Chapter 7

The Amygdala and Anxiety

Sergio Linsambarth, Rodrigo Moraga-Amaro, Daisy Quintana-Donoso, Sebastian Rojas and Jimmy Stehberg

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.68618

Abstract

The amygdala has a central role in anxiety responses to stressful and arousing situations. Pharmacological and lesion studies of the basolateral, central, and medial subdivisions of the amygdala have shown that their activation induces anxiogenic effects, while their inactivation produces anxiolytic effects. Many neurotransmitters and stress mediators acting at these amygdalar nuclei can modulate the behavioral expression of anxiety. These mediators may be released from different brain regions in response to different types of stressors. The amygdala is in close relationship with several brain regions within the brain circuitry that orchestrates the expression of anxiety. Recent developments in optogenetics have begun to unveil details on how these areas interact.

Keywords: amygdala and anxiety, central amygdala, basolateral amygdala, elevated plus maze, anxiety

1. Introduction

Anxiety is a physiological response that we encounter in everyday life. Although we normally associate it with a menace, anxiety can be elicited by a wide range of situations. Usually, we may feel anxious when the outcome of a future situation is uncertain. For example, when we are called to meet our boss, when we are preparing for a date, or when we stand in front of someone we like and we are trying to decide whether to talk to that person or not, even if we do not end up doing so. We also feel anxious when we want to do or have something excessively. Anxiety appears also as a common trait in our stressful daily living activities. When we are stressed, we become nervous, hyperresponsive, and sometimes easy to anger. Anxiety is in fact so common that it is one of the most frequent symptoms in psychiatric and neurological disorders, and it often appears in most chronic diseases.

The usual definitions of anxiety that can be found in Internet are "distress or uneasiness of mind caused by fear of danger or misfortune fear" (dictionary.com); "an uncomfortable feeling of nervousness or worry about something that is happening or might happen in the future" (Cambridge dictionary.org); "the vague, uneasy feeling you get when you're dreading something. Anxiety can also be a permanent state of nervousness that some people with mental illnesses experience, a milder version of panic" (vocabulary.com) or "Anxiety is an emotion characterized by an unpleasant state of inner turmoil, often accompanied by nervous behavior, such as pacing back and forth, somatic complaints, and rumination. It is the subjectively unpleasant feelings of dread over anticipated events" (Wikipedia.com).

Anxiety can also be operationally defined as an emotional response to potential unidentified threats and is characterized by sustained arousal, vigilance, worry, and apprehension that results in specific patterns of defensive behaviors and concomitant autonomic responses [1]. Arguably, in such cases, a threat may not necessarily mean a direct risk to our existence or a menace but may also include situations that threaten our emotional balance, like when we are anxious for meeting someone or when expecting something to happen, whether the outcome of such encounter is expected to be negative or beneficial.

Although we may agree or disagree with any or all the above definitions, clearly, we all have an opinion on what anxiety is from our own experience, as its description is highly subjective. We may also define anxiety based on its differences from fear. According to the American Psychiatric association, "Anxiety is not the same as fear, which is a response to a real or perceived immediate threat, whereas…anxiety is the expectation of a future threat" [2]. Other authors have gone deeper, suggesting that "Fear is defined as short lived, present focused, geared towards a specific threat…facilitating escape from threat; anxiety, on the other hand, is defined as long acting, future focused, broadly focused towards a diffuse threat, and promoting excessive caution while approaching a potential threat and interferes with constructive coping" [3].

When we try to understand the brain circuitry involved in anxiety, we need to distinguish it from the circuitry associated with fear. It is easier said than done, a large number of studies in animals have found that anxiety- and fear-related brain circuitry are quite similar, and much of what we know today of the brain circuitry associated with anxiety is actually extrapolated from that of fear.

