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The dynamic nature of fear engrams in the basolateral amygdala

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Abstract

Great progress has been made in our understanding of how so-called memory engrams in the brain enable the storage and retrieval of memories. This has led to the realization that across the lifetime of an animal, the spatial and temporal properties of a memory engram are not fixed, but instead are subjected to dynamic modifications that can be both dependent and independent on additional experiences. The dynamic nature of engrams is especially relevant in the case of fear memories, whose contributions to an animal's evolutionary fitness depend on a delicate balance of stability and flexibility. Though fear memories have the potential to last a lifetime, their expression also needs to be properly tuned to prevent maladaptive behavior, such as seen in patients with post-traumatic stress disorder. To achieve this balance, fear engrams are subjected to complex spatiotemporal dynamics, making them informative examples of the “dynamic engram”. In this review, we discuss the current understanding of the dynamic nature of fear engrams in the basolateral amygdala, a brain region that plays a central role in fear memory encoding and expression. We propose that this understanding can be further advanced by studying how fast dynamics, such as oscillatory circuit activity, support the storage and retrieval of fear engrams that can be stable over long time intervals.

1. Introduction

A memory engram, as a theoretical concept, is the combined set of physical changes that occur within the brain as a result of a particular experience, and that enable the animal to memorize elements of that experience in order to inform future behavior. Experimental data indicate that these physical changes include synaptic and cell-intrinsic plasticity events that change the functional interactions among a sparsely distributed population of neurons referred to as engram cells (Tonegawa et al., 2015; Josselyn et al., 2015; Mayford and Reijmers, 2015). Memory engrams enable the storage of information without a need for continuous firing of action potentials, thereby supporting long temporal gaps between memory acquisition and memory retrieval. Though engram cells can be silent and stable over prolonged time periods, these periods are interrupted by “offline” modifications that do not require novel experience by the animal. For example, engram cells can become active again during sleep (Chen and Wilson, 2017). Offline modifications can cause a memory to

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rely on different groups of engram cells at different time intervals following memory acquisition, which is referred to as system consolidation (Frankland and Bontempi, 2005). Why the brain would subject memory engrams to offline modification is still unclear, but one likely function is to integrate the specifics of past experiences into more general models of the outside world (Penagos et al., 2017). The dynamic nature of memory engrams has been widely studied in hippocampal and cortical circuits. This review will focus on engrams that rely on circuits located in a subcortical region known as the amygdala, specifically a subdivision of the amygdala known as the basolateral amygdala (BLA). The BLA is connected with a large variety of other brain regions, and thereby contributes to many different brain functions (Janak and Tye, 2015). One of these functions is to support memories of frightening experiences, which are essential to the avoidance of future danger. We will refer to engrams that encode these fear memories as fear engrams. Fear engrams can be studied in the laboratory with the conditioned fear paradigm, a behavioral test in which a neutral conditioned stimulus is presented together with an aversive unconditioned stimulus (for example a tone and a footshock). This type of Pavlovian conditioning results in the formation of a fear engram that causes a fear response whenever the animal is confronted with the conditioned stimulus alone (for example freezing behavior when the tone is played). This review is not intended to provide a comprehensive overview of all the contributions made by BLA circuits to fear engrams, which has recently been done by several excellent reviews (Janak and Tye, 2015; Tovote et al., 2015; Orsini and Maren, 2012; Duvarci and Pare, 2014). Instead, this review will specifically focus on the complex spatiotemporal dynamics of fear engrams that have been observed in the BLA.

