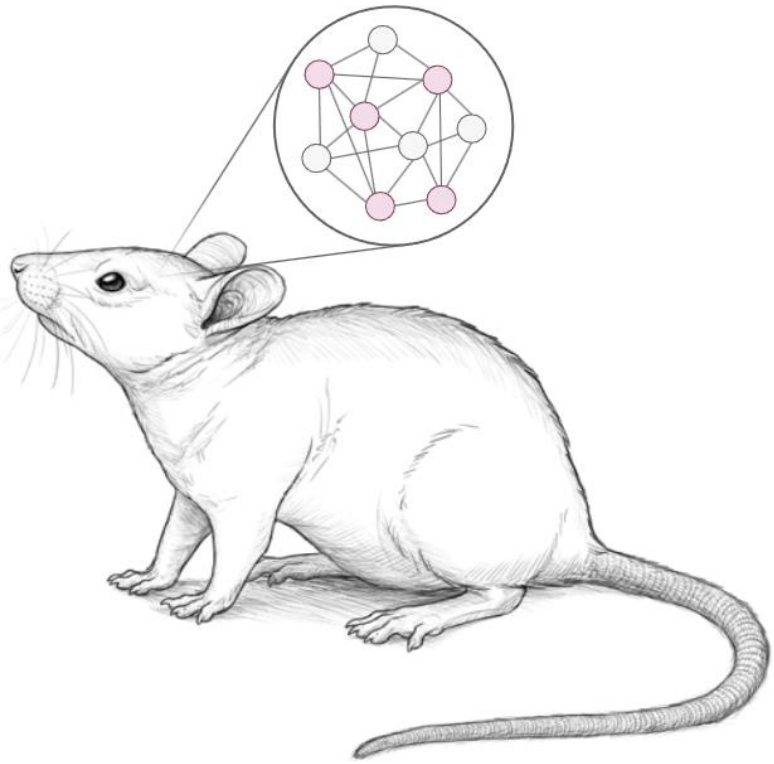

# Memory Linking and Engram Allocation:

## Exploring the Interconnected Mechanisms

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## Abstract

The substrate of memory is thought to be the memory engram; a network of neurons activated by a learning event that undergo changes to contain a memory of this event and which later are reactivated when remembering said event. Several mechanisms controlling neuronal memory engram allocation include plasticity factors and inhibitory mechanisms. The most investigated mechanism of engram allocation is that of differential excitability. The transcription factor CREB increases neuronal excitability, which in its turn increases a neuron's likelihood of being allocated to a memory engram. This mechanism is also implied to be involved in the process of memory linking. Here, two unrelated experiences are integrated and linked together. As a result, the recall of one memory can trigger the recall of the other unrelated memory. Indeed, studies have shown that increased neuronal excitability causes co-allocation of neurons to two engrams, provided there is a limited time-frame between the two events. However, several recent findings suggest neuronal excitability is not the only mechanism at play. The theory of synaptic tagging and capture, as well as inhibitory mechanisms might also partly be responsible for memory linking. Further factors that are suggested to be involved are synaptogenesis and dendritogenesis. In this essay, it is hypothesised that all these mechanisms are linked together by a transcription factor that contributes to all these mechanisms: CREB. This transcription factor is thought to mediate neuronal allocation to memory engrams, and thereby memory linking, via these processes. However, the role of CREB has not sufficiently been investigated in this context to draw such a conclusion. Therefore, it is suggested here that future research focuses around the interplay of all mechanisms proposed to be involved with memory linking, rather than merely looking at one aspect. Further, enlightening more effects of CREB on memory linking is urged.

# Introduction

Memory is omnipresent in our daily lives. From for example remembering the route to work to recognising your colleagues; and from remembering the information your colleagues were telling you, to trying to keep up when doing intricate calculations. Memory is essential in our day to day functioning, something which becomes even clearer when it is impaired. For example, you might have difficulties with aforementioned calculations after a bad night of sleep. Or, when your family member with dementia has a hard time recognising you.

Different areas in the brain seem to be responsible for different aspects of memory (Thompson and Kim, 1996). Multiple of these aspects can be distinguished, including for instance long-term memory, which encompasses for example remembering people and routes. Further aspects are short-term memory, which in this example is used for remembering recent information, and working memory, which here is used when doing calculations (Cowan, 2008). The areas of the brain involved with memory are quite widespread, ranging from a.o. cortical areas to areas from the limbic system. One of the main brain areas responsible for memory functioning is the hippocampus. This region, often referred to as the "seahorse", is required for instance for the encoding of spatial information and the ability to remember specific events, also called episodic memory (Lisman et al., 2017). The amygdala, another area considered to be important for memory, is involved with the emotional aspects underlying learning, such as fear memory (Ehrlich et al., 2009).

The process of acquiring memories and later being able to recall them occurs in multiple steps. Firstly, there is memory encoding or acquisition of the memory. In this step, information from an experience is "transformed" to a memory (Davachi and Wagner, 2002). Secondly, there is memory consolidation. This is the process in which these newly-formed temporary and labile memories are transformed into long-lasting and relatively stable ones so they can be stored for a prolonged period of time (Squire et al., 2015). Lastly there is memory retrieval; when the memory is reactivated and becomes available so that a past event can be recalled again. Further, reconsolidation can also occur; every time a memory becomes reactivated, it is thought to undergo again a process of consolidation in order to be maintained (Alberini, 2005).