A second issue that is critical for understanding the brain areas and mechanisms subserving anxiety is distinguishing anxiety from anxiety-related disorders. Here again, studies in both human and animals tend to extrapolate findings from anxiety-related disorders to anxiety per se. For example, in animals, most of what we know about the circuitry of anxiety comes from studies on fear conditioning, a model for posttraumatic stress disorder (PTSD), while in humans, most studies dealing with anxiety extrapolate their results from anxiety-related disorders, sometimes comparing levels of anxiety in patients with different disorders, including generalized anxiety, PTSD, or phobias.

In the present chapter, we shall try to describe the brain circuitry associated with anxiety per se, based on both human and animal studies. We shall include studies on fear or anxiety-related disorders only in exceptional cases where their findings may be critical for the general understanding of anxiety, or in cases where direct evidence of anxiety is lacking.

2. Amygdala and anxiety

A large number of studies in humans and animals, using a variety of techniques including pharmacology, lesions, and imaging, suggest that the amygdala is a central orchestrator of anxiety responses. As previous chapters of this book deal in more detail with the anatomy and basic circuitry of the amygdala, we shall review succinctly the basic areas of the amygdala, only to the extent necessary to identify those that are believed to be relevant for anxiety.

2.1. The basic anatomy of the amygdala

The amygdala is an almond-shaped structure (hence its name; *almond* in Greek) that is buried deep within the temporal lobe. It was first identified by Burdach in the early nineteenth century. He originally described a group of cells that are now known as the basolateral complex. Subsequently, a large number of structures surrounding the basolateral complex have been identified, what is now dubbed the amygdaloid complex [4]. The nuclei within the amygdaloid complex may be broadly subdivided into the basolateral complex, the cortical nucleus, the medial nucleus, the central nucleus, and the intercalated cell clusters. The regions that appear to be more critical for anxiety are the basolateral amygdala (BLA) within the basolateral complex, the central amygdala (CeA) within the central nucleus, and the medial amygdala (MeA) within the medial nucleus. They differ in terms of cell types, functional organization, and connectivity. The BLA is a cortex-like structure that can be further subdivided into the lateral amygdala (LA), basal amygdala (BA), and basal medial amygdala (BMA) nuclei. The CeA can be further subdivided into the lateral (CEl) and medial central amygdala (CEm) [5]. Broadly, in rodents, it has been suggested that the BLA encodes the threat value of a stimulus, while the central nucleus is essential for the basic species-specific defensive responses associated with fear [6].

2.2. Amygdala studies in anxiety

Earlier studies suggested that the CeA may be involved in processing explicit cue information associated with fear, while less explicit information associated with anxiety may activate the bed nucleus of the stria terminalis (BNST) [7, 8]. The BNST is a component of the "extended amygdala," which plays a critical role in the integration of autonomic and behavioral responses to stress [7, 9]. Given the vast evidence suggesting a role of both areas in anxiety, this view has begun to be contested [10].

Fear extinction studies in animals support the idea that the amygdala plays a central role in the generation and experience of fear that can give rise to anxiety [6, 11]. In general, there is vast evidence that the amygdala plays an essential role in mediating emotions, such as anxiety [12, 13].

Excitotoxic lesions of the CeA [14], BLA [15], and MeA [13] induce changes in anxiety in rodents. Pharmacological studies suggest that activation of the BLA is anxiogenic, whereas its inhibition is anxiolytic [16, 17]. Likewise, pharmacological activation of the CeA and MeA is anxiogenic, whereas their inhibition is anxiolytic [18, 19].

Studies using functional neuroimaging in humans have shown elevated amygdala activity in anxious healthy individuals [20, 21]. Increased amygdala activity has also been reported to unattended fearful faces, which was in turn associated with higher levels of self-reported anxiety [20]. An increment in amygdalar activity has also been correlated with increased activity to neutral faces [22], suggesting that amygdala activity may reflect anxiety levels even in the absence of a threat [23]. In another study, it was reported that subjects with both low and high anxiety show increased amygdala activity in response to attended fearful faces, but only highly anxious volunteers showed increased amygdala response to unattended threat-related stimuli [20].

