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Encoding of fear learning and memory in distributed neuronal circuits

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How sensory information is transformed by learning into adaptive behaviors is a fundamental question in neuroscience. Studies of auditory fear conditioning have revealed much about the formation and expression of emotional memories and have provided important insights into this question. Classical work focused on the amygdala as a central structure for fear conditioning. Recent advances, however, have identified new circuits and neural coding strategies mediating fear learning and the expression of fear behaviors. One area of research has identified key brain regions and neuronal coding mechanisms that regulate the formation, specificity and strength of fear memories. Other work has discovered critical circuits and neuronal dynamics by which fear memories are expressed through a medial prefrontal cortex pathway and coordinated activity across interconnected brain regions. Here we review these recent advances alongside prior work to provide a working model of the extended circuits and neuronal coding mechanisms mediating fear learning and memory.

In the web of cells and cellular processes that make up the brain lies the defining feature of the nervous system, the functional neural circuit. Sensation, action and even our personal memories are produced by connected neurons in distributed neural pathways that transduce outward experiences into perception, give rise to memories and allow us to act on the world in adaptive ways. Understanding these neural circuits and how they encode information is fundamental to understanding brain function. A form of learning called fear conditioning has revealed a great deal about neural circuits, providing one of the best mammalian model systems for studying how sensory information is transformed by the nervous system into memories and ultimately adaptive behaviors[1–7]. This has been particularly true in recent years, when technical advances have allowed researchers to dissect with unprecedented precision the contribution of neural circuits and cellular coding to behavioral learning and memory.

During auditory fear conditioning, a tone (the conditioned stimulus or CS) is paired with an aversive outcome (usually a mild electric shock, the unconditioned stimulus or US). Following learning, presentation of the CS alone generates various visceral and behavioral conditioned fear responses. We use the term fear to refer specifically to these measurable responses that occur in response to threat and not to the conscious feelings of fear (see ref. 8 for a discussion). A brain region called the amygdala, located in the medial temporal lobe, is known to be a key structure in fear learning and memory. On the basis of seminal work, a rough circuit map of sensory inputs to the amygdala and outputs from the amygdala that produce fear responses was developed (reviewed in refs. 1–6). Recent experimental studies, however, have revealed new circuits that project to and from the amygdala and neural coding mechanisms in these circuits that function to trigger and regulate learning as well as produce learned fear behaviors. Here we focus on these distributed circuits that represent, with the amygdala, the key neural substrate for fear learning and memory. We emphasize rodent studies, as much of the circuit analysis has been done in this system.

Neuronal circuits of fear learning

Here we discuss the neural circuits mediating fear learning. Specifically we focus on recent discoveries concerning the circuits that carry auditory and aversive information to the amygdala, on how auditory and nociceptive information is encoded in these circuits, and on how local microcircuits in various brain regions and long-range circuit interactions across brain regions give rise to this neuronal coding. We synthesize these discoveries into an updated working model of the distributed circuits and neural coding mechanisms mediating fear learning.

The role of different amygdala subnuclei in fear learning. Because the amygdala is a central structure in fear conditioning, we first provide a brief review of the current understanding of amygdala function in fear conditioning, as this is important for conceptualizing how learning is implemented across the distributed fear circuit. However, we do not examine the details of the amygdala microcircuit, about which there is a great deal known. For an excellent recent review of the role of amygdala microcircuits in fear conditioning, see ref. 5.

The lateral nucleus of the amygdala (LAn) is the primary sensory input station to the amygdala and is an important site of neural plasticity mediating fear learning[1–4,6,9] (**Fig. 1**). The LAn and basal nucleus of the amygdala (BAn) are cortical-like structures that consist of glutamatergic cells and GABAergic interneurons[9–12], but lack the layered anatomical organization present in the cortex. Single neurons in the LAn receive convergent inputs from both auditory, somatosensory and nociceptive systems[13–15]. From many studies, it has become clear that auditory thalamic and cortical synapses onto LAn neurons

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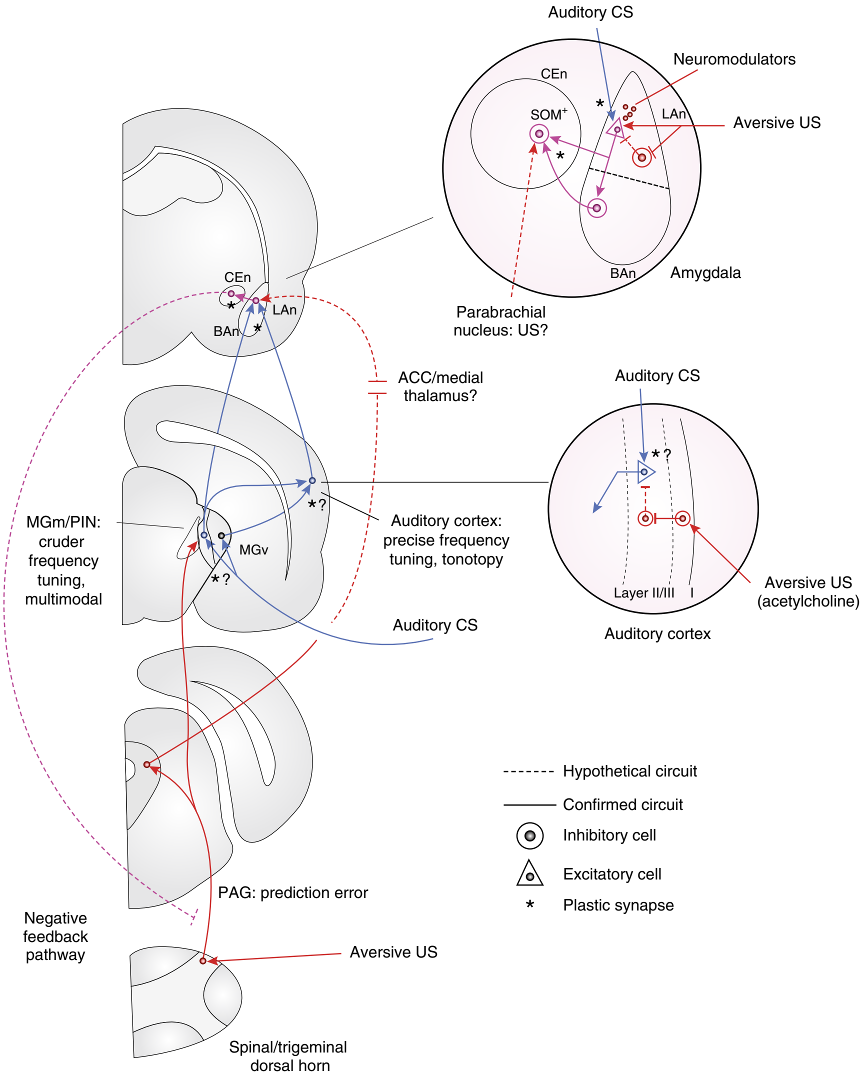

