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Central amygdala cells for learning and expressing aversive emotional memories

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Abstract

Anticipatory defensive responses to an aversive or harmful event depend on memories linking the event with the predictive environmental cues. Extensive evidence indicates that the central amygdala is essential for the acquisition and recall of such memories. The evidence came initially from studies that relied on traditional lesion and pharmacological techniques, and recently from studies in which new methodologies were used to target, record and manipulate neuronal activities with improved precision and specificity. In this review, I will discuss the current understanding of the roles of central amygdala neurons in the learning and expression of defensive behaviors, with a focus on the major neuronal populations identified on the basis of their genetic markers.

Introduction

Animals and humans have the ability to use an environmental cue (also known as conditioned stimulus, or CS) to predict the occurrence of an aversive or harmful consequence (also known as unconditioned stimulus, or US) – on condition that the former is frequently associated with the latter – and to show appropriate behavioral reactions based on the prediction [1-4]. In order to support such ability, which is fundamental for survival and adaptation to the environment, the brain needs to respond to the CSs and USs, form and store memories about CS-US associations, and use the memories to guide the generation of behavioral outputs. These are intricate tasks that require coordinated activities of populations of neurons in distributed brain areas.

Extensive studies indicate that the central amygdala (CeA) plays important roles in the formation of CS-US associations during fear conditioning, and in the expression of conditioned behavioral responses including freezing behavior [5-9]. Transient pharmacological inactivation of the CeA [5,6], or specific inactivation of the lateral division of the CeA (CeL) [7], during fear conditioning blocks the formation of fear memories. Moreover, fear conditioning induces long lasting changes in CeL neuronal activities, causing

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Conflict of interest statement

Nothing declared.

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increased spiking in one CeL population (the “CeL_{ON}” neurons) and decreased spiking in another (the “CeL_{OFF}” neurons) in response to presentations of the CS [7-9]. By contrast, specific inactivation of the medial division of the CeA (CeM) during conditioning has no effect on fear memory formation, although the same manipulation during memory recall blocks the expression of freezing behavior [7]. These findings have led to the notion that the CeL (but not the CeM) is essential for the formation of aversive memories, and that the fear conditioning-induced changes in the responsiveness of CeL neurons to CS presentations, measured as “ON” and “OFF” responses during *in vivo* single unit recording, may facilitate the expression of learned defensive responses.

These findings in the CeL also raise the possibility that CeL neurons may themselves store, or be part of the network that stores, “traces” of aversive memories. The best candidate cellular substrate for memory is experience-dependent synaptic plasticity. However, the detection of fear conditioning-induced synaptic plasticity in the CeL – which is discussed in detail in the following sections – has been challenging until recently, in part because the CeL contains heterogeneous neuronal populations that likely play different roles in the formation, storage and recall of memories.

The CeL contains heterogeneous cell populations

The CeL is a striatal-like structure that contains medium spiny neurons mainly derived from the lateral ganglionic eminence during development [10-13]. These neurons show considerable heterogeneity, a feature that still remains incompletely understood and is currently an area of active research [14]. Nevertheless, it can be partly revealed by the different genetic or neurochemical markers that these neurons express. A partial list of these markers includes somatostatin (SOM) [15], protein kinase C- δ (PKC- δ) [8], corticotropin-releasing hormone (CRH) [16], calcitonin receptor-like (Calcr1) [17], serotonin receptor 2a (Htr2a) [18], neurotensin (Nts) [19,20], and tachykinin 2 (Tac2) [19-23]. Some of these markers label a relatively specific population, while others are more broadly expressed in the CeL. For example, SOM and PKC- δ label two largely non-overlapping populations, which constitute about 80-90% of the entire population in the CeL [8,24]. CRH labels another CeL population located more rostral to SOM⁺ and PKC- δ ⁺ neurons [8,25] that also expresses Nts and Tac2 [19]. The expression of Calcr1 overlaps substantially with that of PKC- δ and to a lesser extent with that of SOM in CeL neurons [17]. Htr2a, on the other hand, does not label a unique population in the CeL, where it is co-expressed with SOM, PKC- δ , CRH, Nts or Tac2 [18,19]. The rapid advance in the development of mouse genetic tools [26,27] has enabled researchers to selectively target some of these cell populations for recording and manipulation, and to reveal previously unknown functions of the CeL. Below I will focus the review mainly on studies that have recorded or manipulated specific populations of neurons in the CeL during fear conditioning. These studies were based on the use of the *Som-Cre*, *Prkcd-Cre*, *Crh-Cre* and *Calcr1-Cre* mouse lines, in which Cre recombinase is selectively expressed in SOM⁺, PKC- δ ⁺, CRH⁺ and Calcr1⁺ neurons, respectively. Of note, many of the manipulation experiments in these studies did not distinguish between the CeL, the capsular part of the CeA (CeC) or the CeM, due to technical difficulty in selectively targeting any one of these structures *in vivo*, even using the specific mouse lines. In such cases I use “CeA” to refer to the cell populations under investigation. As the different subdivisions of the CeA

may have distinct functions (for the CeC, see [28,29]; for the CeM, see above and the following section), one caveat is that such manipulations in the CeA may produce seemingly inconsistent results due to unintentionally but preferentially targeting one or another subdivision / subpopulation (also see latter sections).