In consequence, current evidence suggests that the amygdala is involved in the generation of anxiety, with or without the presence of a threat.

The role of the amygdala in anxiety may be attributed at least in part, to its influence on the hypothalamic-pituitary-adrenal (HPA) axis. Both MeA and BLA are preferentially activated by psychological stressors [24–26]. Lesions of the MeA produce selective deficits in HPA axis responses to psychogenic but not homeostatic stressors [27], while BLA lesions dampen HPA axis responses to restraint stress [28]. The impact of the MeA and BLA on HPA responses is probably mediated by extensive interactions with paraventricular (PvN)-projecting neurons [29].

2.3. Amygdalar involvement in anxiety after stress

Current evidence suggests that both acute and chronic stress can induce anxiety [30, 31] and lesions in the CeA attenuate stress-induced anxiety [14]. This effect may be subserved by increased excitability in the BLA network, possibly mediated by a pronounced reduction in both spontaneous and evoked inhibitory postsynaptic potentials [32], neuronal remodeling of synapses, and dendritic branching in the BLA and MeA [33, 34].

Several studies have reported functional and anatomical changes in the amygdala following acute and chronic stress. Acute immobilization stress in rodents has been reported to increase spine density of principal neurons of the BLA only 10 days after stress, together with an increase in the level of general anxiety [35]. Chronic immobilization stress (e.g., for 10 consecutive days) can lead to greater anxiety-like behaviors in rodents, causing a significant increase in anxiety within 24 h after the cessation of the stress, and more robust and widespread increases in spine density, spanning both primary and secondary dendrites of BLA principal neurons [35], as well as robust dendritic growth in pyramidal and stellate neurons of the BLA [33, 34, 36]. The anxiety elicited by acute stress is also mediated by glucocorticoids (GCs) acting at the BLA, via signaling through nongenomic glucocorticoid receptors and the endocannabinoid CB-1 intracellular signaling pathway [37].

One of the most widely used tests to measure anxiety-like behaviors in rodents is the elevated plus maze (EPM) [38], which measures the avoidance of open spaces during spontaneous exploration. Lesioning studies have shown that permanent lesions of the CeA do not affect anxiety under baseline conditions, but attenuate the anxiogenic effect of restraint stress [39]. Moreover, lesions of the CeA produce "anxiolytic-like" effects on rats as measured by other paradigms that are sensitive to anxiety, including conditioned suppression of drinking (CSD),

conflict and defensive burying [40]. Using optogenetics (a technique that allows the activation or inhibition of specific cell targets or connections in the brain), Tye et al. showed that temporal stimulation of BLA terminals in the CeA was followed by an acute, reversible anxiolytic effect, while temporal inhibition increased anxiety-related behaviors, thus implicating specific BLA-CeA projections in the control of acute anxiety [41]. Stress-induced anxiety induces neuronal remodeling in the amygdala, which appears to be dependent on serine protease tissue-plasminogen activator [42].

2.4. Effects of chronic stress on amygdalar function and anxiety

A large bulk of evidence suggests that chronic stress (e.g., chronic restraint stress) and early exposure to stress (e.g., maternal deprivation) not only induce persistent anxiety even after 21 days from the end of stress [36], but also induce a series of functional and morphological changes in the brain. In the amygdala, changes in neural plasticity and electrophysiological responses including suppression of gamma-Aminobutyric acid (GABA) currents [43], as well as a persistent increase in dendritic arborization of spiny neurons and amygdalar hypertrophy, have been reported as a result of chronic stress, effects are not restored by ceasing the stress [36], but can be restored with short environmental enrichment [44]. Interestingly, some of these changes may be prevented by intra-BLA antagonism of corticotrophin releasing factor (CRF) [45], which is a critical hypothalamic activator of the HPA axis and mediator of stress responses in the brain.

Other brain areas involved in anxiety have also been reported to suffer changes and even damage as a result of chronic stress (reviewed in [46]). For example, in animal models of chronic stress, the hippocampus (HPC) and prefrontal cortex show atrophy, which may lead to memory impairments, whereas the amygdala hypertrophy may lead to increased anxiety and aggression (reviewed in [47]).

