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Dysregulation of aversive signaling pathways: a novel circuit endophenotype for pain and anxiety disorders

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Abstract

Aversive experiences activate dedicated neural instructive pathways which trigger memory formation and change behavior. The strength of these aversive memories and the degree to which they alter behavior is proportional to the intensity of the aversive experience. Dysregulation of aversive learning circuits can lead to psychiatric pathology. Here we review recent findings elucidating aversive instructive signaling circuits for fear conditioning. We then examine how chronic pain as well as stress and anxiety disrupt these circuits and the implications this has for understanding and treating psychiatric disease. Together this review synthesizes current work on aversive instructive signaling circuits in health and disease and suggests a novel circuit based framework for understanding pain and anxiety syndromes.

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Aversive experiences, such as those which are painful, produce strong memories and shape behavior in adaptive ways. For example, getting attacked by a dog while walking in your neighborhood results in detailed, lifelong memories of the experience and emotional responses upon re-exposure to the situation where the attack occurred. These conscious and emotional memories form because aversive experiences are transduced by dedicated neural pathways into 'instructive' signals which alter connectivity in brain networks responsible for storing memories resulting in aversive learning and adaptive changes in behavior.

In some clinical conditions aversive experiences produce disproportionate and dysfunctional emotional responses and memories. In chronic pain syndromes, for example, somatosensory/pain pathways become sensitized resulting in debilitating psychological symptoms [1,2]. In anxiety disorders such as post-traumatic stress disorder (PTSD), chronic stress or trauma can sensitize aversive learning circuits and produce strong, long lasting and incapacitating emotional memories and responses [3,4•,5]. Genetic and experiential factors are the root cause of most psychiatric disorders including chronic pain and anxiety. These factors produce psychiatric dysfunction through actions on specific brain circuits. To understand how psychiatric conditions emerge it is critically important to identify the circuits which mediate normal function and then determine how these systems are disrupted in disease conditions. Related to chronic pain and anxiety disorders, a potential underlying cause could be dysregulation of aversive instructive signaling pathways by genetic and experiential influences. This could result in exaggerated, persistent aversive learning as well as more generalized anxiety and depressed mood, symptoms which are characteristic of pain and anxiety syndromes.

In this review we discuss recent work elucidating the circuit mechanisms of aversive instructive signaling for auditory fear conditioning. We then explore the hypothesis that dysregulation of these instructive signaling circuits underlies chronic pain and anxiety disorders. We focus on fear conditioning because most of the research on aversive instructive circuits comes from this area and these same circuits likely participate in other forms of aversive learning. We note that the circuits underlying fear conditioning mediate only one aspect of the aversive experience [6] and further work on other forms of aversive learning will likely be required to accurately model human emotions/feelings and their psychiatric dysfunction.

The lateral and central nuclei of the amygdala: key sites of plasticity mediating fear learning

Auditory fear conditioning occurs when an auditory stimulus (conditioned stimulus, CS) is associated with an aversive outcome such as electrical shock (unconditioned stimulus, US) [7,8]. Following learning, presentation of the tone alone elicits a set of defensive responses

including behavioral freezing and changes in heart rate and blood pressure. The amygdala has emerged as a critical site of synaptic plasticity mediating fear learning (Figure 1), though there are likely other brain regions in the circuit which undergo plasticity [9–11]. Neurons in the lateral nucleus of the amygdala (LA) integrate auditory information from the thalamus and cortex with aversive nociceptive and neuromodulatory signals. During fear conditioning auditory thalamic and cortical inputs to LA (and possibly the basal nucleus of the amygdala, BA) are strengthened such that tone presentation alone following learning activates LA neurons to produce fear responses through output pathways in the central nucleus of the amygdala (CeA) (for recent reviews see [9,11–13]). Plasticity of LA inputs to the CeA also occurs during fear conditioning [14,15], possibly providing a gating mechanism for parallel plasticity occurring in the LA.