Figure 1 Working circuit model of the fear learning circuit. During fear conditioning, the auditory CS information travels in parallel directly through the MGm/PIN to the LAn or indirectly from the MGv and MGm to the primary auditory cortex and from there, to higher order auditory cortices that project to the LAn. The MGv also projects to various auditory cortices. Auditory pathways appear in blue. US information travels from primary afferent nociceptors to the spinal or trigeminal dorsal horn and from there to the PAG (and other regions). US information is then relayed from the PAG to the LAn through other brain nuclei that may include various midline thalamic nuclei and/or the ACC. A negative feedback pathway from the CEn may inhibit US processing before or in the PAG to set prediction error coding in the US circuit. Insets, microcircuits of the amygdala (top) and auditory cortex (bottom). Top inset, coincident activation of LAn pyramidal neurons by the CS and US in conjunction with US induced inhibition of local interneurons (PV+, SOM+) and US- and/or CS-evoked release of neuromodulators (noradrenaline, dopamine and/or acetylcholine) produce plasticity of CS inputs to pyramidal neurons. This occurs in parallel with plasticity occurring at inputs from the LAn and BAn to the CEl SOM+ neurons, possibly as a result of coincident activation of these cells by nociceptive parabrachial neurons. Bottom inset, US-evoked acetylcholine activates layer I interneurons, which inhibit layer II/III interneurons, which then facilitate auditory CS processing in layer II/III pyramidal neurons. This could provide a mechanism for changes in frequency tuning and possibly tonotopic organization of the cortex specifically at the inputs carrying the CS frequency. Multiple axons emanating from single cells represent output connections from functional classes of neurons, but do not indicate the existence of multiple collaterals from single neurons, which remain an open and interesting question.

are strengthened during fear learning[1–4,6,9,16]. This produces marked changes in auditory coding in LAn neurons. *In vivo* electrophysiological recording studies found that short- and long-latency components of the auditory CS-evoked responses in LAn neurons are potentiated in ~20–30 percent of cells[17–19]. LAn neurons project strongly to the BAn and it is thought that sensory information entering the LAn is then relayed to the BAn[20]. As with LAn cells, BAn neurons also exhibit enhancement of auditory CS-evoked responding during fear learning[21,22]. It is not clear, however, if the learned enhancement of CS processing is a result of local plasticity in the BAn or reflects plasticity occurring in regions afferent to the BAn. Together, these findings support the idea that integration of CS and US information and local plasticity at CS input synapses in the LAn produces an enhancement of phasic neural spiking in response to auditory stimuli after learning, which produces fear memories.

The LAn and BAn both project directly and indirectly to another amygdalar subregion called the central nucleus of the amygdala (CEn; **Fig. 1**) (for a review, see refs. 1–3,5). The CEn is generally thought of as an output structure for fear responses (see below), but one line of research has suggested a role for CEn in fear learning. First, temporary inactivation, NMDA receptor blockade and protein synthesis inhibition in CEn all reduce the acquisition of fear learning[23–25]. Further supporting this idea, recent work found that fear learning produces synaptic potentiation of BAn inputs to neurons in the lateral portion of the CEn (CEl)[26] and LAn inputs specifically to somatostatin-expressing neurons (SOM+)[27]. Notably, inhibition of these SOM+ neurons during learning reduces the acquisition of fear conditioning. Together, these findings suggest that neural activity and synaptic plasticity in SOM+ cells is important for fear learning along with synaptic potentiation occurring in the LAn. This potentiation of LAn-CEl synapses with learning suggests a possible regulatory gating mechanism in which plasticity in CEl allows for plasticity in LAn to be expressed as fear responses.

Auditory circuits and coding mediating fear learning. If the LAn integrates and associates auditory and aversive somatosensory information during learning, what are the circuits that transmit the auditory signals and what kind of information is being integrated in LAn from these auditory input pathways? Thalamic regions such as the medial geniculate nucleus (MG) and posterior intralaminar (PIN) thalamic nuclei, as well as primary, secondary and associative auditory cortices, provide input to the LAn (reviewed in refs. 1–3; **Fig. 1**). Supporting a functional role for these brain regions in fear conditioning, lesions of the MG after learning produce deficits in fear memory expression[28]. Auditory cortex lesions or inactivation also reduce fear conditioning, particularly when complex acoustic stimuli are used[29–33]. Pre-training lesions of either the thalamic or cortical pathway alone, however, have no effect on fear learning, suggesting that compensation occurs by the non-lesioned pathway[28]. Together, this work supports the idea that both auditory thalamus and cortex are important auditory processing areas during fear learning and expression of fear memories (see below), although the conditions that recruit auditory cortex are not entirely clear.

Regarding how information is processed in these circuits, cells in the auditory thalamus and cortex exhibit distinct auditory coding properties (reviewed in refs. 34–36). Briefly, cells in the subnuclei of the auditory thalamus that project to the LAn (medial aspect of the MG, MGm and the PIN) are polymodal (that is, respond to both CS and US) and exhibit a diversity of responses to auditory stimuli, with some cells having sharp and others broad tuning curves[37]. Furthermore, cells in these regions project to both primary and higher order auditory cortical sites[38]. Cortical regions such as the primary auditory cortex and the subnucleus of the thalamus, which projects to the primary auditory cortex (ventral portion of the MG, MGv), have narrow tuning curves and exhibit tonotopy[39]. Notably, CS-evoked responses in neurons in both MGm and the auditory cortical areas are enhanced by learning at the CS frequency, which was paired with shock, with the cortical neurons (and some MGm cells) largely shifting their tuning away from their initial peak frequency toward the CS frequency[34,35,40,41]. Collectively, the neural recording data suggest that the MGv and auditory cortex may be important for the discrimination of fearful and non-fearful auditory stimuli. Furthermore, on the basis of their neural coding properties and their direct access to the LAn, it appears that MGm/PIN cells provide fast, less refined auditory information to the fear system.

Although it is evident that synaptic plasticity at auditory thalamic and cortical inputs to the LAn is important for fear learning, the mechanism by which changes in auditory processing in these areas occurs is less clear. The enhancement of CS responding in MGm neurons, for example, could result from local synaptic plasticity and reflect changes occurring in other parts of the fear circuit. Supporting the local plasticity hypothesis, manipulations of protein synthesis or intracellular signaling in MGm alter behavioral fear learning and discrimination[42,43]. These manipulations affect both MGm/PIN and MGv, however, and it is possible that plasticity in the different thalamic subnuclei may differentially regulate memory strength and discrimination, respectively (as has been suggested by lesion data[44]). Notably, other work has found that learning-induced enhancement of CS responding in thalamic neurons is dependent on activity[41], but not plasticity[45], in the amygdala. Although further work is needed, these findings suggest that learning-induced changes in auditory processing in the auditory thalamus are important for learning and sensory discrimination and that these neural processing changes may be triggered by US or relayed by CS-evoked activation of amygdala neurons.

For auditory cortex, it is also unclear whether the fear conditioning–induced changes in spiking responses of auditory cortex neurons reflect local molecular changes in the cortex or plasticity in other parts of the fear circuit. An intriguing recent study identified a disinhibitory auditory cortical microcircuit that is important for enhancing CS processing in the presence of aversive USs and possibly for regulating neural plasticity in the auditory cortex[33] (**Fig. 1**). The authors found that layer 1 interneurons are activated by basal forebrain cholinergic inputs evoked by aversive foot shock and that this inhibited layer 2/3 parvalbumin (PV$^+$)-expressing interneurons. This produced disinhibition of layer 2/3 pyramidal neurons so that their response to complex auditory CSs was enhanced when it overlapped with the aversive US. To test the function of this circuit, they then used optogenetics, an approach in which light-responsive proteins (opsins) are expressed in specific neural cell types[36,46] and the cells can be manipulated with high temporal precision. They found that overriding the inhibition produced by layer 1 interneurons through optogenetic activation of layer 2/3 interneurons during the shock period of fear conditioning reduced fear learning, as did pharmacological blockade of acetylcholine receptors in the auditory cortex. This suggests that aversive USs activate acetylcholine neurons projecting to the auditory cortex to engage this disinhibitory microcircuit and ultimately enhance CS-evoked activity in pyramidal output neurons in layer 2/3 during fear learning. This permissive mechanism could be used to enhance auditory CS-aversive US associations or simply CS processing and enable plasticity and shifts in frequency tuning in the auditory cortex and/or downstream in the LAn.