3. Neurotransmitters in the amygdala involved in anxiety

Given that stress induces anxiety, stress mediators in the brain, such as CRF, norepinephrine (NA or NE), and glucocorticoids (GCs) can all induce anxiety when microinjected into the amygdala. Other neurotransmitters and modulators known to be involved in anxiety include serotonin, dopamine, GABA, acetylcholine, endocannabinoids, neuropeptide Y (NPY), and orexins. Evidence of their role in anxiety while acting in the amygdala will be summarized succinctly.

3.1. Corticotrophin-releasing hormone

CRF (also known as corticotrophin-releasing hormone, CRH), is a 41-amino acid peptide produced primarily by the paraventricular nucleus of the hypothalamus (PvN) and released onto the medial eminence, triggering the release of ACTH from the pituitary, and activating the HPA axis. CRF is also released in synapses from the PvN innervating stress- and anxiety-associated brain regions, and hence, it mediates endocrine, autonomic, and behavioral responses to stress [48].

CRF microinjections into the amygdala induce anxiogenic effects [49], which appear to be mediated by CRF receptor type 1 and not type 2 [50–52]. The role of the CRF receptor type 2 in anxiety remains controversial [53]. Repeated local infusions of a CRF receptor agonist (urocortin) into the BLA increase anxiety-like behavior in rodents and induce synaptic plasticity [32]. CRF, when injected into the MeA, induces anxiogenic effects, and its antagonism, anxiolytic effects mediated by both CRF type 1 and type 2 receptors [54, 55]. Interestingly, the CeA is one of the brain regions with the highest number of CRF producing cells outside the hypothalamus. While knockdown studies using interference RNA have suggested that these CRF producing cells may have a role in the HPA axis, but not in anxiety-like behaviors [56], CRF overexpression in the CeA of nonhuman primates has anxiogenic effects [57]. Further studies are required to determine the role of CRF-producing neurons in the CeA.

Further evidence of a role of CRF in anxiety comes from studies showing that CRF concentration is markedly reduced in the amygdala after treatment with anxiolytics alprazolam and adinazolam [58], while a significant dose-dependent increase in CRF was found in the amygdala after cocaine injection [59], which is also known to induce anxiety-like behavior in rats [60]. The role of CRF in anxiety does not appear to be mediated solely by the hypothalamic-adrenal axis. In a recent study, selective knockout of hypothalamic CRF without affecting CRF to other brain areas was reported to induce a strong anxiolytic phenotype [61, 62]. Systemic administration of CRF and CRF-receptor 1 agonist, both had anxiogenic effects in the EPM, which was blocked by pretreatment with dynorphin/kappa-opioid receptor antagonist norbinaltorphimine, by a mechanism dependent on CRF receptor 1 signaling [63].

CRF within the extended amygdala has been implicated in the increased anxiety that occurs during prolonged abstinence from chronic opiates, cocaine, ethanol, and cannabinoids, and many of these stress-associated behaviors can be reversed by CRF antagonists administered systemically or into the extended amygdala [64].

3.2. Glucocorticoids (GCs)

Corticosterone (CORT) is the principal GC in rodents. Acute CORT administration can induce immediate anxiogenic effects [62], as do intra CeA microinjections [65]. Acute CORT may induce dendritic hypertrophy of BLA spiny neurons and heightened anxiety 12 days after CORT administration. Acute systemic CORT also induces dendritic atrophy of medial prefrontal cortex (mPFC) pyramidal neurons on day 6, concomitantly with impaired working memory [66], while chronic treatment with CORT also induces increased anxiety [67] and leads to dendritic hypertrophy in the BLA.