Whilst we have an increasing understanding of the brain regions involved with memory and the steps of which memory processes consist, the question of what the neuronal substrate of memory itself is, remains. Or in other words, *how* is memory stored? Currently, one of the most prominent theories is that memories are stored into a network of neurons, a so-called memory engram. With this theory, other old unknowns regarding memory might be resolved. For instance, the processes behind memory-linking remain unknown. In memory linking, two unrelated experiences are integrated and linked together. Consequently, the recall of one memory can trigger the recall of the other unrelated memory. Whilst most often this process is beneficial in daily life, it can become maladaptive in mental disorders such as schizophrenia (Jung and Lee, 2016). It is therefore of utmost importance to investigate the mechanisms behind memory linking, which will be done in this essay within the context of the memory engram. Thus, I aim to answer the following research question: which processes at the level of the memory engram contribute to the phenomenon of memory linking? I will approach this question by looking into which mechanisms are responsible for recruiting neurons into the memory engram. Next, I will argue that these mechanisms also contribute to the observed memory-linking. Firstly, however, it is pertinent to deepen our knowledge of the memory engram itself.

## Discovering the memory engram

In 1904, the German scientist Richard Semon theorised what the neural substrate of memories could be. He believed an experience to activate a subset of cells to undergo changes to contain a memory and to later be activated when remembering said experience. Hereby, he introduced the term "engram", which is akin to a memory trace (Josselyn & Tonegawa, 2020; Tonegawa et al., 2015).

Largely ignored for the next decades, Semon's theory was revived in 1978 by Daniel Schacter and colleagues (Schacter et al., 1978). Who, in their review article, linked the relatively old theory to the modern ideas. More recently, with the discovery of new techniques it became possible to study the memory engram more properly (See also Box 1 for an example of commonly used methodology*)*. One of the first to experimentally observe the memory engram were Reijmers et al. (2007). Using a transgenic mouse model, they investigated whether the same neurons in the basolateral amygdala (BLA) were active during learning and related memory retrieval. Indeed, they successfully located a group of neurons that were active during the experienced fear conditioning training. Subsequently, they observed the neurons activated in the memory retrieval phase, when the mice were subjected to the negative context without receiving an accompanying shock. Further investigation showed that similar neurons were active during both contexts, which entails that the memory engram consists of these neurons (Figure 1). Since then, similar results have been repeatedly found for memory traces across multiple brain regions, including the hippocampus, cortex, and amygdala (Nakazawa et al., 2016; Tayler et al., 2013; Zelikowsky et al., 2014).

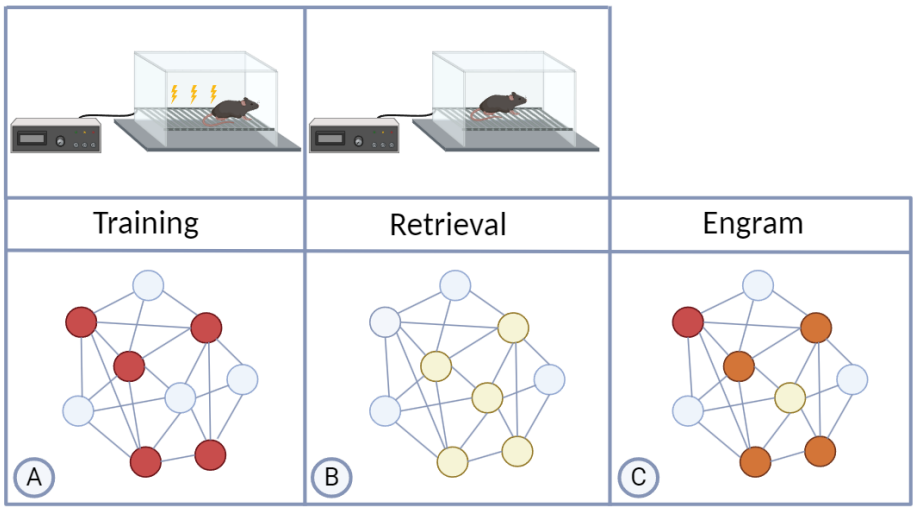


*Figure 1. A memory engram during the object location task.* A) Many neurons are active during the training phase. B) During the retrieval of the memory at a later date, many of these neurons reactivate. C) The neurons active during both phases are thought to comprise the engram of the learning experience (orange neurons). *Created with Biorender.*

Building upon these exciting observations, research can be taken a step further. One such question is, what will occur when these observed processes are disrupted or manipulated? For instance, one study demonstrated that reactivation of engram cells encoding a positive memory could suppress stress-inducing behaviours (Ramirez et al., 2015). Another study showed that mice undergoing a contextual fear conditioning test in training context, do not show a fear response when placed in a neutral and new context 24 hours later. However, when optogenetically stimulating the dentate gyrus engram cells that were present during the training