Although previous studies examined neural coding in different parts of the auditory cortex, a detailed understanding of the functional contribution of individual auditory cortical regions is lacking. An intriguing recent report, however, examined the functional contribution of secondary auditory cortex to fear conditioning[32]. This study showed that specific lesions of secondary auditory cortex reduce the expression of simple tone-evoked fear behaviors when made ~1 month, but not 1 day, after learning. Furthermore, immediate early gene activity was increased in response to auditory CSs specifically at long memory-retention intervals, suggesting that cells in this region are only activated by the CS at this remote memory time point. This work shows that, in addition to the neural changes that occur during the initial fear learning event, the auditory cortex representation is further refined after learning occurs. How this type of dramatic restructuring of the auditory circuit following memory formation occurs is another important open question.

Together, these data demonstrate that, in addition to learning-induced plasticity of auditory input to the LAn, enhancement of CS processing in the auditory thalamus and/or cortex also occurs. Direct or indirect projections of amygdala and basal forebrain cholinergic neurons to auditory thalamus and cortex may facilitate potentiation and/or frequency retuning in these regions. Understanding how information is processed and transmitted to the amygdala during learning by specific cell types in thalamic and auditory cortical areas and how local microcircuits and local plasticity processes in these regions participate in this is critical to understanding fear learning and memory.

Aversive instructive pathways mediating fear learning. There has been a large focus on auditory processing and the role of amygdala plasticity in fear conditioning, but much less is known about the aversive US pathway to the LAn. Understanding this circuit is important as it provides the necessary instructive signal that enables neural plasticity in LAn neurons, resulting in fear memory storage. An aversive US activates many neural processes, including those involved in sensory discrimination and escape responses, as well as instructive signals that trigger the neural plasticity mediating learning. These different processes are partially dissociable at the neural circuit level[47]. Here we focus on the instructive circuits activated by the US (which at least partially overlap with circuits mediating other US-related responses), which trigger neural plasticity and fear learning. As discussed above, PIN neurons respond to both tones and shocks, and early lesion studies have suggested that aversive US information is transmitted to the LAn in parallel through PIN and insular cortex[48]. However, follow-up studies cast doubt on this idea[49,50], and one suggested that the results could be explained by damage to fibers of passage, and not cell bodies, in the PIN[50]. More recently, converging evidence using a variety of approaches identified another region in the midbrain, the periaqueductal gray (PAG), as a potential relay for aversive instructive signals to the amygdala (**Fig. 1**). Although the PAG is known as an output structure for various conditioned fear responses, it receives a strong nociceptive input from the spinal and trigeminal dorsal horn[51]. Furthermore, pairing an auditory CS with direct PAG stimulation, in the absence of an aversive shock US, is sufficient to support fear learning, and this is dependent on activity in LAn neurons[52,53]. Finally, a recent study found that temporary pharmacological inactivation of PAG reduces shock-evoked responding in LAn neurons and the acquisition of fear learning[14]. Demonstrating the importance of shock-evoked activity in LAn cells, activation of LAn pyramidal neurons during the aversive US period is necessary for fear learning to occur and is sufficient, with overtraining or conjoint activation of noradrenergic β-receptors, to produce fear learning and plasticity of CS processing in the LAn[54–56]. Together, this work suggests that, in addition to functioning as an output structure for conditioned fear responses, the PAG relays aversive US instructive signals to the amygdala to produce fear learning.

Although these studies provide evidence that the PAG is part of the aversive US circuit, there are still many important questions remaining. For example, the PAG is a large structure containing many subnuclei and different cell types and it is not clear which of these participate in aversive US processing. In addition, there is no direct pathway from the PAG to the LAn. The PAG may send instructive US information through other regions such as midline thalamic nuclei or the anterior cingulate cortex (ACC) and/or through neuromodulatory systems that do project to the LAn[57–60]. Although teaching signal circuits for reward learning have been elucidated in the basal ganglia[61,62], much less is known about instructive signaling for aversive experiences. Delineating these instructive pathways for fear learning will be an important area of future work, as these pathways may regulate other learning circuits in addition to fear conditioning systems.

Encoding of aversive instructive signals in the fear circuit. In addition to the progress on understanding aversive US instructive pathways to the LAn, theoretical and more recent experimental evidence has shed light on how aversive information is encoded in neurons in the fear circuit. Previous behavioral work demonstrated that with repeated training trials, fear learning reaches asymptotic levels beyond which no further learning occurs despite continued training[63]. Importantly, this asymptote is dependent on the intensity of the aversive US. This type of behavioral finding prompted the creation of theoretical models such as the Rescorla-Wagner[64], temporal difference learning[65] and Pearce-Hall[66] models. These models predict that instructive signals are activated only when there is a discrepancy between what the animal expects based on sensory cues (the CS for example) and the outcome (the aversive US in the case of fear conditioning). Thus, they suggest that, during fear conditioning, neuronal coding of aversive instructive signals should not reflect pure sensory processes, but should instead be modulated by the animal's expectation of whether the US will occur. This provides a theoretical explanation for how learning asymptotes are set through the reduction of instructive signaling as the animal comes to predict the outcome during learning. In these models, this difference between the actual and expected outcome has been termed a prediction error and this type of neural code has been seen in many learning systems, including dopamine neurons in the basal ganglia[62]. It is important to note the distinctions between the different types of models, as they make unique predictions about how prediction errors may be encoded in learning systems. The Rescorla-Wagner[64] and temporal difference algorithms[65] are termed valence-based models because they respond differentially to aversive and rewarding stimuli (that is, the sign of the prediction error is opposite for aversive and rewarding outcomes). In contrast the Pearce-Hall model[66] is an example of an 'attentional' model because it responds equally (in the same direction) to both aversive and rewarding outcomes.

By recording from LAn and BAn neurons, a number of studies found that cells in these regions exhibit firing responses proportional to prediction error[14,67–69]. Thus, neurons in these regions respond robustly to unpredicted aversive USs, but less when the US is predicted by a well-trained auditory CS after learning (**Fig. 2**). Furthermore, distinct populations of amygdala neurons express prediction error-like responses to aversive and rewarding outcomes[67], suggesting that emotional valence can be processed in these cells[67,70,71]. There are, however, some neurons that are not valence specific, responding equally to aversive and rewarding outcomes[67]. Contrary to what has been seen in appetitive procedures[72,73], amygdala cells do not appear to change their firing responses reliably when an expected aversive US is omitted[14,67]. Although further work is required to examine this adequately, this suggests that, for aversive stimuli, LAn neurons do not encode an attentional prediction error (that is, any change in expectation) and only encode a portion of the prediction error (that is, unexpected occurrences, but not omissions, of aversive USs activate these cells).