2. The basolateral amygdala as a storage site for fear memories

Work done by many labs over the past decades has established that neurons in the BLA make critical contributions to the storage and retrieval of fear memories, and therefore presumably to the formation of fear engrams (Janak and Tye, 2015; Tovote et al., 2015; Orsini and Maren, 2012; Duvarci and Pare, 2014). Though previous findings provide strong support for the notion that the BLA can be a storage site for fear memories, this idea is not universally accepted and intensely debated (Fanselow and LeDoux, 1999; Cahill et al., 1999; Paré and Quirk, 2017; Maren, 2003; Maren, 2000; Vazdarjanova, 2000; Pare, 2002). One extreme of the spectrum states that a fear memory is completely and permanently stored within the BLA, while the other extreme states that a fear memory is stored completely outside of the BLA, with the BLA merely modulating extra-BLA storage sites. The truth is likely somewhere in the middle, with the BLA functioning as one of the storage sites of a fear memory for at least part of the memory's lifetime. Though this idea reconciles reports of both impaired and unaffected fear memory acquisition or retrieval following the lesioning or silencing of the BLA (Maren, 2003; Phillips and LeDoux, 1992; Gale et al., 2004; Sananes and Davis, 1992; Do-Monte et al., 2015; Vazdarjanova and McGaugh, 1998), much more work needs to be done to more systematically determine under which conditions the BLA functions as a fear memory storage site, as well as the stability of BLA storage sites over time.

It is important to point out that the BLA is a heterogeneous structure. The BLA contains two adjacent nuclei, the basal amygdala nucleus (BA) and the lateral amygdala nucleus (LA),

which can be further divided into smaller anatomical subdivisions (Duvarci and Pare, 2014; Pitkanen et al., 1997). The BA and LA differ in their connectivity with other brain regions, and as a result make different contributions to fear engrams. For example, the LA plays an important role in engrams for cued fear memories, which result from experiences where a brief unimodal sensory stimulus, the cue, precedes an aversive experience (for example a 20 s tone immediately followed by a footshock). Unimodal cues can activate LA neurons through direct connections between the LA and primary sensory regions in the thalamus and cortex. In contrast, the BA plays an important role in engrams for contextual fear memories, which result from an aversive experience that is not preceded by a cue. In this case, the spatial location (i.e. context) where the aversive experience occurred functions as the conditioned stimulus. To use a spatial location as the conditioned stimulus, the hippocampus has to be involved. The hippocampus is directly connected with the BA, but not the LA, which likely explains the preferential involvement of the LA and BA in cued versus contextual fear engrams, respectively (Calandreau et al., 2005; Nader et al., 2001; Reijmers et al., 2007; Maren and Fanselow, 1995; Xu et al., 2016; Lucas et al., 2016; Onishi and Xavier, 2010).

The reported effects of BLA manipulations on fear memories led to an interest in finding possible engram cells that are located within the BLA. One approach for finding such engram cells is to record the activity of neurons in the BLA in-vivo while the animal performs a fear memory task. This led to the discovery of neurons in both the BA and LA that become more responsive to a tone following its pairing with a footshock (Herry et al., 2008; Quirk et al., 1995; Quirk et al., 1997; Goossens et al., 2003; Schafe et al., 2005; Rogan et al., 1997; Pare and Collins, 2000; Collins and Pare, 2000). Putative engram cells have also been detected in the BLA by imaging the expression of immediate-early genes (IEGs) such as Fos immediately following the acquisition or retrieval of a fear memory (Radulovic et al., 1998; Okuno, 2011). We developed a transgenic reporter mouse that enables the long-lasting expression of a reporter protein under control of the Fos promoter. Our goal was to tag BLA neurons that are active during acquisition of a fear memory, and then determine if these neurons are reactivated during retrieval of that fear memory. The underlying idea is that neurons active during an acquisition trial might be subjected to a form of synaptic and/or cell-intrinsic plasticity that causes these neurons to be recruited into a memory engram, which then results in reactivation of these engram cells when the fear memory is expressed during a subsequent retrieval trial. Using our transgenic reporter mouse, we found that the number of reactivated LA neurons positively correlated with the expression of a cued fear memory, and the number of reactivated BA neurons positively correlated with the expression of a contextual fear memory (Reijmers et al., 2007; Truiche et al., 2013; Davis et al., 2017). The existence of these reactivated BLA neurons is in agreement with the BLA being one of the storage sites of a fear memory, at least under the specific conditions and time-intervals studied.

