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Review

The dynamic nature of fear engrams in the basolateral amygdala

Patrick Davis[a,b], Leon G. Reijmers[a,*]

[a] Department of Neuroscience, Tufts University School of Medicine, Boston, MA, United States
[b] Medical Scientist Training Program and Graduate Program in Neuroscience, Sackler School of Graduate Biomedical Sciences, Tufts University, Boston, MA, United States

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 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.

* Corresponding author.
E-mail address: leon.reijmers@tufts.edu (L.G. Reijmers).

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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; Goosens 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; Trouche 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 too 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;