***Box 1. How to study the memory engram.***

At first glance, studying the memory engram seems to be quite a complex process. Let us thus break it down by looking at one of the earlier studies conducted as an example. When studying the memory engram, it is key to firstly identify which cells are active during formation of a memory and isolate and label them for future manipulation of the engram. Immediate early genes, such as c-fos, can be used to identify which cells are active during memory formation. Reijmers et al. (2007) used this as a basis for their transgenic mouse model, the TetTag mouse (Figure 2). These mice have a transgene for tetracycline transactivator (tTA) which gets transcribed along with c-fos. tTA can trigger the expression of a downstream target gene by binding to its responsive element (TRE). If TRE is then linked to for instance tau-LacZ, which stains the cell blue, the neuron becoming active results in it being stained blue due to the TetTag system. However, seeing as only the activity of neurons during the learning event is of importance, there should be an off and on switch on the system. This can come in the form of doxycycline (dox). When mice are on a dox diet, dox prevents tTA from binding to TRE, thus preventing the staining of active neurons. Just before the learning event of interest, mice can be taken off of the dox diet and afterwards can be taken on the diet again, enabling the staining of neurons active in the learning event (Ramirez et al., 2014; Reijmers et al., 2007). Naturally, the specific approaches vary between studies (such as targeting different immediate early genes) yet they all follow this global concept. Further, this same concept can be used to manipulate neural activity, for instance by adding an optogenetic receptor to TRE. Moreover, neuronal activity during the learning event can be compared with activity of the recalling event, by for instance performing regular immunohistochemistry targeting c-fos.

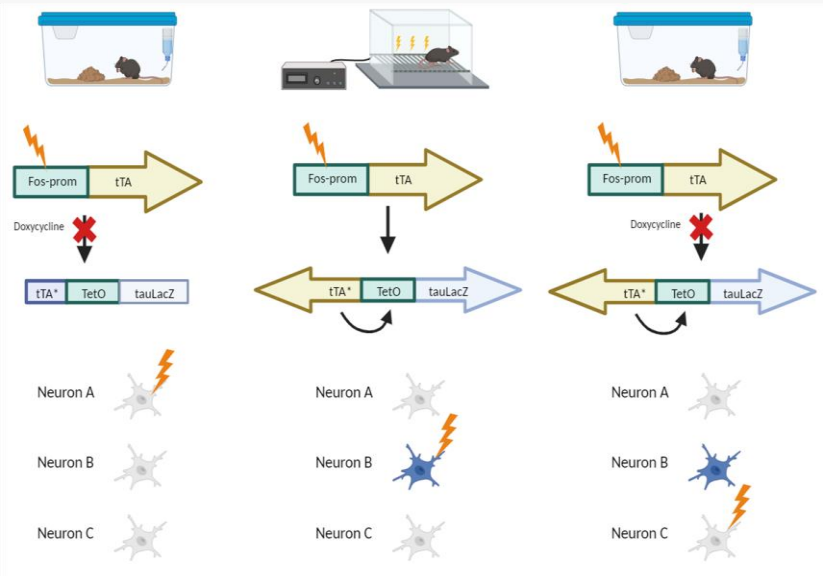


*Figure 2. The TetTag system.* When in the home cage, TetTag mice are on a doxycycline diet that suppresses the tagging of neurons with tauLacZ. During the learning event, they are taken off the doxycycline diet and tagging of the activated neurons is occurring. Afterwards, when being put on the diet again, the neurons activated during the learning phase will continue to express tau-LacZ because the feedback loop maintains its own activation (tTA keeps activating TetO), as can be seen in neuron B. *Adapted from Reijmers et al. (2007), altered with Biorender.*

context, mice suddenly showed freezing behaviour in the neutral context (Liu et al., 2012). Similar results have been obtained by a.o. Roy et al. (2016), Ramirez et al. (2013) and Ryan et al. (2015). Thus, reactivating engram cells causes animals to remember the conditions under which the cells were activated for the first time. Or in other words, reactivation of the cells stimulates memory retrieval. On the other hand, silencing the engram cells seems to prevent this (Han et al., 2009).

Whilst in this essay, the focus will be mostly upon memory engrams of fear memories, memory engrams also can contain information for other aspects of cognition, such as social recognition and object location (Karaca et al., 2021; Okuyama et al., 2016). Further, studies of the memory engram encompass a broad range of contexts, including but not limited to Alzheimer's, sleep deprivation and addiction (a.o. studied in Bolsius et al., 2023; Hsiang et al., 2014; Perusini et al., 2017).

We can conclude from this section that the theory of memory engrams can deepen our knowledge on the topic of memory. After all, we can study memory in a different light now that we realise that memories are stored, consolidated and retrieved within memory engrams.

## The mechanisms behind engram allocation

One of the questions that seems to flow logically from the discovery of the memory engram is that of which factors are involved with recruitment or allocation of the neurons to the engram. After all, only a specific subset of neurons from a region are allocated to the memory engram, whereas others are not (Miry et al., 2021). Many different factors have been proposed to be influential in engram allocation, such as neural age or location (Chawla et al., 2013; Erwin et al., 2020). Another one is synaptic plasticity. Specifically, changes in neural connections are an important aspect of memory (Ramirez and Arbuckle, 2016) and might therefore be a promising factor involved with engram allocation. It has, for instance, been suggested by Jeong et al. (2020) that long-term potentiation (LTP) is essential. When in axonal connections from the thalamus and auditory cortex to the lateral amygdala LTP was optogenetically induced, the receiving neurons were preferentially recruited into the memory engram. Moreover, it has been demonstrated that neurons can compete for the relevant proteins required for LTP when there is reduced protein synthesis (Fonseca et al., 2004). This same competition has been observed by (Sajikumar et al., 2014), who also showed that synapses potentiated by memory induction compete with each other for plasticity related proteins. From these findings, it is thus hypothesised that there is competition between neurons regarding plasticity factors, resulting in selective engram allocation.