An interesting example of the dynamic nature of fear engrams is a process known as reconsolidation. Blocking protein synthesis in the BLA immediately after the retrieval of a cued or contextual fear memory can impair future retrieval of the memory (Nader et al., 2000; Mamiya et al., 2009). These findings indicate that under certain conditions a retrieved fear memory has to be reconsolidated by a process that requires the synthesis of proteins that

are presumably used to re-stabilize synaptic connections of BLA neurons that are part of the fear engram. It has been proposed that reconsolidation provides engrams with the ability to update a stored memory when new information becomes available (Besnard et al., 2012; Hardt et al., 2010). The ability to dynamically update memory engrams after their initial storage makes sense, especially in the case of fear memories that can have severe consequences when expressed under inappropriate conditions. The idea that interfering with the reconsolidation of a retrieved fear memory might lead to its erasure has inspired efforts to develop it into a treatment for anxiety disorders such as post-traumatic brain disorder (Kroes et al., 2016).

3. Manipulating fear engrams

To consider a brain region as a storage site for a memory, it is not sufficient to find neurons within that brain region that become more responsive following the acquisition of the memory. For example, this pattern of activation could just indicate that these neurons are involved in processing of sensory input or behavioral output that is associated with the memory test, especially when using simple Pavlovian memory tests with defined conditioned stimuli and responses. Though this can be ruled out to some extent by using proper controls, definitive confirmation that a brain region functions as a storage site requires the demonstration of some type of synaptic or cell-intrinsic plasticity that 1) occurs during or following memory acquisition, and 2) is essential for behavioral expression of the memory. These two criteria appear to be met for fear engrams in the BLA: 1) synaptic plasticity has been observed in both the BA and LA following acquisition of contextual and cued fear memories, respectively (Yeh et al., 2006; Girardeau et al., 2017; Nonaka et al., 2014; McKernan and Shinnick-Gallagher, 1997; Rosenkranz and Grace, 2002), and 2) blocking or reversing acquisition-induced synaptic plasticity in the LA can impair the retrieval of a cued fear memory (Rumpel et al., 2005; Kim and Cho, 2017; Mao et al., 2006; Doyere et al., 2007; Nabavi et al., 2014), while post-acquisition manipulations predicted to interfere with plasticity within the BLA can impair the retrieval of a contextual fear memory (Sacchetti et al., 1999; Maren et al., 2003; Kochli et al., 2015). It is likely that the retrieval deficits observed in these studies result from direct interference with fear engram storage sites located within the BLA.

The observed pattern of repeated BLA neuronal activity during fear memory acquisition and retrieval combined with the observed synaptic plasticity in the BLA during fear memory encoding strongly support the involvement of BLA neurons in the storage of fear memories. If correct, then it can be expected that following the encoding of a fear engram, the BLA contains a group of neurons that contribute to the behavioral expression of the fear memory. The ability to directly test this prediction was made possible by the development of genetic tools that can target neurons with a defined activation history (Reijmers et al., 2007; Han et al., 2007). These genetic tools enable the expression of chemogenetic or optogenetic receptors specifically in a group of neurons that are active during the acquisition of a memory, so that this group can be reactivated or silenced during a later retrieval trial. This approach assumes that at least a fraction of neurons activated during the acquisition of a memory will become part of the engram for that memory. This assumption appears correct for neurons located in the BLA that are active during acquisition of a fear memory, as the

specific manipulation of only these neurons in either LA or BA is sufficient to intervene with the retrieval of a cued or contextual fear memory, respectively (Han et al., 2009; Redondo et al., 2014; Zhou et al., 2009; Rashid et al., 2016). Similar results were obtained with the manipulation of neurons in several brain regions outside of the BLA that are also active during fear memory acquisition (Tanaka et al., 2014; Garner et al., 2012; Cowansage et al., 2014; Liu et al., 2012). Combined, these studies have enabled the tracing of fear engrams across the BLA and other brain regions, providing support for a distribution of storage sites across the brain.

4. Spatiotemporal dynamics of fear engrams

The assignment of BLA neurons to a fear engram does not appear to be hard-wired. For example, the encoding of one fear engram in the BLA can temporarily impact the encoding of a subsequent fear engram through a process called memory allocation (Rogerson et al., 2014). For example, it was found that two different cued fear engrams encoded 6 h apart include a larger number of shared LA neurons as compared with two different cued fear engrams encoded 24 h apart (Rashid et al., 2016). A similar finding was made for contextual fear engrams in the hippocampus (Cai et al., 2016). Apparently, there is an underlying process that can actively determine if a memory is allocated to a specific group of neurons based on the activation history of that group. By allocating two memories to a partially overlapping group of neurons, it might make it easier for an animal to remember that two events are related to each other.