3.3. Norepinephrine (NE)

NE (also known as noradrenaline) is produced primarily by the nucleus coeruleus (LC) in the pons, although it is also produced in several other areas of the pons, medulla, and thalamus. Noradrenergic innervation from the LC is widespread, releasing NE in all stress and anxiety-related areas and inducing arousal and anxiety. In fact, acute restraint stress activates NE release in stress-related limbic regions, such as the CeA and MeA, lateral BNST, mPFC, and

lateral septum, and microinjections of adrenergic antagonists into those regions affect both stress-induced release of NE and anxiety [68]. Adrenergic manipulation in the amygdala has effects on anxiety [69] and adrenergic antagonists injected into the extended amygdala block the increased anxiety induced during prolonged abstinence from chronic opiates, cocaine, ethanol, and cannabinoids [64].

3.4. Serotonin

There is ample evidence of a role for serotonin in anxiety. Serotonin is produced in the dorsal raphe nucleus (DRN) and enhances fear and anxiety. Serotoninergic DRN projections activate a subpopulation of CRF neurons in the BNST via 5-HT 2C receptors in mice, engaging a CRF BNST inhibitory microcircuit that silences anxiolytic BNST outputs to the ventral tegmental area (VTA) and lateral hypothalamus [70]. In parallel, the PvN also receives serotonergic innervation from the median raphe nuclei in the midbrain, activating the HPA axis, with concomitant release of glucocorticoids [71], by activating serotonin 2A receptors on PvN neurons [72]. The anxiogenic effects of serotonin are further supported by the anxiogenic side effects of SSRI antidepressants that inhibit serotonin uptake [70].

Despite the clear role for brain serotonin in anxiety, studies reporting a role of serotoninergic transmission in the amygdala in modulating anxiety are somewhat inconclusive. Although the microinjections of serotonin receptors 3, 4 and 1A agonists and antagonists into the rodent BLA have no effects on anxiety, as measured using the EPM [73], compounds acting as 5-HT 3 receptor subtype antagonists microinjected into the BLA produce anxiolytic effects [74, 75]. Further support for a role of serotonin at the amygdala in anxiety comes from studies reporting that animals showing more anxious behavior in the EPM show greater serotonin content in the right amygdala [76].

3.5. Dopamine

Dopamine is another neurotransmitter that has long been associated with anxiety (for a review see [77]). Dopamine is produced by the VTA and the substantia nigra and appears to be critical within the reward system for motivation and anxiety. Both the VTA and substantia nigra release dopamine to all brain regions involved in anxiety (including the amygdala) via the mesolimbic, mesocortical and nigrostriatal dopaminergic systems (for a review, see [78]). Studies reporting a role for dopamine in the amygdala mediating anxiety suggest region specificity. Dopamine receptor D1 and D2 agonists show anxiogenic effects, while D1 and D2 antagonists induce anxiolytic effects when microinjected into the BLA [79] (but see Ref. [80]), yet they show no effects on anxiety when microinjected into the CeA [81].

3.6. GABA

The most commonly prescribed and well-known anxiolytics are benzodiazepines (BDZs), which are GABA agonists. GABA is the main inhibitory neurotransmitter in the brain and is released primarily by interneurons and astrocytes, which are widely distributed throughout the brain.

Microinfusions of GABA agonists have anxiolytic effects when injected into the BLA [15, 82–84] and CeA [85, 86], while microinjection of GABA antagonists produces anxiogenic effects when microinjected into the BLA [16, 82]. Microinjection of GABA agonists into the CeA produces anxiolytic effects in the social interaction test, which measures the interaction of unfamiliar rats [16]; however, a study by Zarrindast et al. [86] reported that intra-CeA injection of a $GABA_A$ receptor agonist had anxiogenic effects, while $GABA_A$ receptor antagonist had anxiolytic effects in the EPM.

In general, current evidence suggests that endogenous GABA acts at the BLA to inhibit anxiety responses [16]. However, the amygdala, or at least the CeA, does not appear to be critical for the anxiolytic effects of BZDs, as the anxiolytic effects of acute BZDs and barbiturates, such as chlordiazepoxide, phenobarbital, and carbamazepine, which are all GABA agonists, are not affected by CeA lesions [40].