Besides mechanisms that allocate neurons to an engram, there are also mechanisms that prevent them from being recruited. Memory engrams are quite consistent in size per memory, a feature that might be the result of inhibitory interneurons (Giorgi and Marinelli, 2021). Silencing interneurons in the amygdala, for example, increases the size of the memory engram. Interestingly, the size of the engram does not correlate with the strength of the memory (Morrison et al., 2016). Similarly, inhibitory mechanisms in the dentate gyrus have also been found to limit engram size (Stefanelli et al., 2015). Here, the granular cells recruited in the engram laterally inhibit neighbouring cells, preventing their allocation. This occurs via stimulation of somatostatin positive interneurons, which via GABAergic signalling inhibit the surrounding granular cells (Stefanelli et al., 2015).

Thus, it seems that both plasticity factors, as well as inhibitory processes are involved with the allocations of neurons to a memory engram. The former increases the chances of the

neuron itself being allocated to the engram, whilst the latter decreases the chances of a neighbouring neuron being allocated to the engram. Additionally, another third mechanism is posed to be influential, which will be addressed in the upcoming section.

## The role of differential excitability in engram allocation

Two of the possible mechanisms responsible for engram allocation have been discussed in the previous paragraph. However, by far the most well-established theory for engram allocation is the theory concerning neural excitability. This states that neurons differ in intrinsic excitability and those neurons with a higher excitability have a higher chance at being recruited to the memory engram (Miry et al., 2021). One of the earlier pieces of evidence for this theory has been provided by Han et al. (2007). They were able to experimentally manipulate the excitability of lateral amygdala neurons and it was shown that as a consequence, these neurons were more likely to become part of a fear memory engram. Following up on this study, it became clear that the memory of the fear event becomes blocked after selectively deleting the relatively more excitable neurons by means of apoptosis (Han et al., 2009). The research group of Han and colleagues was not the only one to find the link between intrinsic excitability and likelihood of engram allocation, rather, these findings have been consistently repeated since then (Gouty-Colomer et al., 2015; Yiu et al., 2014; Zhou et al., 2014). The importance of relative excitability is not merely limited to the amygdala. Instead, it has been found to be at least also true for the hippocampus (including the CA1 region and dentate gyrus), cortex and insula (Matos et al., 2019; Park et al., 2016; Sano et al., 2014; Sekeres et al., 2012).

The factor that seems to be the most influential on the intrinsic excitability of a neuron is cAMP-response element binding protein, also known as CREB. CREB is a transcription factor and can bind to various target genes including neurotransmitters, growth factors, transcription factors and metabolic enzymes. Consequently, CREB has been known to be involved in processes such as neuronal development, neural plasticity, proliferation and differentiation (Sakamoto et al., 2010; Wang et al., 2018). In addition to these functions, CREB is able to modulate excitability of neurons via a.o. decreasing voltage-gated K+ currents by regulating the transcription of K+ channels (de Armentia et al., 2007; Dong et al., 2006). This has been observed for example using whole-cell recording techniques (Zhou et al., 2009). Moreover, CREB overexpression leads to a smaller afterhyperpolarisation, suggesting enhanced excitability of CREB overexpressed cells as well (Lisman et al., 2018). From this, CREB seems to be a likely candidate involved with engram allocation due to differential excitability. Indeed, Han and colleagues already showed back in 2007 a possible involvement of CREB in engram allocation; neurons artificially made to overexpress CREB were 3 times more likely than their neighbours to be included in the memory engram (Han et al., 2007). Consistent with this, when Kir2.1 (which is an inwardly rectifying K+ channel that causes decreased action potential firing) is coexpressed with CREB overexpression, the effects of CREB on memory performance are attenuated (Yiu et al., 2014). This further seems to indicate that CREB may enact its effects through excitability. It must be mentioned that one study, however, seemed to find no such relation between CREB expression and engram allocation (Lacar et al., 2016). Nevertheless most studies point towards the involvement of CREB (*e.g.*, Hsiang et al., 2014; Sano et al., 2014; Yiu et al., 2014). This is further supported by the study of Park et al., who also found CREB to increase neuronal excitability (Figure 3). Additionally, they found that chemogenetically silencing neurons overexpressing CREB results in attenuation of the memory expression, whilst silencing neurons that do not have this overexpression did not alter memory expression (Park et al., 2016). Moreover, overexpressing CREB regulated transcription

coactivators (CRTCs), which are able to potentiate CREB function, has been shown to enhance memory consolidation and reconsolidation, further suggesting the involvement of CREB with engram allocation (Sekeres et al., 2012).