Though the manipulation of fear engrams has provided support for at least partial localization of fear engrams within the BLA, they do not necessarily address the temporal stability of these engrams. The reason is that fear engram manipulations are typically done at one time-point following the acquisition of the fear memory, leaving unanswered if the same manipulations would have the same effects at other time-points. There is reason to expect that fear engram manipulations could have different effects at different time-points after acquisition based on a reported temporally restricted role for the BA in the storage of a cued fear memory (Do-Monte et al., 2015). However, other reports indicate a more permanent role for the BLA in both cued and contextual fear memory storage (Gale et al., 2004; Maren et al., 1996). Interestingly, a recent study found support for a permanent role of the BA in contextual fear memory storage, but with a dependence on different BA input pathways over time. Specifically, entorhinal cortex input activated the BA engram cells during the first week following acquisition, while at later time-points the prefrontal cortex drove the BA engram cells (Kitamura et al., 2017). This resembles a system consolidation-like process that over time modifies certain components of the engram (Frankland and Bontempi, 2005). Further studies are needed to determine the temporal stability of BLA engram cells and participating synaptic connections over time periods of days and weeks following acquisition.

Fear engrams in the BLA are also subjected to fast dynamics that play out over milliseconds and seconds. These fast temporal dynamic can be detected with an electrode that is implanted in the BLA to record changes in the local-field potential (LFP). LFP recordings revealed that the retrieval of both cued and contextual fear memories is accompanied by LFP

oscillations in a 3–12 Hz range within the BLA (Pare and Collins, 2000; Seidenbecher et al., 2003; Pape and Pare, 2010; Likhtik et al., 2014; Cambiaghi et al., 2016; Popa et al., 2010). These oscillations could reflect neuronal activity in BA and/or LA, since the single-electrode LFP recordings used for these studies cannot localize the LFP source to a single BLA subdivision. Similar LFP oscillations as observed in the BLA are also observed in the hippocampus and prefrontal cortex during fear memory retrieval (Seidenbecher et al., 2003; Likhtik et al., 2014; Cambiaghi et al., 2016; Popa et al., 2010). The observed LFP oscillations in the BLA and other brain regions reflect the coordinated and synchronized activity of groups of neurons, which is predicted to support fast information transfer between BLA and these other brain regions. Though the reports of LFP oscillations associated with fear memory retrieval are correlative, it is likely that oscillatory neural activity makes causal contributions to fear memory retrieval by facilitating information flow through the fear engram. Artificial induction of a 4 Hz oscillation in the prefrontal cortex of naïve mice can synchronize BLA activity and trigger fear behavior (Karalis et al., 2016), and manipulating neuronal activity in the prefrontal cortex during specific phases of an endogenous 4 Hz LFP oscillation interferes with cued fear memory retrieval (Dejean et al., 2016). Whether manipulating oscillatory neural activity in the BLA can also impact fear memory retrieval has not yet been tested. In addition to supporting fear memory retrieval, oscillatory neural activity might also support fear memory consolidation (Girardeau et al., 2017; Popa et al., 2010).

While initial studies of fear engram-related LFP oscillations in the BLA focused on a single type of LFP oscillation in the 3–12 Hz range that correlated positively with fear memory expression, recent studies have observed two different types of BLA LFP oscillations in this frequency range that show opposite correlations with fear memory expression. We previously found that parvalbumin-expressing (PV) inhibitory interneurons in the BA might suppress the activation of the BA fear engram following extinction of a contextual fear memory (Trouche et al., 2013). To directly test this hypothesis, we chemogenetically silenced BA PV-interneurons following contextual fear memory extinction, which as predicted resulted in increased activation of putative BA fear engram neurons and increased behavioral expression of the contextual fear memory (Davis et al., 2017). The same PV-interneuron manipulation increased BLA LFP oscillations in the 3–6 Hz range, while decreasing BLA LFP oscillations in the 6–12 Hz range. This finding is in agreement with another report of two different types of BLA LFP oscillations in these same frequency ranges with opposite relationships to fear memory expression (Cambiaghi et al., 2016). Furthermore, we found that the 3–6 Hz and 6–12 Hz LFP oscillations in the BLA can compete with each other through a mechanism that depends on PV-interneurons in the BA, which might reflect the existence of two different types of neuronal ensembles in the BA that differentially participate in the 3–6 Hz and 6–12 Hz LFP oscillations (Davis et al., 2017).