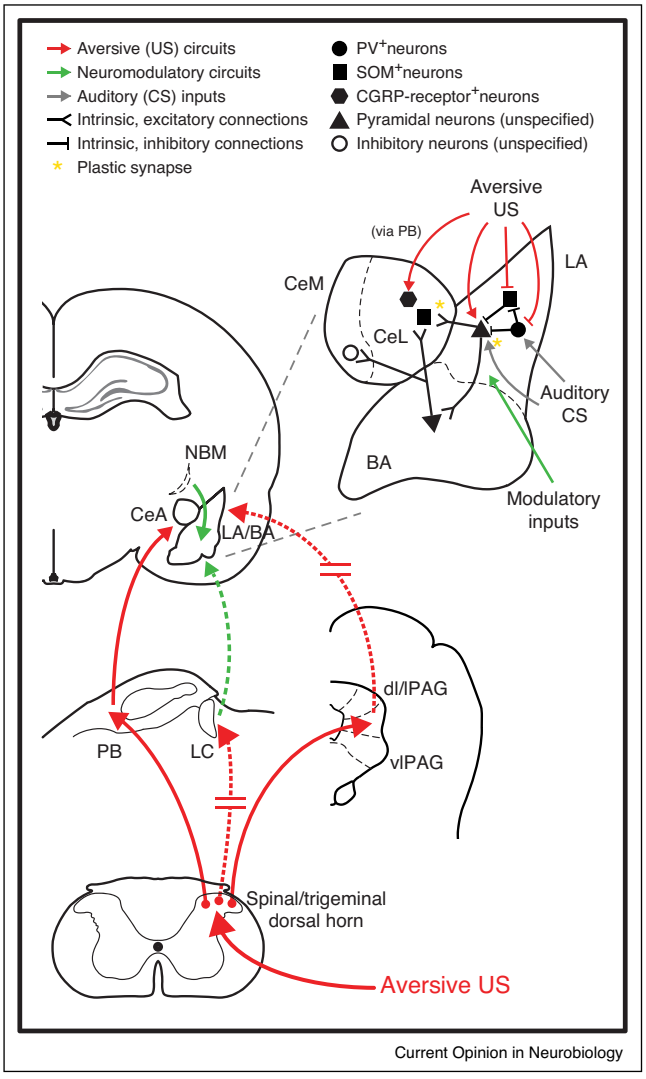
Instructive signals in the lateral amygdala for fear learning

Because plasticity mediating fear conditioning occurs in the LA, it is important to consider the signals within this nucleus which initiate plasticity and fear learning (Figure 1). In LA pyramidal neurons, aversive shock-evoked activity is necessary for strengthening auditory (and olfactory) synapses in LA as well as fear learning and local GABAergic mechanisms are important in regulating this process [16–18]. However, under normal learning conditions activation of LA pyramidal neurons is not sufficient to produce fear conditioning or plasticity unless β-adrenergic receptors (β-ARs) are co-activated [16,19]. Together, this suggests that parallel depolarizing and noradrenergic signals trigger neural plasticity in LA and fear learning.

In addition to noradrenaline, other neuromodulators such as acetylcholine and dopamine are important in fear learning [20••,21]. However, the mechanisms through which neuromodulators regulate plasticity are not known and could include direct modulation of intracellular signaling in pyramidal neurons and/or heterosynaptic control of pyramidal cell activity through local GABAergic networks [22,23]. Furthermore, it is unclear where many of these signals originate or what kinds of information they transmit to LA. Targeted manipulations of cellular level processes and recordings of amygdala projecting neuromodulatory cells could help determine the information conveyed to the amygdala by these neuromodulatory systems and how they control plasticity in LA.