3.7. Acetylcholine

Acetylcholine is produced by several areas in the brainstem collectively known as the mesopontine tegmentum area, the basal forebrain, the basal nucleus of Meynert, and the medial septal nucleus. The mesopontine tegmentum innervates the locus coeruleus, raphe nucleus, basal ganglia, and basal forebrain [87]; the basal nucleus of Meynert innervates the cortex and the medial septal nucleus projects to the hippocampus and cortex. Cholinergic agonist nicotine microinjected into the CeA induces anxiogenic effects, while intra-CeA injection of mecamylamine, a selective nicotinic acetylcholine receptor antagonist, produces anxiolytic effects [86].

3.8. The endocannabinoid system

Considerable evidence suggests that cannabinoids are anxiolytics and modulate the behavioral and physiological responses to stressful situations [88, 89]. There is vast evidence of a role of endocannabinoids in anxiety acting particularly in the amygdala. The primary constituent of cannabis, tetrahydrocannabinol (THC), when microinjected at low doses into the BLA produces anxiogenic effects [90]. Likewise, other cannabinoids agonists microinjected into the CeA induce anxiolytic effects [91], while cannabinoid antagonists induce anxiogenic effects [92].

3.9. Neuropeptide Y (NPY)

NPY is primarily produced by the arcuate nucleus of the hypothalamus and is released into the PvN. Local injection of NPY into the PvN increases acutely the release of CRF [93]. NPY and its receptor agonists microinjected into the CeA and the BLA have anxiolytic effects [94–96], while microinjection of NPY antagonists has anxiogenic effects [97]. The ablation of the Y2 gene—which encodes for the NPY receptor type 2—both in the BLA and CeA results in an anxiolytic phenotype, whereas deletion in the MeA or in the BNST has no effects on anxiety [98].

NPY also has an important role in feeding [99] and has been associated with stress-related obesity and metabolic syndrome [100].

4. Interactions between neurotransmitter and neuromodulatory systems in anxiety

It is critical to think of the brain as a dynamic system that is always changing and compensating to keep a balanced state, which may change over time with ageing, or chronic stress and could eventually lead to a pathological allostatic balance.

When we think of a neurotransmitter, we have the tendency to think of an individual synapse receiving a particular neurotransmitter and expressing receptors for this neurotransmitter only. In fact, the picture is much more complex, as a single neuron in cortex may participate in more than 10,000 synapses and receive different neurotransmitters, from glutamate, GABA, serotonin, or dopamine, to different peptides like orexins or neuropeptide Y. It may also respond to endocannabinoids, signaling fatty acids or microRNAs. In addition, released neurotransmitters can activate receptors located at the plasmatic membrane of neurons outside synapses. Moreover, astrocytes may also release their own transmitters (known as gliotransmitters) onto synapses, including glutamate, D-serine, and glycine, which are required for normal synaptic transmission and synaptic plasticity (for a review, see Ref. [101]).

It is assumed that single neurons may integrate information from several neurotransmitter systems by expressing different receptors in various locations of their cytoplasm. For example, a neuron may express receptors for some neurotransmitters in distant dendrites and for others located close to its soma, so that the effect of the activation of each receptor differentially influences the firing outcome (by spatial or temporal summation).

Interactions between neurotransmitter systems may not only occur at the single neuron level but may also take place between different neuronal populations, which may receive predominant inputs from different neurotransmitter systems. Alternatively, interactions may also take place not only at the site where neurotransmitters are released, but also at the site where they are produced. For example, chronic stress and chronic CORT administration both have anxiogenic effects. In a recent study, it has shown that chronic CORT treatment induces an increase in serotonin synthesis at the dorsal raphe nucleus, effect that is mediated by CRF within the nucleus [102].

There are many other relevant interactions between stress-mediators. Chronic CORT treatment mimicking chronic stress induces an increase in mRNA and protein levels of NE transporter (NET) and dopamine β-hydroxylase (DBH) in the locus coeruleus, amygdala and hippocampus, suggesting increased NE synthesis [103].