This type of expectancy-modulated aversive coding in amygdala neurons raises an important question. How are prediction errors encoded by the fear circuit and what function does this serve for behavior? Interestingly, prediction error coding is also seen in PAG neurons in rats[14] and humans[74], suggesting that inhibition of expected aversive US processing occurs before the signal arrives in the LAn (**Figs. 1** and **2**). Consistent with this, an early conceptual model and previous work[59,75–78] suggested that a negative feedback pathway from the CEn to the PAG functions to inhibit US instructive signaling when the US is expected, thereby setting prediction error coding in the fear circuit (**Fig. 2a**,**b**). According to this idea[78], following learning, the strengthened CS inputs to the amygdala activate a negative feedback pathway from the CEn to the PAG. This would serve to inhibit aversive US processing when it is expected and give rise to prediction error coding in PAG and LAn neurons (that is, larger responses to unpredicted compared with predicted shocks). The inhibition of US processing could occur either directly in the PAG, through activation of a descending analgesia circuit that inhibits pain processing at the level of the spinal cord, and/or through refinement of US processing at various stages of the circuit. Possibly related to this, other recent work suggested that the ACC may be important in refining prediction error coding in the amygdala during learning[68,79], although how ACC neurons contribute to this process is not clear. Given that aversive US-evoked activity in LAn neurons is important for triggering fear learning[54,55,80], the CEn-PAG circuit mechanism could set the amount of learning that occurs at a given US intensity (that is, the learning asymptote) by regulating the amount of LAn neuronal depolarization evoked by the US during training. These circuit mechanisms remain to be tested, however, and understanding how prediction error coding is constructed by the fear circuit is a critical open question. Answering this question may help to explain how adaptive fear learning levels are set and how dysregulation in these circuits could be a predisposing factor for pathological fear disorders.

Another possible mechanism for modulating aversive instructive signaling is through local interneurons in the LAn and/or through neuromodulatory networks (**Fig. 1**). Recent work[55] using a technique called optogenetic identification, in which light activation is combined with *in vivo* physiology, identified specific cell populations expressing opsin proteins on the basis of their responses to light and then examined the neural coding properties of these cells[81–83].

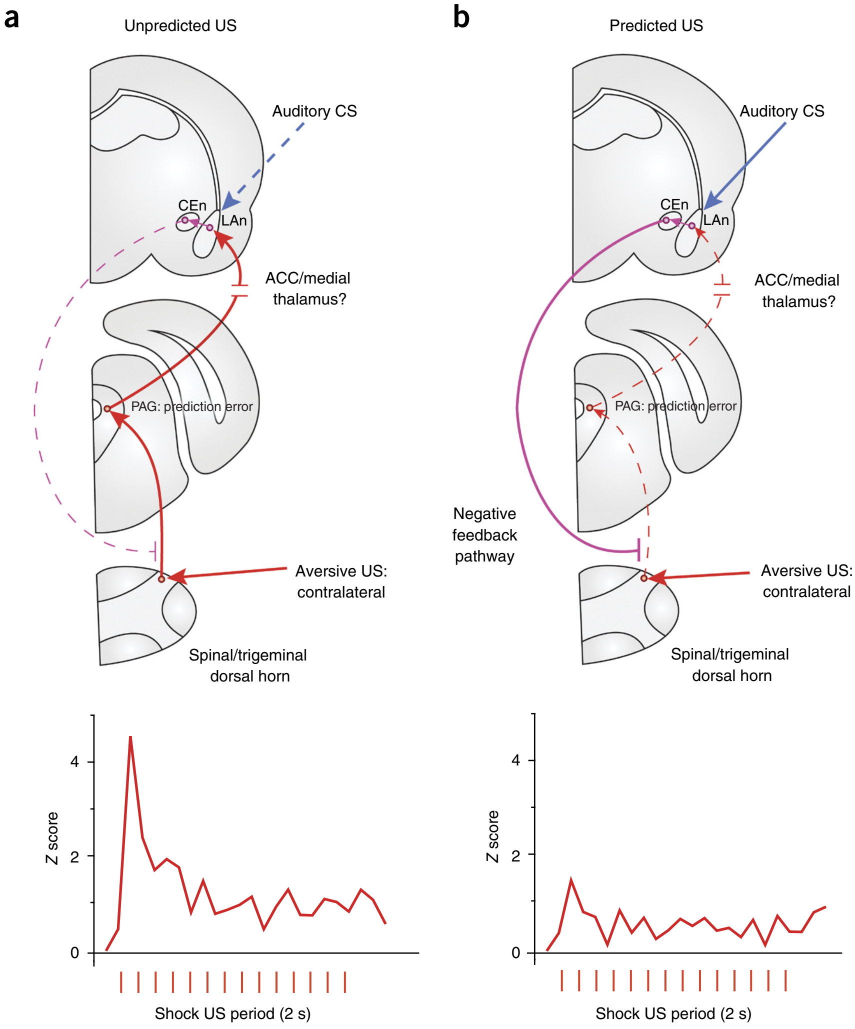

Figure 2 Hypothetical circuit construction of prediction error coding during fear learning. Prediction error coding in LAn neurons is characterized by a larger US-evoked neural firing rate response to unpredicted shocks compared with shocks that are predicted by the CS (actual data, bottom). (**a**) According to the working model presented (top), unpredicted shock USs strongly activate LAn neurons through a pathway that includes the PAG (red line). This is because the CS (dashed blue line) is either not present or its inputs to the amygdala are not strong enough to drive a negative feedback pathway (dashed purple line) that could inhibit US processing. (**b**) However, when the US is predicted by a well-trained CS (filled blue line), whose onset occurs before US onset, it activates this negative feedback pathway from the amygdala (filled purple line) to inhibit US processing at the level of or before the PAG. This results in larger shock responses to unpredicted compared with predicted shocks as seen in peri-event time histograms (PETHs). PETHs represent the *Z* score–normalized shock-evoked response (*y* axis) of prediction error coding neurons in the LAn during a 2-s, pulsed eyelid shock US (*x* axis) (adapted from ref. 14).

In this study, the authors used optogenetic identification of two different types of amygdala cells, parvalbumin (PV$^+$) and SOM$^+$ interneurons, to show that, in contrast with most pyramidal cells in LAn, these interneurons are inhibited by aversive USs during fear learning. Furthermore, the authors found that optogenetic inhibition of these interneurons during behavioral learning facilitated the shock US–evoked activation of LAn and BAn pyramidal neurons as well as fear memory formation. Notably, local interneurons and intracellular signaling networks that are important for plasticity in LAn are regulated by neuromodulators such as dopamine or noradrenaline. These neuromodulatory systems[84,85] respond to aversive and/or rewarding outcomes and project to the amygdala. Furthermore, dopamine neurons encode prediction errors[62]. Together, the modulation of different interneuron subtypes by aversive USs suggests a mechanism through which neuromodulatory systems[86–88] could regulate LAn pyramidal cell activity and, ultimately, fear learning. It will be important in the future to determine how LAn interneurons and neuromodulatory systems projecting to the LAn encode information during fear memory formation and how they contribute to amygdala neural coding, plasticity and behavioral learning.

Learning circuits summary. Although previous work has provided a wealth of information on the circuits and sites of neural plasticity mediating fear learning, new studies have substantially extended our knowledge of these pathways and uncovered new circuits and coding mechanisms that are engaged during memory formation. In the auditory CS system, recent work discovered local and long-range circuit mechanisms that may regulate changes in frequency tuning of auditory cortical cells during learning and found that the cortical representation of auditory CSs is further refined after learning has occurred. In studies of the aversive US circuit, recent work has revealed a previously unknown midbrain PAG pathway that may relay US information to the amygdala to trigger fear learning. In addition, several studies found that aversive US information processing in neurons in the US circuit is negatively modulated by the expectation of the US, providing a potential circuit mechanism for setting the strength of fear memories. Although many important questions remain, these studies provide new ideas and avenues for exploration to the field of fear conditioning. Leveraging modern technical advances and traditional approaches, fear researchers are poised to make great leaps in understanding the circuit and neuronal coding mechanisms of this important associative learning system.