We found that silencing BA PV-interneurons after contextual fear memory extinction not only increased the activation of putative fear engram neurons and 3–6 Hz LFP oscillations within the BLA, but also increased the activation of putative fear engram neurons located in the prelimbic region of the prefrontal cortex (Davis et al., 2017). This was accompanied by an increased lead of BLA 3–6 Hz oscillations over prefrontal cortex 3–6 Hz oscillations,

indicating increased information flow from BLA to prefrontal cortex in the 3–6 Hz frequency range. Interestingly, the flow of information between BLA and prefrontal cortex during fear memory expression is bidirectional, and the dominant direction can go either way depending on the conditions (Davis et al., 2017; Kitamura et al., 2017; Karalis et al., 2016; Lesting et al., 2013; Burgos-Robles et al., 2017; Klavir et al., 2017). This bidirectional flow of information illustrates the complex spatiotemporal dynamics that underlie the activation of brain-wide fear engrams that include the BLA.

5. Future directions

The studies discussed above demonstrate that fear engrams in the BLA and connected brain regions are supported by complex spatiotemporal dynamics. To advance our understanding of these incompletely understood dynamics, future studies will need to employ methods for observation and manipulation that combine high temporal resolution with high spatial and/or cell-type resolution. Though extracellular electrophysiological recordings enable the observation of in-vivo neuronal activity with high temporal resolution, they do not enable a definitive determination of the identity of the recorded neurons. A method known as optogenetic tagging or “opto-tagging” combines electrophysiological recordings with optogenetic identification of the recorded neurons. In an opto-tagging experiment, a light-sensitive receptor is expressed in a genetically defined group of neurons that are located around the electrode, for example PV-interneurons. This enables the use of light during electrophysiological recordings to confirm that a recorded neuron is a PV-interneuron. Use of the opto-tagging method enabled the discovery of highly specific and temporally restricted recruitment of specific types of BLA interneurons during the encoding of a cued fear engram (Wolff et al., 2014). In addition to targeting genetically defined cell-types, the opto-tagging method can also be targeted to projection-defined groups of neurons. This approach enabled the discovery of a specific role for BA neurons projecting to the prelimbic prefrontal cortex in controlling the behavioral expression of a cued fear engram when rats are presented with conflicting information (Burgos-Robles et al., 2017). Future studies employing opto-tagging of highly defined BLA neurons (defined by cell-type, projection target, synaptic input, synaptic output, or activation history) will be essential to addressing critical questions concerning the finer temporal interactions among the different types of BLA neurons that participate in fear engrams. The BLA contains a large variety of different interneurons and projection neurons that differ in their synaptic connectivity within BLA microcircuits as well as with other brain regions (Janak and Tye, 2015; Tovote et al., 2015; Orsini and Maren, 2012; Duvarci and Pare, 2014). It is still unclear how this large variety of BLA neurons coordinate their activity to 1) trigger synaptic and cell-intrinsic plasticity during fear engram encoding, and 2) drive fear engram expression under the appropriate circumstances.