A recent study showed that delta opioid receptors and CRF co-localize in close proximity to NE-containing fibers in both BLA and CeA. In yet another example of such interactions,

the dopamine receptor agonist, SNC80, significantly attenuates the anxiogenic effects of α2 adrenergic agonist yohimbine, as measured in the rat on the elevated zero maze [104].

5. Different anxiety responses associated with different stress circuitries

To understand the different underpinnings associated with stress responses, some studies have suggested that different neurotransmitter systems may have differential roles in stress responses depending on the state of the animal or the type of stress. Recently, Smith et al. reported that while CRF receptor 1 antagonists have anxiolytic effects and allow escape in previously submissive animals, α2-adrenoreceptor antagonists have anxiogenic effects and hinder escape in nonsubmissive escaping mice [105].

Results obtained in a study using fMRI in rats receiving intravenous yohimbine, which induces stress and anxiety [106, 107], showed that the brain activity pattern found after yohimbine, which included activation of limbic structures including prefrontal, cingulate, orbito-frontal, and retrosplenial cortices, CeA, ventral hippocampus, BNST, and the shell of the nucleus accumbens, could be strongly attenuated by a α2-adrenoceptor agonist and by a dopamine (DA) D1 receptor antagonist [108]. Moreover, pretreatment with a CRF1R antagonist inhibited yohimbine-induced activation in the amygdala, striatum, and cingulate cortex, while an orexin type-1 receptor antagonist inhibited the response in fronto-hippocampal regions as well as the extended amygdala [108]. In summary, it appears that the behavioral choices in response to stress are the result of an interplay between different neurotransmitter systems in different brain areas involved in stress responses and anxiety.

6. Other brain areas within the circuitry of anxiety

6.1. Hippocampus (HPC)

The HPC is usually subdivided into ventral (vHPC) and dorsal (dHPC). There is a wide range of evidence showing that both regions of the HPC are central in the regulation of anxiety. This role appears to be mediated via several pathways, including direct amygdala-HPC and PFC-HPC interactions and regulation of the HPA axis. There are direct interconnections between the HPC and the amygdala (reviewed in [109]) and between the HPC and other limbic areas involved in anxiety [110]. Optogenetic activation of BLA-vHPC synapses increases anxiety, while their inhibition decreases anxiety [111]. This amygdalar-HPC interaction has been proposed as pivotal also for the regulation of emotions and cognition (reviewed in [112]).

Numerous studies link the hippocampus with inhibition of the HPA axis [113, 114], thus decreasing the release of glucocorticoids in response to stress and anxiety. Hippocampal activation decreases glucocorticoid secretion in rats and humans [115, 116], whereas HPC damage increases basal and stress-induced glucocorticoid secretion [113, 114]. Notably, lesion effects are most pronounced during the recovery phase of stress-induced glucocorticoid secretion, implicating the HPC in the termination of HPA responses. The inhibitory effects of the HPC

on the PvN are subserved by a relatively circumscribed population of neurons in the ventral subiculum [117] and lesions of this area result in increased corticosterone release following psychogenic but not systemic stressors [117], consistent with a context-specific modulation of stress responses.

The HPC also influences autonomic tone, as hippocampal stimulation decreases heart rate, blood pressure, and respiratory rate in awake rats, effects that are blocked by mPFC lesions [118].

Both vHPC and dHPC have been implicated in the action of several drugs that affect anxiety, including nicotine [119–121]. Nicotine elicits anxiety in mice as measured in the EPM [122]. In fact, mice deficient in the α5 nicotinic acetylcholine receptor (α5−/− mice) show high levels of anxiety in the EPM and in the dark-light box compared to WT. Interestingly the study showed that the reexpression of the α5WT gene in the VTA and the HPC of α5−/− mice restored WT levels of anxiety [123].