Another important point to consider is that these intra-amygdala signals likely regulate plasticity in specific populations of LA/BA neurons. Recent studies have demonstrated functionally distinct aversive, rewarding and safety cell populations in the BA [24–28]. Furthermore, varying levels of intracellular signaling molecules such as CREB and cellular excitability can modulate

Figure 1



Working model of aversive signaling pathways to the amygdala for fear learning. During fear conditioning, auditory and aversive-nociceptive pathways converge in lateral amygdala (LA) pyramidal neurons (*inset*). Auditory input synapses are strengthened (denoted by yellow *) through a parallel hebbian/neuromodulatory mechanism involving activity in the auditory inputs, depolarization of postsynaptic pyramidal neurons by the shock and noradrenaline signaling. Aversive US information (*left image*) originating from peripheral nociceptive receptors reaches spinal or trigeminal dorsal horn, and is then relayed to PAG, LC, PB, and other brain regions. The PAG and PB may act as relays or modulators of aversive signals to LA/BA and CeA, through either monosynaptic (PB–CeA) or multi-synaptic connections (PAG–LA/B). In addition, the neuromodulatory inputs (from LC and NBM) onto LA/BA networks, along with local inhibitory interneurons (PV^+ and SOM^+ interneurons, *inset*), regulate the sensory-evoked activity and plasticity of LA/BA pyramidal neurons. Dotted lines indicate hypothetical functional/anatomical circuits, solid lines indicate established functional/anatomical pathways. LA/BA: lateral and basal nuclei of the amygdala; CeA: central nucleus of amygdala; CeL: central nucleus of amygdala, lateral division; CeM: central nucleus of amygdala, medial division; PV^+: parvalbumin-expressing; SOM^+: somatostatin-expressing; NBM: nucleus basalis of meynert; dl/l PAG: dorsolateral/lateral subregion of the periaqueductal grey; vlPAG: ventrolateral subregion of periaqueductal grey; LC: locus coeruleus; PB: parabrachial nucleus.

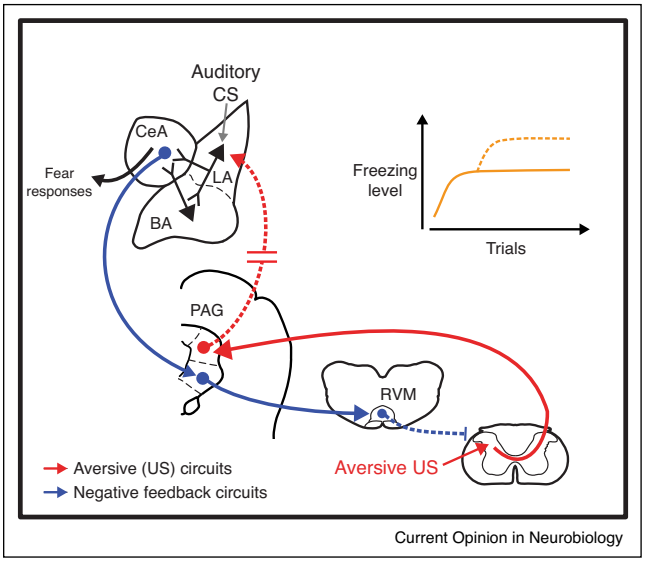
which neuronal populations in the LA are recruited during fear learning to produce fear responses at memory recall [29–32]. This cell-to-cell variability could be due to randomly fluctuating basal levels of CREB/excitability and/or result from cell type specific regulation of CREB expression/excitability by extrinsic factors such as neuromodulators. Differences in these processes between cells could modulate which neurons respond to shocks and tones and/or their ability to undergo plasticity. It will be important in future work to determine the mechanisms which define whether a given cell population participates in aversive, reward or safety learning and how intracellular signaling and extrinsic inputs contribute to plasticity in defined amygdala cell populations.

Aversive instructive signaling pathways to the LA are coordinated with negative feedback systems

The neural pathways which convey aversive shock information to depolarize LA neurons are not completely elucidated [33–35]. However, there is evidence that the midbrain periaqueductal grey (PAG) is important as a relay or modulator of aversive US signals to the LA [36,37••] (Figure 1). The PAG receives dense innervation from the spinal cord dorsal horn and inactivation of the PAG reduces shock-evoked responding in LA neurons and fear learning [36,38]. Furthermore, pairing stimulation of the dorsolateral/lateral subregion of the PAG (dl/lPAG) as a US with an auditory CS is sufficient to produce fear learning and this effect is dependent on neural activity in the LA [39]. However, the subregions/cell types in PAG responsible for this and the final afferent pathway(s) to the LA are not known.

