ifenprodil prevented the deterioration of memory retrieval.

AMPAR-mediated memory retrieval

As mentioned in the previous section, AMPAR density is one of the key variables that modulate synaptic strength and plasticity. AMPARs are multi-subunit proteins coded by genes Glu1-4. Each AMPAR is made up of two identical heterodimers. In the hippocampus, most of the AMPARs are GluA1/2 or GluA2/3 heterodimers [24]. Previous studies such as [25] have indicated that long-tailed AMPARs, coded by GluA1 and GluA4, are exocytosed in an activity-mediated manner during learning and synaptic strengthening. Short-tailed AMPARs, coded by GluA2 and GluA3, are dynamically recycled at the synapse in the absence of synaptic activity <inset citation>. Hence, most of the research pertaining to AMPARs' role in synaptic plasticity is focused on GluA2 and GluA1.

In order to study the role of AMPAR endocytosis on memory retrieval, Lopez et al. used $GluA2_{3Y}$. $GluA2_{3Y}$, a mutation of the wild type (WT) GluA2 subunit, which inhibits the endocytosis of AMPARs containing GluA2. $GluA2_{3Y}$ contains mutations in three tyrosine residues, where the tyrosines are mutated to alanines. In WT animals, [26] showed that the phosphorylation of tyrosine in GluA2 is essential for its endocytosis. The $GluA2_{3Y}$ mutation inhibits activity-dependent AMPAR endocytosis while maintaining the constituent and basal rates of AMPAR endocytosis. Infusion of $GluA2_{3Y}$ with RPI and DMSO (RPI control) prevented the disruption of memory retrieval, indicating that blockage of AMPAR endocytosis prevents forgetting or the loss of memory retrieval. Scrambled versions of the $GluA2_{3Y}$ gene were infused in the animals as a control for $GluA2_{3Y}$.

Lopez et al. then showed that protein synthesis inhibition decreased the levels of GluA1 at the postsynaptic size while the levels of GluA2 remained unaffected. They showed that this decrease in GluA1 density could be alleviated by the infusion of $GluA2_{3Y}$. They found that RPI applied in the absence of memory retrieval did not cause this decrease in GluA1 levels. As the density of GluA1 levels is mediated by memory retrieval, they hypothesized a causal role of NMDAR activity-mediated AMPAR trafficking.

Extending this study, Dong et al. analyzed the role of PKMζ in memory retrieval. PKMζ is a protein kinase that is misallocated to the neurofibrillary tangles in Alzheimer's disease [27]. In order to understand the potential mechanism by which PKMζ affects memory retrieval, Dong et al. used PKMζ inhibitor ZIP. They found that ZIP inhibits AMPAR recycling, and infusion of $GluA2_{3Y}$ alleviated this. Dong et al. also used Alzheimer's mice model APP23/PS45 in order to study the role of AMPAR endocytosis in forgetting in Alzheimer's. The mice were trained to perform the Morris Water Maze, and the amount of time spent in the target quadrant was used as an estimate of recovery from disrupted memory retrieval. They found that infusion of $GluA2_{3Y}$ resulted in significant memory recovery than the control.

Relation between forgetting and engram accessibility

The reduced retrievability of memory that was once successfully encoded or consolidated using natural cues is referred to as forgetting. In experiments, forgetting can be studied by applying the same cues that were used during encoding, and the extent of retrievability can be measured. Natural forgetting is often considered a feature of the brain as it enables efficient storage and retrieval of essential information. Pathological forgetting spans from hypermnesia to severe amnesia. Individuals with Hypermnesia have an impaired natural forgetting process. These individuals possess extraordinary memory prowess and have difficulty forgetting past events. On the other hand, individuals affected by severe amnesia have extreme difficulty remembering prior events. Natural forgetting lies at the midpoint between these two extremes, enabling us to forget insignificant details while retaining essential information. An approach to better understanding how they differ would be to study their molecular underpinnings.

It is widely accepted in the neuroscience community that variation in the accessibility of an engram varies the extent of memory retrieval [28], [29], [30]. When access to an engram is hindered, memory retrieval is disrupted. As discussed above, the work of Ryan et al. [5] provides evidence backing this principle. When the synapses onto an engram were disrupted, the animals were no longer able to retrieve the memory corresponding to fear conditioning. But, direct activation of the engrams was able to reinstate memory retrieval. These engrams, which are not activated by the presentation of natural cues, but their direct activation results in memory retrieval, are referred to as silent engrams.

The very nature of these silent engrams hints at their possible role in the early stages of natural forgetting. When the natural cue is no longer able to evoke the expected response, the observed behavioral output will correspond to forgetting. Extending the cue extinction study in Nabavi et al. [16], natural forgetting is also proposed to be mediated by the repeated presentation of the conditioned stimuli (e.g., tone) in the absence of the unconditioned stimuli (e.g., shock). When this is performed, the conditioned stimuli no longer have the significance associated with the unconditioned stimuli. This would result in the decoupling of the conditioned stimuli and the unconditioned stimuli and subsequently result in forgetting. While the study of silent engrams is still at its infancy, it does propose a promising approach to understanding natural forgetting.

CONCLUSION

Memory is defined as the process of encoding, consolidating, and retrieving prior information, for future utilization. In the evolutionary sense, memory is essential for developing adaptive behavior in organisms. In order to better understand the concept of memory, engrams and the physical substrates of memory, are studied. Recent advances in genetic and molecular tools available have enabled the identification of neurons that are active during learning. Subsequent advances in the field of optogenetics and imaging enabled the epigenetic tagging and activation of engram neurons. This opened the possibility of understanding fundamental mechanisms that govern the different memory processes, such as encoding, consolidation, and retrieval. Subsequent studies described key physiological attributes which can be used to differentiate between engram and non-engram cells. Finally, studies describing the potential molecular mechanisms underlying these physiological changes paved the way to understanding memory retrieval and forgetting and also presented possible treatments for rescuing memory retrieval in Alzheimer's [31].

Future Direction

While a lot of studies have focused on memory processes such as memory consolidation and retrieval, very little is known about memory storage. The work of Ryan et al. [5] showed that while memory consolidation is protein synthesis-dependent and is hampered during induced amnesia, memory storage is resistant to protein synthesis inhibition and persists through induced amnesia. The exact molecular mechanisms underlying memory storage in these silent engrams are still unknown. The mechanisms mediating the transition of active engrams into silent engrams are still yet to be identified. All the studies discussed in this review paper use rodent models such as mice and rats. The works that studied memory retrieval in Alzheimer's also employed rodent models. Hence, a key challenge to be addressed in the field is the translation of findings from rodent models to humans. Almost all the studies discussed in the review employ fear conditioning as the main task on which the animals are trained. While rodent fear conditioning is quick and efficient, it limits the learning to the amygdala and hippocampus. The studies need to be scaled up to the complexity of human memory formation. It will be interesting to study how the distributed engram ensembles interact with each other and if there are any differences in the inter- and intra- engram interactions. The information gained about the mechanisms underlying engram functioning can be used to develop efficient reinforcement learning agents and deep learning architectures.

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