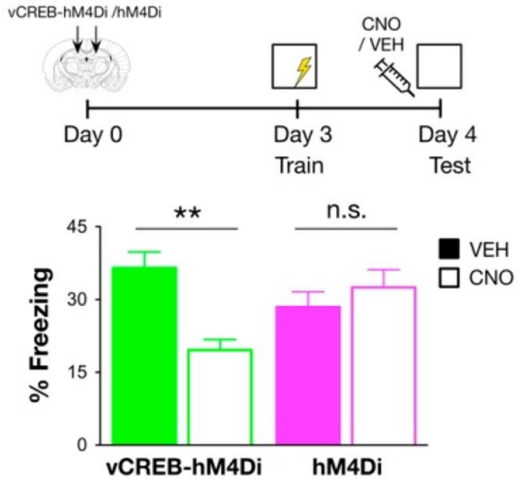


*Figure 3. CREB overexpression results in engram allocation.* Pre-test chemogenetic signalling of neurons overexpressing CREB during training decreases subsequent freezing behaviour indicating an impaired memory (CNO in mice expressing vCREB-hM4Di). Silencing a similar number of random neurons (expressing hM4Di without vCREB) does not disrupt freezing behaviour. These findings suggest a preferential allocation of neurons overexpressing CREB to an engram. *Adapted from Park et al. (2016).*

To conclude from this section, research seems to indicate that the main mechanism involved in engram allocation is the intrinsic differences neurons have regarding their excitability, which seems to be driven by CREB expression. Following this, the possibility arises of the involvement of CREB with memory linking. In the upcoming sections, this possibility will be explored. Firstly, though, we will revisit the topic of memory linking in itself.

## Memory linking within the memory engram

Now that we have discussed the mechanisms behind engram allocation, it is time to re-introduce the phenomenon of memory linking. As briefly mentioned in the introduction of this essay, memory linking occurs when two unrelated experiences are integrated and linked together, due to the interrelated nature of memories (Seghal et al., 2018). As a result, the recall of one memory can trigger the recall of the other memory. In practice, an example of memory linking is the creation of false memories, which has been shown in mice by amongst others the study of Lau et al. (2020). They showed that if mice are exposed to a neutral stimulus after a cued fear conditioning test, the mice also develop a fear response when being presented with this neutral stimulus later.

At the level of the memory engram, it is thought that memories are linked due to co-allocation of neurons; they become a constituent of both engrams. In other words, the engrams of linked memories share neurons (Rashid et al., 2016). Whether two memories can become linked depends for a part on the intermission between the two events they encode. Two events with a close temporal proximity become linked via co-allocation of neurons to overlapping engrams. On the other hand, when the events are more distant from each other, neurons are not co-allocated but are recruited to separate engrams (Jung et al., 2023 ; Rashid et al., 2016).

Memory linking does not only occur due to a short time interval in between two experiences; a similar emotional valency attributed to two experiences can also cause them to become linked. This has been shown for instance with Pavlovian conditioning. Mice performed a conditioned taste aversion test and four days later an auditory-cued fear conditioning test. When being presented with the aversive taste later, mice showed freezing behaviour as if they were presented with the auditory stimulus of the fear conditioning test. Thus, the two negative events became linked to one another (Yokose et al., 2017).

In practice, memory linking can be thought of as an aspect of relational memory, the type of memory that is concerned with associations between items and events (Avery et al., 2019). Whilst thus memory linking has its advantages, it may also become maladaptive. The linking of information can be inappropriate or indiscriminate. For instance in patients with post-traumatic stress disorder or schizophrenia, impairments in relational memory can decrease the patient's functioning and quality of life (Jung and Lee, 2016). Likewise, psychotic patients and people suffering from major depression seem to have deficits in this type of memory (Avery et al., 2019; Nemeth et al., 2016). Whilst admittedly memory linking is a small component of relational memory, it is crucial to understand its underlying processes nevertheless.

## Differential excitability and memory linking

In the previous paragraphs, the problem of memory linking was defined, as well as the role of differential excitability within the memory engram. However the main question of this essay still remains to be addressed. That is, which processes at the level of the memory engram contribute to the phenomenon of memory linking?

The main hypothesis is that differential excitability is of utmost importance in the process of memory linking. More specifically, differential excitability lies at the basis of the co-allocation of neurons to memory engrams. Research from Rashid et al. (2016) seems to confirm this theory (Figure 4). In this study, mice received two distinct auditory conditioned stimuli (CS) at two events either with a long (27h) or short (6h) intertraining interval, with CS1 being negative and CS2 being neutral. When optogenetically exciting neurons and thereby artificially co-allocating neurons to both CS1 and CS2, mice showed freezing behaviour when hearing CS2, indicating that the memory of CS1 has become linked to that of CS2. This, however, was only found when the intertraining interval was short. T he same was repeated but instead of artificially exciting neurons directly, CREB levels were virally overexpressed. Again, mice showed freezing behaviour when presented with CS2 after learning events of both stimuli, indicating that CREB overexpression resulted in neuronal co-allocation and consequent memory linking. These findings, along with the notion that learning temporarily increases the excitability of neurons, (Oh et al, 2003; Thompson et al., 1996) lead the authors to suggest that after the first learning event, the neurons allocated to the memory engram have temporarily a higher excitability, which increases their chances of being recruited into a second memory engram, resulting in co-allocation.