A relatively new method that enables the observation of fear engrams with high spatiotemporal resolution is in-vivo calcium imaging. Though the temporal resolution of calcium imaging is not yet at the level of electrophysiological recordings due to temporal limitations of the calcium-reporters, its spatial resolution is superior to extracellular electrophysiological recordings. An initial limitation of calcium imaging was the need for relatively large microscope objectives, which limited its in-vivo use to the imaging of

superficial cortical circuits. This limitation has now been resolved with the development of miniaturized microscopes that can be implanted in deeper brain structures. These miniscopes have recently been used for the longitudinal imaging of neural activity patterns in the BLA during cued fear memory encoding, extinction, and retrieval (Grewe et al., 2017). This longitudinal imaging led to the discovery that the storage of cued fear engrams is associated with changes in the activity patterns of BLA neurons that are more complex than suggested by models based on simple Hebbian plasticity, with both increases and decreases in conditioned stimulus responses contributing to the fear engram. Future studies can employ in-vivo calcium imaging to further refine our understanding of fear engrams at the ensemble level, and to more precisely localize sources of functional heterogeneity within the BLA. Functional heterogeneity has been reported among circuits located in different areas within the LA (Quirk et al., 1995), and circuits located within the anterior versus posterior subdivisions of the BA (Kim et al., 2016). For example, though there is strong experimental support for the co-existence of pro-fear and pro-safety neurons within the BA, it is still unclear to what extent these two types of neurons are intermingled or spatially separated (Duvarci and Pare, 2014; Herry et al., 2008; Kim et al., 2016; Senn et al., 2014).

Methods that enable high spatiotemporal resolution will also be needed to understand if and how the encoding and retrieval of fear engrams in the BLA is supported by the various oscillatory patterns of synchronized activity that have been detected within the BLA. Specifically, which neurons in the BA and LA are active during different types of oscillatory activity, and how does synchronized oscillatory activity support the contribution of these neurons to fear engrams. One unique benefit of optogenetic manipulations that has so far been underutilized is the ability to promote or inhibit specific patterns of neuronal activity. As discussed above, the retrieval of fear memories is associated with synchronous activation at specific frequencies that are reflected in LFP oscillations. Optogenetic methods are uniquely suited to determine whether these oscillatory activities have any causal significance to fear engram functions, or are merely epiphenomena. Recent studies have started to employ optogenetic manipulations for this purpose, but still need to be extended to the manipulation of BLA engrams (Karalis et al., 2016; Dejean et al., 2016).

6. Concluding remarks

In this review we discussed studies that have identified various spatiotemporal dynamics that govern fear engrams in the BLA. These dynamics are not yet completely understood. For example, it is still unclear whether the storage of a fear memory can be supported by the exact same BLA neurons and synaptic connections across the lifetime of the memory, and how different patterns of synchronized neuronal activity can promote or suppress fear memories. Critically, very little is known about how different spatiotemporal dynamics that govern BLA fear engrams relate to one another; that is, how do the fast dynamics of circuit oscillations support the optimal formation and expression of fear engrams that can be stable over long time intervals? Current BLA fear engram models tend to consider engram cells as binary on-off switches, which is unlikely to be the case. Also, they do not adequately explain how single BLA neurons can participate in multiple fear engrams, which seems necessary for enabling sufficient coding capacity. We propose that current fear engram models can be improved by incorporating emergent circuit properties such as oscillatory activity. Those

who study spatial engrams in the hippocampal circuit have advanced this concept greatly, with many decades of work revealing that the activity of individual hippocampal place cells has precise and complex relationships with oscillatory activity of hippocampal circuits such as theta rhythms. By way of example, place cells fire in a particular sequence with clearly delineated phase relationships to theta cycles while an animal is using spatial memories to navigate an environment, and disruption of this relationship disrupts memory stability (Burgess and O'Keefe, 2011). An enhanced understanding of the relationships between fear engrams and circuit oscillations will be critical for understanding the true mechanism of fear memory formation and retrieval. Studying these relationships will be aided by the recently developed methods that we discussed under "Future directions". These methods can reveal the dynamic endogenous activity patterns of defined BLA fear engram components, which can then be specifically promoted or suppressed to achieve precise engram manipulation. Though we focused this review on fear engram components located within the BLA, it is important to keep in mind that these components will have to be studied within the broader context of brain-wide fear engrams. We anticipate that the increasing appreciation and understanding of fear engrams as dynamic engrams will pave the way for improved treatment options for patients suffering from post-traumatic stress disorder, as well as reveal broader principles that also apply to other types of memory engrams.

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