SOM-expressing and CRH-expressing neurons in the CeL

As a first step to deal with the issue of heterogeneity among CeL neurons when assessing their synaptic changes in response to fear conditioning, a recent study used the *Som-Cre;Ai14* mice, in which SOM-expressing (SOM⁺) CeL neurons express the red fluorescence protein tdTomato and thus can be visually identified for electrophysiological recording in acute brain slices [24]. This strategy led to the discovery of robust synaptic plasticity in CeL neurons induced by fear conditioning (Fig. 1). Specifically, the excitatory synaptic transmission from the basolateral amygdala (BLA) onto SOM⁺ neurons is potentiated, whereas that onto SOM-negative (SOM⁻) neurons (which are mainly PKC- δ ⁺ neurons) is weakened by fear conditioning [24]. This result was subsequently replicated by follow-up studies [30,31]. Interestingly, shock alone was sufficient to induce potentiation of excitatory synaptic transmission onto SOM⁺ CeL neurons, measured as miniature excitatory postsynaptic currents, but was insufficient to induce potentiation of the synapses onto these neurons driven by the BLA-CeL pathway [23] (and unpublished observation from our lab). Consistent with this result, more recent studies, which employed in vivo bulk calcium imaging [32] or single unit recording combined with optogenetic tagging [33] methods, demonstrated that SOM⁺ CeL neurons show excitatory CS responses after fear conditioning, and that the responses correlate with freezing behavior [33].

These studies also show that inhibition of SOM⁺ CeA neurons during fear conditioning using chemogenetic [24,31], optogenetic [24] or molecular [34] methods, which can abolish the fear conditioning-induced potentiation of excitatory synapses onto SOM⁺ CeL neurons [24,31], impairs the expression of conditioned freezing behavior. Moreover, these studies provided evidence that optogenetic or pharmacological activation of SOM⁺ neurons in the CeA promotes freezing behavior, or biases against active defensive responses [24,31-33]. In summary, SOM⁺ CeL neurons undergo synaptic potentiation during fear conditioning, are recruited during fear memory recall and facilitate the expression of conditioned freezing behavior or other passive defensive behaviors (Fig. 1). These findings strongly suggest that these neurons constitute an important element of the circuitry that supports the formation and expression of aversive memories.

In light of previous findings in the CeA circuits [35,36], SOM⁺ CeL neurons can potentially influence freezing behavior via their inhibitory interactions locally within the CeL and the resulting disinhibition of the CeM [7,24], a structure that has been shown to control, through interactions with the midbrain periaqueductal gray (PAG), the expression of freezing behavior [9,33,37-41] (see Fig. 1). Interestingly, SOM⁺ CeL neurons also send direct projections to the PAG [30], providing another potential pathway for the control of freezing behavior or other passive defensive behaviors (Fig. 1).

CRH⁺ neurons are another CeL population that undergoes fear conditioning-induced potentiation of excitatory synapses [25] (Fig. 1). These neurons are required for the learning

but not expression of conditioned freezing behavior, in particular in situations where the US (shock) intensities are low. This effect of CRH⁺ CeL neurons is likely mediated by their local release of the neuropeptide CRH, and by CRH modulation of synaptic plasticity in SOM⁺ neurons [25]. Interestingly, CRH⁺ CeL neurons are also important for the expression of active defensive responses [33]. In situations where both freezing and active responses (such as “flight”) can be observed in the same animals after fear conditioning, the activity of CRH⁺ neurons correlates with and is required for the flight (but not freezing) behavior. Furthermore, artificial activation of these neurons promotes flight over freezing behavior in conditioned mice [33]. These studies indicate that CRH⁺ CeL neurons have multiple functions that can be engaged in different circumstances (i.e., mild vs. intense threats, or imminent vs. remote threats). Alternatively, but not mutually exclusively, CRH⁺ CeL neurons are heterogeneous, with one subpopulation being critical for learning of freezing behavior through local interactions with SOM⁺ neurons, and another being critical for promoting flight responses via long-range projections to the midbrain periaqueductal gray (PAG) [25,33].