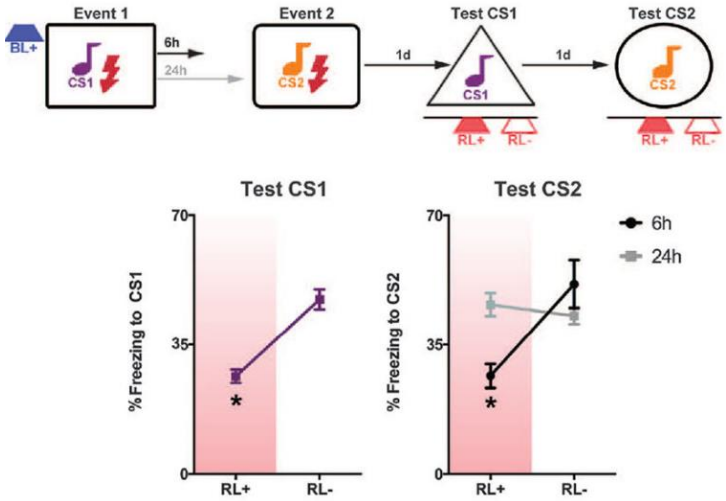


*Figure 4. Memory linking due to increased excitability* Exciting neurons before CS1 paired with a negative stimulus allocates them to the engram of engram 1 and of engram 2, if engram 2 occurs 6 hours, but not 24 hours, later. This can be deducted from the freezing behaviour that occurs when the neutral stimulus is presented. *Adapted from Rashid et al. (2016).*

Lau and colleagues (2020) provide further evidence for memory linking resulting from co-allocation. Similarly as in the previous study, mice were exposed to a negative CS1 and a neutral CS2. Shortly after the presentation of CS1, the allocated neurons were optogenetically inhibited, preventing the temporary increase of excitability after a learning event. As a result of this, when CS2 was presented after 6 hours and later recalled, no freezing behaviour was shown by the mice, indicating that memory linking did not occur. Further, when the optogenetic inhibition did not transpire, memory linking was observed. This suggests that the co-allocation to engrams encoding different events might result in the linking of these events. Interestingly, older mice have a lower intrinsic baseline excitability in the CA1 region of the hippocampus (Oh et al., 2010). On this premise, Cai et al. (2016) found that consequently, aged mice show less memory linking compared to young mice. When virally increasing the excitability before the two learning events, the aged mice now did show freezing behaviour when re-exposed to the second, neutral, event. Thus, this study also reiterates the importance of neuronal excitability.

Not only has this importance been experimentally investigated *in vivo,* computational studies are likewise consistent. With their rate-based computer model, Delamare and colleagues showed that when the initial excitability of a subset of neurons is enhanced, the allocation to memory engrams is biased towards these neurons. Further, when they inhibited the subpopulation of neurons with an enhanced excitability, freezing behaviour during memory recall of a neutral memory was decreased, suggesting a decreased link to the negative memory (Delamare et al., 2022). Additionally, Kastellakis et al. (2016) showed with their computational model that differential excitability is involved with the pairing of weak memories with strong memories.

Thus, from the discussed studies above, it becomes clear that differential excitability indeed is at the basis of memory linking. However, one must ask whether differential excitability alone can be held fully accountable for memory linking or whether there might be different processes involved as well. An implication of the idea that memory linking occurs due to overlapping neuronal populations is that when silencing neurons within these engrams,

memories for all events encoded by these neurons ought to be impaired. Yet, it has been shown that linked memories can be selectively silenced (Abdou et al., Yukose et al., 2017). What possible explanation could there be for this? It seems that in addition to overlapping neurons, memory engrams sharing dendrites might be at play during memory linking as well. Xu et al. (2023) looked at spine loss during fear extinction. If spine loss occurred on an overlapping neuron, but not on overlapping dendrites, fear extinction was specific. That is, for only one stimulus. When the dendritic branches in which spine loss occurred were overlapping, however, fear extinction was generalised. This seems to suggest that in memory linking, overlapping dendrites also seem to be at play.

In addition to this, computer models emphasise that besides differential excitability, other factors are involved with engram allocation. From the model of Delamare and colleagues it becomes clear that regulation of inhibition, and thereby inhibitory cell types, is also an important factor influencing memory linking. If levels of inhibition are too great, memory linking is prevented from taking place (Delamare et al., 2022). Furthermore, the model of Kastellakis et al. (2016) suggests the significance of plasticity related proteins (PRPs). PRPs are generated after a strong LTP event and required for synaptic tags in neurons. That is, lasting higher protein levels that make future LTP events more likely. Both high PRPs levels in the soma of the neuron as well as neuronal excitability influenced the linking of weak memories with stronger ones.

Of course, these computer models have the limitation of being too simplified from nature. Therefore, it is of utmost importance that these suggested mechanisms also hold to be true *in vivo.* Indeed, a study of Frey and Morris (1997) is in line with the earlier proposed idea of Kastellakis, the so-called synaptic tagging and capture (STC) idea. In their experiment, they show that usually weak stimulation of a neuron does not lead to LTP, unless this neuron has already established LTP up to 3 hours before. This posited the idea that LTP initiates a creation of a short lasting synaptic tag (to which later PRPs are recruited) at the potentiated synapse, making it more likely that LTP takes place at that synapse again within the next few hours (Frey and Morris,1997; Govindarajan et al., 2011). Through this, STC could also be associated with memory linking. STC makes repeated engagement of synapses in multiple memory engrams more likely, thus contributing possibly to memory linking as well.