During learning, aversive responses in LA and PAG neurons become inhibited as the auditory cue comes to predict the occurrence of the shock [36,40] (Figure 2). While the dl/lPAG may be important for transmitting aversive information to the LA, a descending feedback pathway from the CeA to the ventrolateral PAG (vlPAG) negatively regulates this ascending aversive signaling circuit to inhibit predicted aversive responses [37••]. This CeA-vlPAG pathway engages a specific population of vlPAG cells which project to a pain modulatory brainstem region called the rostroventromedial medulla (RVM). Auditory CS activation of this circuit controls the maximal strength of fear learning (learning asymptote) that occurs with extended training. These results are consistent with other pharmacological studies implicating endogenous analgesia systems in fear conditioning and specifically in controlling the strength of fear learning [41–43]. Together, this suggests that the learning dependent inhibition of signaling in dl/lPAG and LA occurs through the engagement of descending pain modulatory systems and conditioned analgesia which then sets the strength of fear memories.

Figure 2



Predictive cues recruit a feedback circuit which calibrates the strength of fear learning. During fear conditioning, shock-evoked neuronal responses decrease in dl/lPAG and LA/BA neurons, thereby reducing the ability of the aversive US to produce learning. After a certain amount of training animals reach the learning asymptote where further tone-shock pairings do not produce more learning (i.e. higher freezing responses) (solid line, *upper-right inset*) unless the shock intensity is increased (dashed line, *upper-right inset*). This occurs because the auditory predictive cue recruits a feedback pathway through CeA–vlPAG–RVM (blue lines) which functions to inhibit aversive instructive signaling circuits (red lines) and set behavioral learning asymptotes. Inhibition of this feedback pathway leads to excessive fear responding to the predictive cues (dashed line, *upper-right inset*) much like increasing the shock intensity. Dotted lines indicate functionally hypothetical circuits in the context of fear learning. RVM: rostroventromedial medulla.

Neuromodulatory pathways to the LA

While many neuromodulatory systems have been implicated in fear conditioning using anatomical tracing and pharmacological approaches, the functionally relevant circuits which provide direct neuromodulatory signals to modulate LA/BA networks and plasticity are not well understood. In the case of noradrenaline, the LA receives input from various brainstem noradrenaline centers including the locus coeruleus (LC) [44] (Figure 1). LC lesions in some conditions reduce fear conditioning, but the evidence for this is somewhat mixed [44]. Stimulation of LC inhibits LA/BA neurons in anesthetized animals, though these effects are dependent on which amygdala ARs are activated and the method of stimulation [45,46]. For example, in some conditions LC stimulation or direct noradrenaline application can excite or disinhibit pyramidal neurons [45–49] and activation of optically sensitive β-ARs can directly excite them [50]. These discrepancies in behavioral and physiological experiments could be resolved using cell type specific and terminal manipulation techniques in awake, behaving animals.

Using an optogenetic circuit based approach, a role of ACh innervation of LA was recently explored [20••] using cell type specific manipulations of nucleus basalis of meynert acetylcholine (NBM-ACh) inputs to the BA (Figure 1). The authors found that inhibition of the terminals of NBM-ACh cells in the BA throughout auditory fear conditioning reduced fear learning. Stimulation of these ACh inputs did not enhance learning, but did make fear memories more resistant to extinction. In addition they found that optical stimulation of ACh-BA terminals prolonged excitation of BA pyramidal neurons. This suggests that the NBM-ACh inputs to BA may enhance the excitability of BA neurons and thereby facilitate fear learning.