Neuronal circuits and mechanisms of fear expression

The canonical view of circuits supporting fear behavior posits that the CEn has a critical role in fear expression (**Fig. 3a**). However, recent data collected using refined approaches, such as optogenetic manipulations and large-scale recordings of neuron activity and local field potential (LFP), have extended this view. These data identified, in addition to the BAn-CEm pathway, a complementary circuit composed of neurons in the CEl directly projecting to the ventrolateral part of the periaqueductal gray (vlPAG) that can regulate fear behavior. A second pathway, which participates in fear expression, was identified between the dorsal part of the medial prefrontal cortex (dmPFC, which includes the ACC and the prelimbic area (PLc)[89]) and the BAn, In this BAn-dmPFC circuit, the development of neuronal oscillations and synchrony, along with the recruitment of specialized neuronal

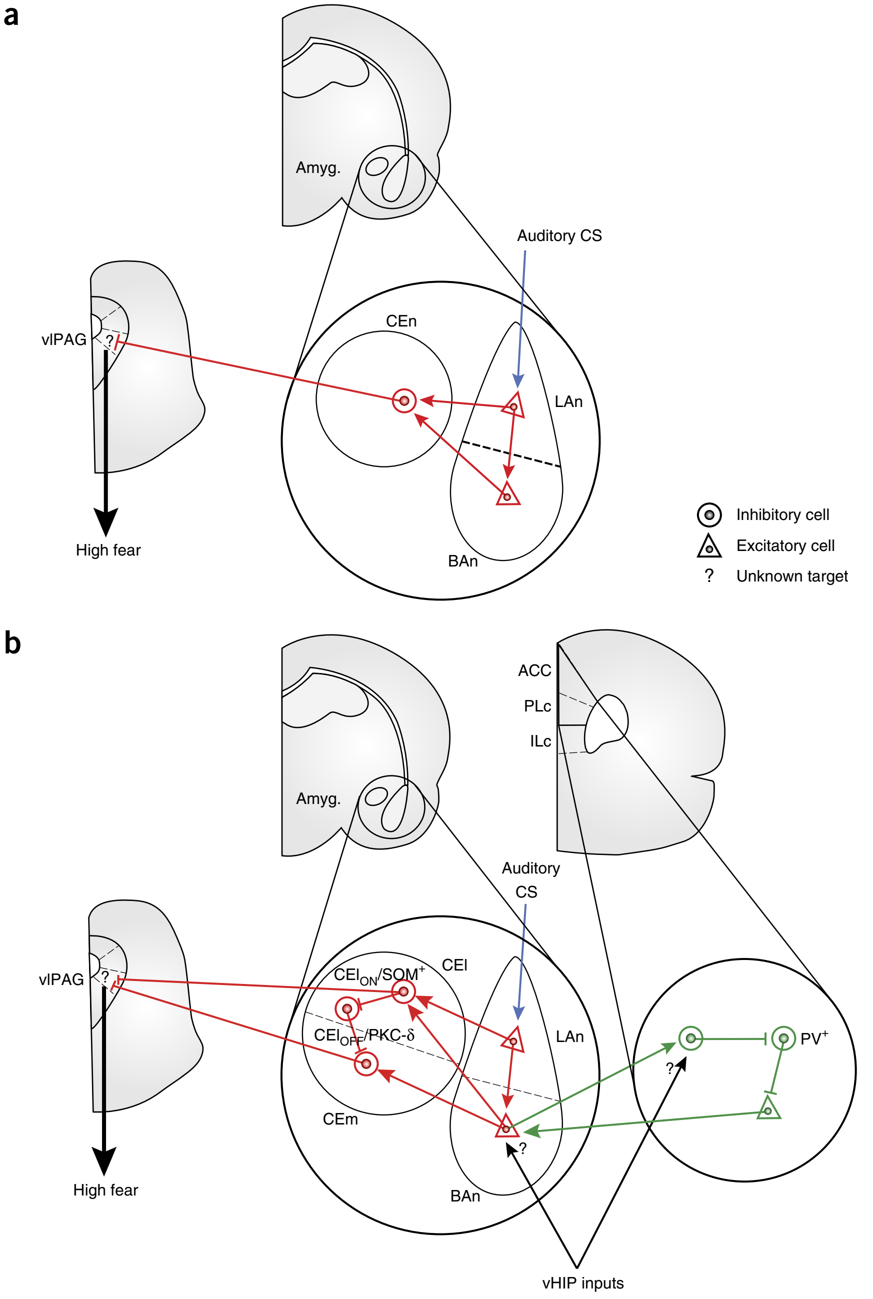

Figure 3 Classical and updated circuit model of fear expression. (**a**) In the classical model, presentations of conditioned tones (CS) following fear conditioning induce the reactivation of LAn and BAn excitatory neurons that project to the CEn. Fear behavior is thought to be mediated by the activation of long-range inhibitory interneurons projecting to the vlPAG. (**b**) The refined and expanded circuit model of fear expression contains two main neuronal pathways. The first (red lines) is recruited following fear conditioning by presentations of CS that lead to the activation of LAn and BAn excitatory neurons. LAn neurons project directly to CEl$_{\mathrm{ON}}$ neurons (labeled CEl$_{\mathrm{ON}}$/SOM$^+$ in the schema), which inhibit CEl$_{\mathrm{OFF}}$ neurons (labeled CEl$_{\mathrm{OFF}}$/PKC-δ in the schema), thereby disinhibiting CEm neurons activity. An increase in neuronal activity in CEm neurons is thought to regulate vlPAG neuronal activity to drive fear behavior. In this circuit, BAn neurons (potentially BAn fear neurons) can project to both CEl$_{\mathrm{ON}}$ cells and CEm neurons to regulate fear responses. CEm and CEl$_{\mathrm{ON}}$/SOM$^+$ cells then project to the vlPAG to control fear expression. The second neuronal circuit (green lines) relies on a dmPFC disinhibitory network (comprising the ACC and PLc) that could be recruited by BAn excitatory inputs and/or modulated by ventral hippocampal inputs (vHIP). In this circuit, the disinhibition of dmPFC excitatory neurons projecting to the BAn through the inhibition of parvalbumin-expressing local interneurons (PV$^+$) is associated with fear expression. Question marks indicate that the cell type or function of the targeted neurons has not yet been identified. For the sake of clarity, auditory inputs pathways have been simplified in the schema, but correspond to those described in **Figure 1**, and only the major connections between CEl$_{\mathrm{ON}}$, CEl$_{\mathrm{OFF}}$ and CEm have been illustrated, although reciprocal connections between CEl$_{\mathrm{ON}}$ and CEl$_{\mathrm{OFF}}$ and direct connection between CEl$_{\mathrm{ON}}$ and CEm have been described (for a complete picture of local CEn circuits, see refs. 5,9,92). Multiple axons emanating from single cells represent output connections from functional classes of neurons, but do not indicate the existence of multiple collaterals from single neurons, which remain an open and interesting question.

populations, allows for the precise control of activity to drive fear expression. Here we discuss the recent studies that have contributed to the refinement and expansion of the classical model of neuronal circuits and mechanisms involved in fear expression.