## CREB, the common denominator?

From the evidence provided in the last sub-section, it becomes increasingly more likely that the idea of differential excitability alone is insufficient in providing a full explanation for memory linking. Instead, theories regarding inhibition or synaptic tagging (and thereby synaptic plasticity) have been proposed. Rather than being mutually exclusive, I propose these theories to be mutually inclusive; interacting with one another to ultimately cause memory linking. At the centre of this interaction, CREB is a likely candidate. Earlier, the role of CREB in causing differences in intrinsic neuronal excitability has been discussed at length. However, as mentioned very briefly, CREB is involved with many more processes. A disadvantage from several of the studies mentioned in earlier sections (such as Han et al., 2007; Hsiang et al., 2014 and Sano et al., 2014) is that they artificially manipulated CREB levels without monitoring the excitability of the neurons investigated. Consequently, it cannot be said with confidence that the observed memory effects are due to CREB changing the excitability or CREB's effects on other processes, or an interplay of the two.

One of the additional observed effects of CREB concerns itself with synaptic plasticity. CREB is able to alter dendritic spine morphology, as well as increase the number of dendritic

spines (Sargin et al., 2013). This is not only the case for the amygdala, as Sargin et al. have investigated, CREB has also been proven to be involved with spine formation in the hippocampus (Leuner et al., 2003; Yiu et al., 2011). As mentioned in the previous section, dendritic spines also play a role with memory linking. From this, it seems likely that CREB could mediate memory linking not only through increasing excitability, but also through stimulating dendritic spine overlap of multiple memory engrams by increasing dendritic spine formation.

Additionally, acute expression of CREB can cause LTP (Marie et al., 2005). Moreover, CREB initiates transcription of plasticity related genes (Redondo et al., 2010). It has been suggested that as a result of this, CREB might also be involved in the assembly of PRPs Okuda et al., (2020), although it ought to be mentioned that as of today, this has not been experimentally tested yet. As an additional consequence, the plasticity related genes could be distributed cell-wide, priming synapses for the capture of LTP Barco et al., 2002. Via these three (proposed) functions of CREB with synaptic plasticity, its protein levels might influence STC. For memory linking, this would entail that after a first learning event, CREB is able to promote a second learning event through these mechanisms. That is, CREB could recruit PRPs to the soma of the neurons, thus promoting allocation to a second engram. Further, by "capturing" a second LTP event, CREB can increase the probability of being allocated to a second engram, thus promoting memory linking.

Further, CREB function can also be linked to the inhibitory processes associated with engram allocation and suggested in memory linking. Phosphorylated CREB stimulates transcription of the somatostatin gene (Gonzalez and Montminy, 1989). As mentioned when discussing the mechanisms behind engram allocation, interneurons in the hippocampus are somatostatin positive. In fact, the same holds for one class of cortical interneurons (Riedemann, 2019). Somatostatin is able to lower dendritic spine density, as well to depress excitatory postsynaptic currents (Tallent and Siggins, 1997; Hou and Yu, 2013). To some extent because of this, GABAergic interneurons can inhibit neurons that will not be recruited to the memory engram. Following this reasoning, CREB might be able to influence inhibition of neurons excluding the memory engram through means of GABAergic interneurons and somatostatin. As the computer model of Delamare et al. (2022) stated, inhibiting neighbouring neurons might also be of influence in memory linking. Whilst no study has been performed yet to confirm this, possibly CREB is involved through this pathway in preventing disallocation, thereby stimulating co-allocation and thus stimulating memory linking.

With this reasoning, the interesting question also arises whether there are other transcription factors that could potentially mediate the same effects as described for CREB. Whilst this has not been examined yet, we can speculate on the matter nonetheless. One possible candidate might be transcription factor 4 (TCF4), despite being relatively little investigated in the context of memory. TCF4 has been implicated to be involved with synaptogenesis, dendritogenesis, and LTP (Badowska et al., 2020; Li et al., 2019). Further, TCF4 is thought to be of importance for the regulation of neuronal excitability. Deficits in excitability have been observed in mice heterozygous knock-out for the TCF4 gene (Rannals et al., 2016). Besides this, the transcription factor has been found in GABAergic interneurons (Kim et al., 2020). These functions make TCF4 a candidate for memory-linking research, in a similar manner as for CREB. However, as mentioned, this has no empirical foundation yet and future research ought to be conducted to solidify the role of TCF4 in memory linking.

From this, it is suggested that transcription factors mediate the mechanisms involved with engram allocation and, consequently, memory linking. Whilst the role for TCF4 remains insecure, the one of CREB is more established: CREB is an intricate molecule, involved with many

cellular functions and several of CREB's functions have the possibility of being involved with memory linking (for an overview, see Figure 5). However, it must be emphasised that although there is indirect research pointing towards the involvement of the aforementioned proposed functions of CREB in synaptic plasticity and inhibition with memory linking, no such studies directly investigating this relationship have been performed as of today. What is more, most research focussing on memory linking merely concerns looking at one proposed mechanism at a time. These are two limitations of the research being conducted currently and here I would like to suggest for future research to tackle these limitations. I hereby propose the focus to be shifted towards research investigating the interplay between for example synaptic plasticity and differential excitability in memory linking, and the role CREB may play herein. As has been conducted on several instances already, computer models might be a suitable start for this. However, the interplay between the proposed processes involved with memory linking and CREB ought to also be investigated *in vivo*.