Aversive instructive signaling pathways to the CeA

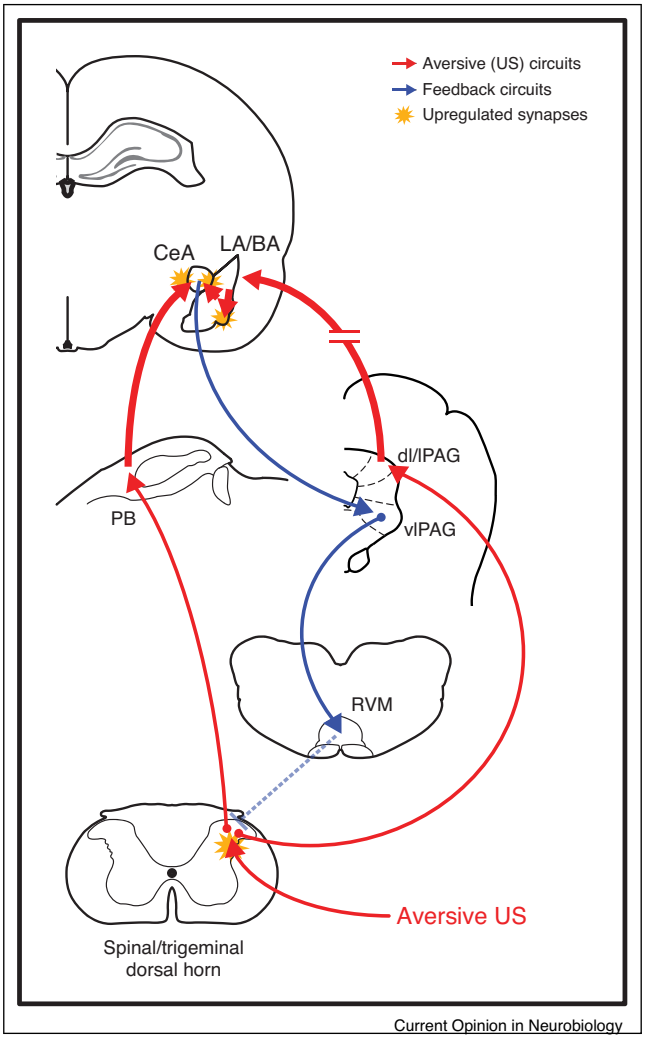
Related to the neural circuits conveying aversive shock information to the CeA, classical anatomical/physiological work demonstrated a nociceptive specific pathway from the superficial layers of the spinal cord dorsal horn to the parabrachial nucleus (PB) and from there to the lateral/capsular portion of the CeA [51] (Figure 1). Two recent studies [52••,53••] directly manipulated this pathway and found that it is important for fear learning. Stimulation of PB neurons (and, specifically, calcitonin gene-related peptide (CGRP) expressing PB cells) is sufficient as a US to produce context and tone fear conditioning. Furthermore, inactivation of PB cells reduces fear learning. One of these studies [53••] also examined the cell types in the CeA which receive PB input and identified a population of CGRP-receptor expressing neurons that overlap with other known CeA cell types (protein kinase C-δ (PKC-δ) in the more posterior CeA and a bit with SOM expressing cells). Stimulation or inhibition of these cells produced or reduced, respectively, fear learning. What is still unclear is whether PB inputs regulate local plasticity in CeA SOM cells and, if so, what information is conveyed by the inputs that are strengthened.

Together the current data support the idea that multiple parallel circuits convey aversive information to different subregions of the amygdala (Figure 1). A spinal-PB pathway is important for conveying aversive shock information to the CeA and a multicomponent set of pathways including the PAG and neuromodulatory systems transmit aversive information to the LA.

Disruption of aversive instructive signaling and feedback circuits in chronic pain conditions

Chronic pain syndromes are associated with emotional suffering and avoidance behaviors as a result of a sensitized pain system. This sensitization is due in part to neuroplastic changes occurring at the level of the spinal cord [54]. However, given the affective nature of chronic pain symptomology, another contributing factor could be changes occurring specifically in aversive instructive signaling pathways and in the feedback systems that control these circuits [2,55,56•] (Figure 3). Supporting this idea, synaptic strengthening of PB and LA/BA inputs to the CeA as well as changes in excitability of CeA neurons occurs in various rodent chronic pain models [57–59]. Possibly related to the involvement of CGRP expressing PB inputs to CeA in fear learning, blockade of CGRP receptors in CeA following induction of chronic pain reversed the plasticity at PB-to-CeA synapses [60]. Chronic pain models also induce synaptic potentiation of LA-to-BA synapses and enhanced responsiveness of BA