CEn-PAG circuits controlling fear memory expression. Although the focus of this review is not related to CEn local circuitry in fear expression, which has been extensively described[5], it is important to summarize these findings to understand recent data on CEl long-range projections contacting the vlPAG that might be involved in the control of fear. The CEn, which is composed of two main nuclei, the CEl and the central medial amygdala (CEm), is thought to be a relay between the BAn and hypothalamic, midbrain and brainstem systems[90,91]. In this model, the LAn is the key site of CS-US association during fear conditioning[3] and projections from the LAn and BAn directly or indirectly through GABAergic intercalated neurons to the CEn control the activity of CEm output neurons (**Fig. 3a**)[5,20,90]. Recent studies using reversible inactivation with the GABA$_{\mathrm{A}}$ receptor agonist muscimol have revealed a dichotomy in CEn functions, with the CEl being involved in fear acquisition and the CEm being more closely related to fear expression[23,92]. Slice physiology experiments and extracellular recordings during behavior have refined this model by showing activity-dependent plasticity at BAn to CEl synapses during auditory fear conditioning[26], that CEl inhibitory neurons activated during CS presentations (CEl$_{\mathrm{ON}}$ neurons) inhibit protein kinase C-delta (PKC-δ)-expressing CEl neurons[92,93] (CEl$_{\mathrm{OFF}}$ neurons), that CEl$_{\mathrm{OFF}}$ neurons tonically inhibit CEm output neurons[93] and inhibition of CEl$_{\mathrm{OFF}}$ neurons facilitates tone-evoked responses in CEm neurons[93], and that CEm output neurons might regulate conditioned fear responses via projections to the vlPAG[93,94] (**Fig. 3b**). More recently, it was demonstrated that a class of CEl SOM$^+$ interneurons is important for fear expression[27]. In this study, the authors revealed that optogenetic inhibition of CEl SOM$^+$ cells suppresses fear expression, whereas their optogenetic activation drives unconditioned fear.

Interestingly, recent data have indicated that long-range projection neurons from CEl to the PAG or the paraventricular nucleus of the thalamus (PVT) are likely to be involved in fear expression[95]. In this study, the authors first used retrograde tracers to demonstrate that a subset of CEl neurons project to the PAG, PVT or both. Next, they showed that 80% of these long-range projecting CEl neurons expressed SOM, whereas only 20% expressed PKC-δ (CEl$_{\mathrm{OFF}}$ neurons). Notably, fear conditioning enhanced synaptic transmission onto PAG or PVT-CEl projecting neurons and optogenetic activation of CEl SOM$^+$ neurons elicited inhibitory currents in the vlPAG.

These data suggest that, in addition to the CEm-vlPAG pathway, CEl SOM$^+$ output neurons modulate conditioned fear behavior through direct projection to the vlPAG (**Fig. 3b**). Although these studies have extended our knowledge on CEn circuits mediating conditioned fear behavior, several questions remain to be answered. In particular, it is not clear which circuits and elements are targeted by CEl or CEm output neurons at the level of the vlPAG and how these circuits encode the onset, offset and duration of conditioned freezing. Moreover, because mammals display heterogeneous behavioral responses to threatening stimuli, it would be of general interest to understand how the switch between different fear strategies is achieved in the amygdala, PAG, brainstem and hypothalamic circuits.

Bidirectional control of fear expression in BAn-dmPFC circuits. In addition to the classical CEn-PAG pathway, recent studies identified circuits containing the dmPFC and the BAn, which can be modulated in a bidirectional manner during fear expression (**Fig. 3b**). In the amygdala, the formation of CS-US associations during fear conditioning is thought to occur in the LAn and is mediated by distinct neuronal populations (for a review of these circuits, see ref. 5). In summary, during and following fear conditioning, these neurons display short-latency phasic firing increases in response to presentations of conditioned tones[17–19]. Interestingly, similar populations of neurons have been identified in the BAn whose activity correlates with fear expression[21]. Two main types of BAn neurons have been described, the first type, fear neurons, display phasic tone-evoked responses that correlate with high fear states, and the second type, persistent neurons, exhibit long-lasting evoked activity following fear conditioning that does not correlate with fear states[21,22]. Although the function of these BAn neurons is unclear, the transient or sustained increase in activity of these cell populations might directly or indirectly regulate fear responses via projections to CEm neurons[96], represent a storage mechanism for fear memories, or act as relay neurons to transmit fear-related information to cortical structures[3].

In support of the last hypothesis, it has been shown that projections to subregions of the mPFC emanating from the BAn and the ventral hippocampus (vHIP) strongly influence fear expression and inhibition. In an elegant study, one group observed that inactivation of the BAn using muscimol decreased spontaneous and tone-evoked firing of putative excitatory neurons located in the dmPFC (notably in the PLc), but had no effect on PLc putative inhibitory interneurons[97]. In contrast, vHIP inactivation had no effect on putative excitatory neurons, but decreased spontaneously occurring spikes from PLc putative inhibitory interneurons. Moreover, tone-evoked activity was enhanced in PLc putative excitatory neurons following vHIP inactivation[97]. Although these data suggest that BAn projection neurons contact PLc excitatory neurons and vHIP-projecting neurons directly contact PLc inhibitory neurons, it is possible that more complex circuits composed of different classes of inhibitory interneurons could be involved[33,82,98]. Interestingly, vHIP inactivation performed 24 h after fear conditioning decreased fear behavior, as evidenced by increased lever pressing for food. In contrast, during fear extinction, vHIP inactivation increased fear behavior[97]. Although the connection between vHIP projections and prefrontal interneurons has not been anatomically established, these results suggest that modulation of PLc inhibitory circuits regulates fear expression (**Fig. 3b**). These data raise interesting questions about how modulation of the same hippocampal input to PLc neurons could mediate opposite behavioral outcomes. For instance, distinct subsets of hippocampal neurons could be recruited at different time points during behavior, or various local PLc inhibitory circuits might be differentially engaged during fear expression and fear inhibition, as the authors suggested[97].

Distinct projections onto subregions of the mPFC might also contribute to the selection of appropriate behavioral responses by balancing of neuronal activity between prefrontal subregions involved in fear expression (the PLc area of the dmPFC) or fear inhibition (the infralimbic area, ILc). This hypothesis was recently supported by a study[83] showing that fear neurons of the BAn targeting the PLc subdivision of dmPFC are active during fear expression (**Fig. 3b**). In contrast, extinction neurons projecting to the ILc, a region involved in fear inhibition[83], are recruited and exhibit cell type–specific intrinsic plasticity during fear inhibition[83]. By using optogenetic approaches targeting BAn-PLc or BAn-ILc pathways, combined with extracellular recordings, the authors observed that fear and extinction neurons[21] were recorded exclusively among PLc- and ILc-projecting BAn neurons, respectively, that optogenetic inhibition of PLc-projecting BAn neurons during extinction facilitated fear inhibition, and that optogenetic inhibition of ILc-projecting BAn neurons during extinction facilitated fear expression[83]. Finally, using slice recordings from retrogradely labeled PLc- and ILc-projecting BLAn neurons, the authors observed that PLc-projecting BLAn neurons displayed bursting activity after fear expression similarly to BAn fear neurons recorded *in vivo*. Moreover, increased bursting activity and broader spike widths were observed in both ILc-projecting BAn neurons and identified extinction neurons *in vivo* during fear inhibition. Together, these data suggest that the plasticity of action potential waveforms in subpopulations of BAn projection neurons determine the expression and inhibition of fear behavior, likely by switching the balance of activity between PLc- and ILc-output neurons or promoting plasticity at specific BAn synapses onto PLc or ILc neurons.