PKC- δ -expressing and Calcrl-expressing neurons in the CeL

The two markers PKC- δ and Calcrl label an overlapping population in the CeL, in particular in its caudal locations [17]. Observations from optogenetic or molecular manipulations, targeting either Calcrl⁺ [17] or PKC- δ ⁺ [34,42] neurons in the CeL, indicate that the activity of these neurons is required for fear conditioning or learning reinforced by aversive stimuli, and that activation of these neurons is sufficient to drive the formation of artificial aversive memories. Notably, PKC- δ ⁺ CeL neurons seem to provide a “teaching signal” and control fear learning through the BLA during fear conditioning, wherein these neurons encode prediction errors, convey US information indirectly to the BLA and are essential for the synaptic plasticity underlying learning in BLA neurons [34] (Fig. 1). Anatomical tracing experiments indicate that neuromodulator systems, including the dopaminergic and cholinergic systems, may connect PKC- δ ⁺ CeL neurons with the BLA [34,42]. Nevertheless, how exactly the teaching signal is transmitted from PKC- δ ⁺ CeL neurons to the BLA remains to be resolved.

The Han et al [17] study also demonstrates that neurons expressing calcitonin gene-related peptide (CGRP) in the parabrachial nucleus (PBN), which provide nociceptive or aversive US information, send direct excitatory inputs onto Calcrl⁺ neurons in the CeL (Fig. 1) and are required and sufficient for driving aversive learning. These results are consistent with the finding that nonspecific inhibition of PBN neurons impairs fear conditioning, while activation of the PBN-CeA pathway is sufficient to drive fear learning [28]. Together, results from these studies support a model in which CGRP⁺ neurons in the PBN transmit nociceptive US signals to Calcrl⁺ or PKC- δ ⁺ CeL neurons in the CeL, which in turn process and convey these signals indirectly to the BLA to instruct synaptic plasticity and learning during fear conditioning [34] (Fig. 1).

Interestingly, results from recent studies indicate that the CeA provides a feedback control over the PAG pain-modulatory neurons, which constitute another potential source of the aversive US to the BLA during fear conditioning [43,44] (Fig. 1), and thus regulates aversive

memory strength [43]. The identity of the CeA neurons providing this feedback signal has yet to be defined.

The identities of CeL_{ON} and CeL_{OFF} cells

The CeL_{ON} cells and CeL_{OFF} cells were initially identified based on their firing properties in fear-conditioned animals [7,9]. An intriguing hypothesis has been that these functionally defined classes can be mapped onto genetically distinct populations. Indeed, it has been shown that SOM⁺ CeL neurons are activated [32,33] whereas PKC- δ ⁺ CeL neurons are inhibited [8] by the CS after fear conditioning. These results are consistent with the notion that SOM⁺ and PKC- δ ⁺ CeL neurons are “ON-cells” and “OFF-cells”, respectively.

Alternatively, or additionally, the ON-cells and OFF-cells are not simply determined by their genetic identities, but also by their functional interactions with input neurons and local neurons within the CeL. This alternative view raises the possibility that ON-cells and OFF-cells can be either SOM⁺ or PKC- δ ⁺ CeL neurons. Indeed, it has been recently shown that a substantial fraction of PKC- δ ⁺ CeL neurons (~27%) are activated by the CS after fear conditioning and meet the criteria to be ON-cells [34]. This result, together with those mentioned above [32,33], indicate that both SOM⁺ neurons and PKC- δ ⁺ neurons can act as ON-cells during fear conditioning.

Could both populations also act as OFF-cells? It seems likely, given that neurons in the CeL are GABAergic in nature and make substantial local inhibitory connections with each other [8,24,25,45]. In particular, SOM⁺ neurons and PKC- δ ⁺ neurons frequently form connections with neurons in their own classes (i.e., SOM⁺→SOM⁺ and PKC- δ ⁺→PKC- δ ⁺ connections are frequent), but rarely form connections with neurons outside of their own classes (i.e., SOM⁺→PKC- δ ⁺ and PKC- δ ⁺→SOM⁺ connections are rare) [45]. Thus, if a SOM⁺ or PKC- δ ⁺ neuron is activated by a CS, and thus “selected” as an ON-cell, some of the postsynaptic SOM⁺ or PKC- δ ⁺ neurons, respectively, could be inhibited and thus become OFF-cells. It has been shown that PKC- δ ⁺ neurons can indeed be OFF-cells [8]. Whether SOM⁺ neurons can also be OFF-cells remains to be determined.