Figure 3



Aversive instructive signaling pathways are disrupted in chronic pain conditions. In ascending pain pathways (red lines), rodent chronic pain models produce synaptic potentiation of the parabrachial nucleus (PB)-to-the central nucleus of the amygdala (CeA) synapses, lateral/basal nucleus of the amygdala (LA/BA)-to-CeA synapses and LA-to-BA synapses. Chronic pain conditions also upregulate pro-nociceptive RVM neurons which ultimately reduce rostroventromedial medulla (RVM)-to-spinal cord descending analgesia systems (dotted pale blue line), potentially reducing the effectiveness of predictive negative feedback systems (blue lines) to control learning strength.

neurons to innocuous and noxious stimuli [56•,61]. Although the effects on fear conditioning were not assayed, chronic pain induction produced hyperalgesia as well as increased vocalizations and anxiety-like behavior [61–63]. These effects were blocked when the enhanced synaptic strength in the LA-BA and PB-CeA synapses was pharmacologically reversed, directly linking the pain induced synaptic changes in the amygdala to clinically relevant behavioral alterations.

Chronic pain induced dysregulation of descending pain modulatory systems could also underlie psychological aspects of pain syndromes (Figure 3). The PAG-RVM-spinal cord circuit contains pro and anti-nociceptive cell populations [64]. The balance of activity across these opposing cell populations has been proposed to control the sensitivity of nociceptive processing in the spinal and trigeminal dorsal horn neurons [64,65]. Pain facilitatory RVM neurons expressing the mu-opiate receptor are necessary for the maintenance of chronic pain [66], suggesting that chronic pain conditions preferentially engage pro-nociceptive RVM subcircuits. If the feedback systems regulating prediction error coding recruit this circuit as evidence suggests [37••], chronic pain induced shifts from negative to positive feedback in this system would serve to enhance aversive instructive signaling and increase the strength of aversive learning.

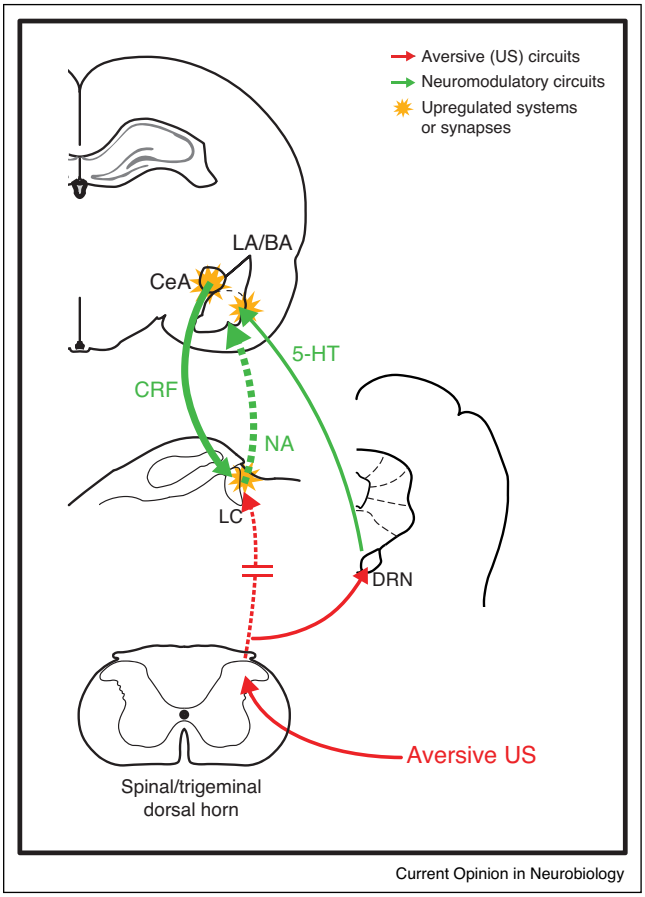
Dysregulation of aversive instructive circuits by stress and anxiety

Anxiety syndromes are characterized by exaggerated and persistent aversive learning as well as dysregulation of amygdala-associated networks following traumatic experiences or chronic stress [3,4•]. Similar to chronic pain syndromes, a causal factor in the development and maintenance of anxiety disorders could be disrupted aversive instructive signaling circuits. No studies to date have examined whether the PAG/PB circuits or negative feedback systems associated with them are dysfunctional in anxiety disorders. However, given the importance of these systems in initiating and modulating the strength of fear memories, an examination of this question is critically important.