Mechanistically, activity-dependent plasticity, such as long-term potentiation (LTP) and long-term depression (LTD) could control the expression and inhibition of conditioned fear behavior in PLc and ILc-output neurons. For instance, studies performed in rodents and non-human primates used artificial stimulation protocols to induce LTP or LTD in prefrontal regions during fear extinction[99,100]. These studies showed that LTP-inducing stimulation in the rodent ILc or LTD-inducing stimulation in the monkey dorsal anterior cingulate cortex (dACC, an analog of the rodent PLc) both facilitate fear inhibition during extinction[99,100]. Together with studies presented above, these data suggest the existence of parallel pathways regulating fear expression. The first pathway connects BAn to CEn and can directly control fear expression after conditioning (**Fig. 3b**). The second pathway originates from distinct sets of neurons in the BAn and projects either to the PLc or the ILc, where it can, depending on the target, strengthen or reduce fear expression (**Fig. 3b**). This BAn-mPFC pathway, which involves cortical processing of emotionally relevant information, could be important in ambiguous situations in which animals have to select between two behavioral outcomes (fear expression versus fear inhibition). In addition, long-term synaptic plasticity at BLAn inputs to the different mPFC subregions could alter the balance of this system toward fear or non-fear states in a more persistent manner. This form of behavioral control on fear expression is thought to be mediated by reciprocal inputs from the PLc or ILc to the BAn[82,101,102]. However, the precise neurons and structures involved are still largely unknown and will require further investigation.

Prefrontal-amygdala coding mechanisms of fear expression. What are the coding mechanisms allowing for the precise control of fear responses in mPFC circuits? Although numerous data indicate a role of PLc and ILc in fear expression and fear inhibition, respectively[3,103,104], it is not clear how neuronal changes occurring in these regions are translated to downstream structures involved in fear expression. A potential mechanism might be long-range neuronal synchronization of spiking and oscillatory activity between mPFC and BAn circuits contacting CEm output neurons. Indeed, coordinated oscillation of neuronal activity across brain areas represents a form of neuronal synchrony that might increase synaptic strength through coincident pre- and postsynaptic activation and simultaneous convergence of multiple inputs[6,105,106]. Neuronal synchrony can therefore coordinate and enhance the effect of input signals and strengthen information transmission to downstream targets, such as the Ban, and from there possibly to the CEn (**Fig. 4**). An elegant study showed that the synchronization of spiking activity between dACC and BAn during fear expression using a partial reinforcement extinction task in non-human primates is associated with long-lasting fear expression[102]. Following tone-odor conditioning, between 25–30% of the neurons in the dACC and BAn displayed tone-evoked responses. Interestingly, neuronal responses in the dACC developed before, and therefore predicted, fear behavior, whereas BAn neuronal responses followed fear behavior. Moreover, correlational analyses performed between pairs of recorded neurons in the dACC and BAn revealed an increased correlation between dACC and BAn spiking activity during the acquisition phase of the task, which predicted long-term fear expression[102].

The data reviewed above reported changes in firing activity of mPFC and BAn neurons which correlates with fear expression. This form of neuronal coding is referred as rate coding and implies that precise firing patterns of neurons are less important than their average firing rates. Another mechanism that has largely been unexplored in the field of aversive memory is the contribution of temporal coding of information to control fear expression. Temporal coding refers to the firing of local groups of neurons that can cooperate and synchronize, thereby forming temporary functional neuronal cell assemblies (**Fig. 4**). In this form of coding, precise timing of firing is important, although average firing rates can remain unaltered. The main advantage of temporal coding, as compared with classical rate coding, is its dynamic range of plasticity. In temporal coding, neurons can rapidly switch between different neuronal assemblies according to external sensory or internal inputs. Moreover, the organization of spiking activity in temporal patterns can dramatically increase the coding capacity[107]. In addition, in temporal coding, oscillations are known to be critical for binding neuronal assemblies, organizing the spiking activity of neurons and coordinating neuronal activities in remote structures. Recent work has suggested that mPFC-BAn oscillatory coupling might be important during discriminative fear learning[108]. Indeed, in animals trained to discriminate an aversive (CS^+) from a safety (CS^-) CS, mPFC and BAn LFPs synchronize in the theta range (4–12 Hz). In contrast, animals displaying fear generalization to the CS^- did not exhibit increased LFP synchrony. Interestingly, directionality analyses suggested that mPFC LFP oscillations precede BAn oscillations during fear discrimination. Although these data did not address whether mPFC and BLAn oscillations are necessary for the formation of neuronal assemblies, they highlight the importance of oscillations in mPFC and BLAn circuits for the selection of appropriate behavioral outputs.

To the best of our knowledge, no study has directly explored whether firing sequences in the mPFC, BAn or CEn could support encoding of fear behavior, although temporal coding of information has been demonstrated in several sensory and memory systems. For instance, coding of spatial information by the coordinated activity of neuronal ensembles has been described in the hippocampus, where navigation pathways are encoded in the sequential firing of place cells[109,110]. Episodic memory recall and future behavioral choices are also represented in the formation of neuronal assemblies in the hippocampus and mPFC[111,112]. In the context of anxiety behavior, mPFC neurons display increased firing in the open arms of the plus maze when the animal is anxious. These anxiety-modulated mPFC neurons are modulated by vHIP theta, suggesting a mechanism by which fear-related assemblies in the mPFC might be modulated[113]. Recent work indeed suggests that hippocampal theta allows the formation of behaviorally relevant prefrontal neuronal assemblies. Prefrontal neuronal assemblies display synchronous activity when theta coherence was enhanced between hippocampus and mPFC, and this synchronous activity was increased during learning[111]. Similar analyses performed during the development of fear responses could potentially reveal new coding mechanism for fear behavior. For example, consolidation of fear behavior in distributed neuronal networks, including the BLAn and mPFC, could be achieved by

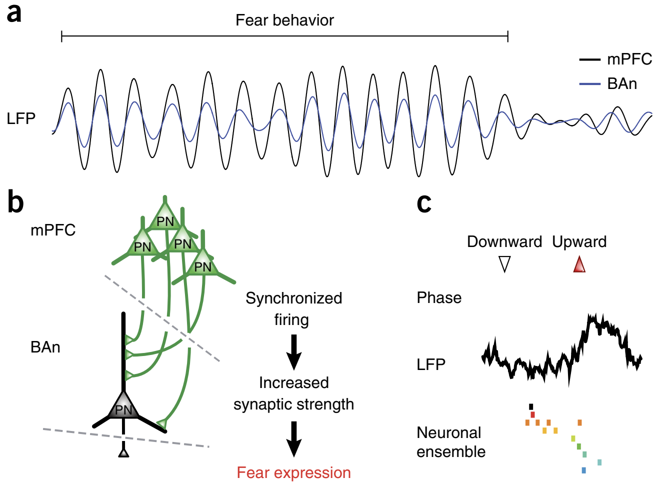

Figure 4 Neuronal mechanisms of fear expression. Two main neuronal mechanisms may coexist in prefrontal-amygdala networks to allow the expression of conditioned fear behavior. (**a**,**b**) First, neuronal synchronization of spiking activity and/or local field potential (**a**) between connected structures might increase synaptic strength through coincident pre- and postsynaptic activation and simultaneous convergence of multiple inputs. Neuronal synchrony can therefore coordinate and enhance the effect of input signals from the mPFC and strengthen information transmission to the BAn and from there possibly to the CEn to ultimately gate fear responses (**b**). (**c**) Second, the formation of temporary synchronized and coordinated neuronal assemblies in response to the development of neuronal oscillations might be an important mechanism for encoding fear expression in a flexible manner. With this mechanism, neurons phase-locked to different phases of the oscillation will be sequentially activated, which may represent a necessary condition for fear expression. PN, principal neurons. Colored dots represent individual neurons firing sequentially in the neuronal assembly.

neuronal sequence replay during post-learning sleep, when BLAn and mPFC theta synchronizes[114]. Because pathological fear memories are thought to rely on abnormal memory consolidation processes, a better understanding of these mechanisms could enable precise therapeutic interventions in pathological conditions such as anxiety disorders.