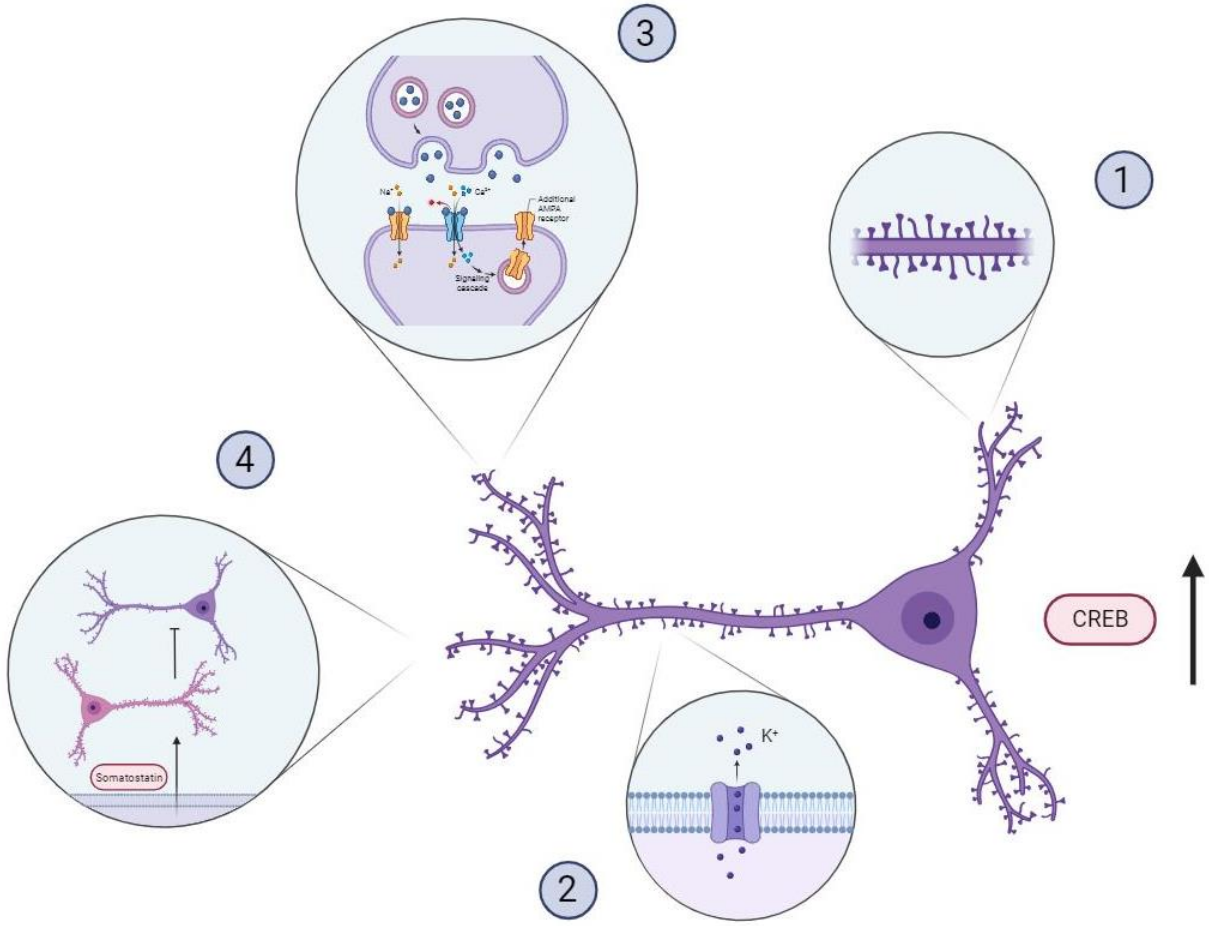


*Figure 5. The four mechanisms through which CREB is proposed to control memory linking*. 1) CREB stimulates synaptogenesis and dendritogenesis. 2) CREB increases neuronal excitability, increasing the likelihood of being allocated to the memory engram. 3) CREB is involved with LTP and the assembly of PRPs. 4) CREB induces somatostatin production, which stimulates GABAergic interneurons to inhibit neighbouring neurons. *Created with Biorender.*

## Conclusion

In this essay, the question of which processes on the level of the memory engram were contributing to memory linking, was central. After having provided information regarding the

context of this discussion, the memory engram and memory allocation, evidence was discussed elucidating that differential excitability seems to be the main process responsible for memory linking. More specifically, higher CREB levels were found to induce higher intrinsic neuronal excitability, causing a greater chance of engram allocation. After a first learning event, excitability remains relatively high, resulting in the neurons also being allocated to the memory engram of a second learning event. When taking a closer look, however, it appears that other processes might also be involved with memory linking. These include dendritic spine formation, inhibition of non-engram cells and synaptic tagging and capturing. These processes appear to be mutually inclusive. One of the factors enabling this mutual inclusivity is CREB, which seems to be functionally involved in all four processes involved with memory linking proposed in this essay. However, research as of today has neither focussed yet on the interaction of all processes contributing to memory linking, nor on CREBs interconnected role in this. It is thus suggested that this interconnectivity will be a pillar of future research. Of course, this essay has been limited as well; memory engrams are extensive and intricate and many of its mysteries have not yet been elucidated to us. Subsequently, numerous variables not discussed in this essay might also be of influence on memory linking. Naturally, future research also ought to aim at uncovering those. After all, the mechanisms could be the key to discovering what causes memory linking to become indiscriminate, hopefully relieving those suffering from maladaptive memory linking in the future.

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