Sensitization of the noradrenaline system in anxiety disorders has received relatively more attention [4•,67,68]. Anxiety disorder patients exhibit increased sensory evoked (and possibly basal) noradrenaline release and heightened noradrenaline levels during sleep relative to wakefulness [69–72]. Consistent with the idea that the noradrenaline system is sensitized in anxiety disorder patients, administration of an antagonist to the α-2-AR (a noradrenaline autoreceptor) which can increase ongoing noradrenaline release, produces strong anxiety symptoms in anxiety disorder patients, but not in healthy controls [73].

A sensitized noradrenaline system could directly contribute to anxiety disorder pathology by over-engaging amygdala dependent fear learning and consolidation/reconsolidation mechanisms during traumatic experiences and sleep (Figure 4). Supporting this idea, augmenting β-AR signaling in the LA/BA increases fear learning, fear memory consolidation/reconsolidation and anxiety related behaviors [16,50,74–77]. If the LC is important in supplying noradrenaline to the amygdala and in triggering fear memories, then an upregulation in LC-noradrenaline activity could produce exaggerated and persistent fear learning. Paralleling the human condition, various stressors induce higher tonic firing in LC neurons and, in some cases, increase aversive stimulus evoked responses [78,79,80••]. This stress-induced enhancement of LC responsivity could arise through

Figure 4



Stress induced dysregulation of neuromodulatory circuits controlling fear and anxiety. Stress increases neuronal activity in the locus coeruleus (LC) through recruitment of corticotropin-releasing factor (CRF)-positive neurons in the central nucleus of the amygdala (CeA) and CRF release in the LC. Increases in NA release could exaggerate fear learning by enhancing plasticity in the lateral/basal nucleus of the amygdala (LA/BA). Stress upregulates the dorsal raphe (DR)-serotonergic system by increasing the expression of 5-hydroxytryptamine (5-HT) 2-receptors in the LA/BA leading to heightened fear memory consolidation following stress. Dotted lines indicate hypothetical functional/anatomical circuits, solid lines indicate established functional/anatomical pathways.

CeA-corticotropin-releasing factor (CRF) innervation of LC. CRF administration in awake, behaving animals is accompanied by increased LC activity and stress-elicited LC activation requires CRF signaling [78,81,82]. Suggesting a functional role for enhanced LC-noradrenaline signaling during anxiety through CeA-CRF activity, an interesting recent study showed that stress-induced enhancement of anxiety occurs through an increase in LC-tonic activity mediated by CeA-CRF innervation of LC [80••].

Another recent study [83••] implicated the serotonin system in heightened fear learning following stress (Figure 4). The authors found that stress produced an increase in fear learning and 5-HT2-receptor expression, but not serotonin release, in the LA/BA. The stress induced increase in fear learning was dependent on 5-HT2-receptor signaling in LA/BA and shock-evoked activity in serotonin neurons in the dorsal raphe nucleus. Interestingly, this system did not appear to be important for fear learning under normal learning conditions in the absence of stress.

Conclusions

Our understanding of the neural circuitry of aversive instructive signaling during fear conditioning is rapidly expanding due to modern, circuit based technical approaches. These studies suggest that parallel neuromodulatory and excitatory pathways to distinct subregions of the amygdala trigger fear learning and that these systems are coupled with feedback mechanisms which regulate the strength of fear memories. Under conditions of chronic pain, stress or anxiety, these aversive signaling circuits can become dysregulated leading to exaggerated and persistent aversive emotional memories and potentially to debilitating psychiatric dysfunction. Fully elucidating these aversive instructive systems and understanding how they are altered in disease conditions could lead to novel approaches for the treatment of chronic pain and anxiety disorders.

Conflict of interest statement

Nothing declared.

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