Role of mPFC local circuit connectivity during fear expression. Beyond the possibility that neuronal assemblies encode fear information, it is critical to consider the structural framework that could support such coding. In particular it is important to understand what kind of excitatory neurons and inhibitory interneurons form cell assemblies in the mPFC, how the changes in activity of those projection neurons are constrained by local interneurons, and what is their remote or local connectivity. Over the past years, a strong corpus of data established that distinct subpopulations of interneurons play a critical role in the control of cortical activity[115]. In the hippocampus, it has been shown that PV^+ and SOM^+ interneurons, which provide perisomatic and dendritic inhibition onto principal neurons, respectively, differentially regulate the firing sequences of pyramidal neurons[116]. Interestingly, it was recently demonstrated that inhibitory axo-axonic cells in the mPFC and BAn change their firing in response to noxious stimuli, suggesting that they might be involved in processing aversive informations[11,117]. Moreover, one group observed changes in tone-evoked neuronal responses in putative prefrontal fast-spiking interneurons following fear conditioning[118], and the genetic ablation of NMDA receptors from PV^+ mPFC interneurons blocked associative fear learning[119].

Recent data identified a class of prefrontal inhibitory neurons that controls the activity of BAn-projecting neurons to regulate fear expression (**Fig. 3b**)[82]. In this study, the authors used single-unit recordings and optogenetic manipulations of physiologically defined neuronal classes to demonstrate that the dmPFC contains a disinhibitory microcircuit that is required for fear expression. PV^+ interneurons, the central element of this circuit, were phasically inhibited during CS presentations. This inhibition produced a disinhibition of dmPFC pyramidal neurons, likely by suppressing ongoing perisomatic inhibition. Behaviorally, optogenetic inhibition of prefrontral PV^+ interneurons elevated fear behavior under baseline conditions, whereas their optogenetic activation reduced conditioned fear responses. Interestingly, tone-evoked inhibition of PV^+ interneurons was causally related to the resetting of theta oscillations, a neuronal mechanism that synchronizes prefrontal projections neurons. Finally, using antidromic stimulations, it was found that prefrontal pyramidal neurons exhibiting CS-evoked phasic excitation (that is, putative disinhibition) preferentially project to the BAn (**Fig. 3b**). These results provide the first demonstration that prefrontal PV^+ interneurons mediate two complementary mechanisms (disinhibition and synchronization) to coordinate and enhance the activity of projection neurons to drive fear expression[82]. It would be of great interest in the future to identify whether changes in activity of prefrontal PV^+ interneurons during fear expression is associated with the recruitment of particular neuronal assemblies. All together, these studies suggest that distinct types of local inhibitory interneurons regulate the activity of cortical neurons involved in the control of fear behavior by promoting neuronal synchronization. These data also raise the possibility that the regulation of distinct subpopulations of prefrontal inhibitory interneurons might represent new therapeutic strategies for regulating pathological fear behavior. Nevertheless, additional studies are required to understand which neuronal elements in the BAn are targeted by prefrontal output neurons and whether or not they differ from the BAn neurons involved in fear acquisition. Moreover, the output circuits directly controlling behavioral fear expression will need to be identified.

Expression circuits summary. In recent years, it has become clear that multiple circuits comprising the CEn, dmPFC, BLAn and PAG regulate fear responses. First, this recent work has extended our view of the circuits mediating fear expression. These data have expanded our knowledge about the role of the LAn/BAn-CEm pathway during fear expression and have identified the local circuitry involved. Second, this work allowed the anatomical identification of non-canonical neuronal circuits, composed of specific cell populations, such as the neuronal pathway between CEl SOM^+ neurons and PAG, which can directly regulate fear expression. Third, important mechanisms allowing fear expression have also been identified. These mechanisms include the development of neuronal oscillations, which are instrumental for the recruitment of dedicated cell populations and the local and long-range synchronization of spiking activity in the dmPFC and the BAn, ultimately gating fear expression. Despite these important findings, several key questions related to the requirement of multiple circuits for controlling fear behavior, the conditions under which these circuits are recruited and whether or not they work in parallel remain to be addressed in future studies.

Conclusion

Recent technical developments such as optogenetic identification and manipulation of specific neuronal elements, genetic rodent models and large-scale recordings of neuronal populations have considerably increased our capacity to dissect and understand the function of dedicated neuronal circuits regulating fear behavior. The emerging model of the neuronal circuits involved in fear behavior suggests the existence of parallel collaborative neuronal circuits and mechanisms involved in the acquisition or expression of learned fear behaviors. First, in addition to the activity-dependent plasticity that develops in the LAn and BAn during fear conditioning, recent studies have demonstrated a potentiation of LAn and BAn to CEl synapses during fear learning, suggesting a potential CEl gating mechanism for fear behavior. Moreover, it appears that thalamic and cortical sensory regions display activity-dependent plasticity during fear learning that could lead to the sharpening of frequency tuning curves toward fear-conditioned tones, a potential mechanism allowing fear discrimination. Other studies have revealed that a nociceptive pathway through the PAG to the LAn supports an aversive teaching signal critical for fear learning that could be regulated by long-range amygdala-PAG circuit interactions, LAn and BAn local interneurons, and/or neuromodulatory mechanisms. Second, recent studies have revealed that fear expression could depend on multiple parallel neuronal circuits. One circuit directly modulates fear behavior through connections between the LAn, BAn and CEm output neurons. In the CEl, SOM^+ neurons also project to the vlPAG, where they can directly regulate conditioned fear responses. Another circuit relies on the projections of distinct sets of BAn neurons to the PLc area of the dmPFC and to the ILc, and possibly the development of long-term synaptic plasticity or intrinsic plasticity mechanisms at BAn inputs to these subregions. Finally, in mPFC-BAn circuits, the recruitment of specialized neuronal populations such as PV^+ interneurons, the development of neuronal oscillations and the synchronization of prefrontal output neurons contacting the BAn are potential neuronal mechanisms that could allow for the precise regulation of fear expression.

The conditions in which the different neural circuits and mechanisms mediating fear acquisition and expression are selected are still largely unknown, but could depend on the complexity of the behavioral task, the strength of the CS and US inputs activated during conditioning, internal states, or environmental situations that may impose the selection of distinct neuronal circuits to produce an appropriate behavioral output. From a clinical standpoint, it is clear that dysfunction in associative processing in amygdala and prefrontal neuronal circuits are at the core of pathological fear behavior occurring in anxiety disorders such as post-traumatic stress disorder. Understanding the precise plasticity and neuronal mechanisms occurring in dedicated neuronal elements and across distributed circuits during fear behavior will be instrumental for the development of new therapeutic strategies for these psychiatric conditions.

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COMPETING FINANCIAL INTERESTS

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