The selection of ON- or OFF-cells during a behavioral event is unlikely to be purely stochastic, but more likely to be shaped or guided by experience. As exemplified by findings in fear conditioning [24,25,30,31], learning may induce strengthening or weakening of the excitatory synaptic transmission onto CeL neurons, with the degree of such plasticity being heterogeneous. As a consequence, the neurons that undergo a higher degree of synaptic potentiation would have a higher chance to be activated by CS and therefore selected as ON-cells.

Beyond aversive memories

Recent studies indicate that individual CeA neuronal “types”, which were targeted based on the aforementioned genetic markers, or their projection targets, have diverse behavioral roles. For example, Htr2a⁺ CeA neurons (which partially overlap with SOM⁺ CeA neurons [18,19]) have been shown to play an important role in controlling the hierarchy between innate and learned fear [46]. Interestingly, optogenetic activation of SOM⁺ CeA neurons [19], or Htr2a⁺ CeA neurons [18], induces appetitive behaviors. This function of the Htr2a⁺

neurons is mediated by long-range projections to the brainstem parabrachial nucleus (PBN) [18]. In addition, SOM^+ CeA neurons projecting to the bed nucleus of the stria terminalis (BNST) play an important role in the generation of anxiety state [23]. Thus, these studies indicate that SOM^+ CeA neurons are involved in functions beyond the learning and expression of defensive behaviors, and that the divergent functions of these neurons can be, at list in part, determined by their distinct local or long-range projection targets. Similarly divergent functions have also been described for $PKC-\delta^+$ CeA neurons, which, in addition to having a critical role in the formation of aversive memories [8,34,42], also participate in the regulation of feeding [47] and anxiety-related behaviors [48]. Finally, a recent study shows that CeA neurons drive predatory hunting behaviors through projections to brainstem areas [49], although the genetic identities of these neurons remain unknown. For the many other functions of the CeA circuits, please see a recent review by A. Luthi and colleagues [14].

Concluding remarks

Recent years have witnessed rapid progress in our understanding of how the central amygdala contributes to the learning and expression of aversive memories. Such progress has greatly benefited from the development of new neuroscience tools, including molecular and mouse genetic tools, viral tools, optogenetics, chemogenetics, and new in vivo recording techniques. On the other hand, recent studies have also opened up important new questions. For example, what is the relationship between the memories in the BLA and those in the CeL, in light of the vast literature on the essential roles of the BLA in the formation of aversive memories? How does the CeL coordinate with other components of the aversive memory acquisition and expression system, including the BLA, the medial division of the central amygdala (CeM) and the periaqueductal gray (PAG), to guide active versus passive defensive responses? How exactly do distinct CeL circuits orchestrate behaviors driven by aversive stimuli and those driven by appetitive stimuli? Addressing these questions will require new technologies that allow identifying and targeting bona fide CeL cell types (e.g., see [26]), and monitoring as well as control of CeL neuronal activities with improved spatial and temporal resolutions (e.g., see [50,51]).

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Figure 1. An updated circuit model for fear conditioning.

Shown are the circuits involved in the conditioning phase (left) and the recall phase (right) during Pavlovian auditory fear conditioning. For clarity, only key known components of the circuitry are shown. One source of US signals during the conditioning is the PBN, in which neurons provide nociceptive signals to the amygdala [17,28]. It has been shown that midbrain dopamine neurons play an important role in fear learning, and that CeA neurons preferentially innervate GABAergic neurons over dopamine neurons [52]. Therefore, it is possible that PKC- δ^+ or Calcr1 $^+$ CeL neurons drive disinhibition of dopamine neurons in response to US, which in turn instruct learning in the LA (as indicated by the question mark). Alternatively, or additionally, neurons in the substantia innominata or the retrorubral field may also mediate the function of these CeL neurons [34,42]. Another source of US signals during the conditioning phase for the LA is the PAG [43,44], which is indirectly connected with the LA (as indicated by the question mark). During the recall phase, SOM $^+$ or CRH $^+$ CeL neurons promote either passive or active defensive behaviors in response to the CS, through their connections locally (within the CeL or with neurons in the CeM), or through long-range projections to the (PAG) [24,25,30,31,33]. The arrows that are thickened in the recall (right) as compared with the conditioning (left) indicate connections known to be strengthened after fear conditioning. Besides the BLA, neurons in the CeL also receive major synaptic inputs from the paraventricular thalamus (PVT) [31,33], insular cortex (IC) [33,53] and the ventral hippocampus (vHipp) [33,54], which play distinct roles in behavior. Other abbreviations: LA, lateral amygdala; BA, basal amygdala; CeL, central amygdala,

lateral part; CeM, central amygdala, medial part; PAG, periaqueductal gray; PBN, parabrachial nucleus.