The HPC appears to mediate the effects of many other drugs in anxiety. Cholecystokinin (CCK) administration causes anxiety [124] and the injection of the CCK8S isoform into the dHPC has anxiogenic effects in the EPM [125]. The serotonin agonist meta-Chlorophenylpiperazine (mCPP) is another drug that produces anxiogenic effects in rodents when administered intraperitoneally [126] and shows anxiogenic effects when injected directly into the HPC [127]. Substance P (SP) is an endogenous neurokinin known to have effects on anxiety [128, 129] and the HPC contains a high density of SP containing fibers [130]. Injection of SP into the dHPC has anxiolytic effects [131]. Anxiolytics such as BZDs also show anxiolytic effects when injected into the HPC [132] and decreased BZD receptor binding in the HPC and PFC has been reported in patients with anxiety disorders [132].

6.2. Medial and lateral prefrontal cortex

Both medial and lateral prefrontal cortices (mPFC and lPFC, respectively) have direct connections with limbic structures involved in anxiety [133] including dense reciprocal connections with the amygdala and HPC [134].

The mPFC is a complex cortical structure with different subregions that may contribute to anxiety and stress responses. In general, lesions of the mPFC decrease anxiety in rats exposed to the EPM and the social interaction test [133, 135, 136]. The prelimbic mPFC preferentially inhibits HPA axis responses to psychogenic stressors [137–140]. It also regulates glucocorticoid secretion, particularly its duration. Inhibition of the prelimbic mPFC or local injection of NE enhances heart rate responses to psychological stimuli [141]. The infralimbic PFC is involved in initiating autonomic and HPA responses to psychogenic stimuli [140, 142]. Electrical stimulation of the ventromedial mPFC (including the infralimbic cortex) increases blood pressure in awake rats, while lesions or inactivation of the ventromedial PFC inhibits conditioned cardiovascular responses [143, 144] without affecting baseline heart rate or blood pressure, suggesting that it may be selectively involved in stress-induced cardiovascular regulation [145]. In a recent study using multi-site neuronal recordings with terminal optogenetic stimulation, it was shown that inhibition of the vHPC inputs to the mPFC induces anxiolytic effects [146].

Some studies have reported lateralization in the role of mPFC in anxiety. Activation of the right mPFC has been shown to increase anxiety, while its inhibition induces anxiolytic effects, whereas inhibition of the LmPFC elicits anxiogenic effects in a model of social defeat [147].

Imaging studies in humans support the notion that the mPFC is involved in anxiety. During sustained anxiety and high trait anxiety, amygdala activity has been shown to be positively coupled with dorsomedial PFC activity [148].

6.3. Paraventricular nucleus of the hypothalamus (PvN)

As stated earlier, the PvN is crucial for the regulation of the HPA axis, and hence, it is pivotal in stress responses and anxiety. The PvN secretes a number of factors or hormones that are released into the medial eminence to trigger the activation of specific neurosecretory cells in the pituitary, including CRF, which triggers the release of ACTH, which in turns activates glucocorticoid release from the adrenal cortex. It also releases other hormones, such as vasopressin and oxytocin through the neurohypophysis. Interestingly, these hormones also act as neurotransmitters and are released at synapses in limbic areas that are innervated by the PvN. Brain and peripheral oxytocin are released in response to stress and HPA activation (reviewed in [149]), release that is modulated by corticosterone [150], and it may have a role in gastric reflexes and penile erection [151]. Although it has not been proven so far, oxytocin may contribute to anxiety-related erectile dysfunction and, in an interplay with arginine vasopressin (AVP), to stress-induced gastric motility disorders [152]. Brain AVP acts synergistically with CRF on the pituitary, stimulating the release of ACTH and regulating the HPA axis. AVP is synthesized in the PvN and supraoptic nuclei of the hypothalamus and is involved in stress responses via HPA regulation (for a review see [153]). It is also released to the hypothalamus and limbic system (including the amygdala) and is involved in stress responses and anxiety. Notably, brain AVP and oxytocin appear to have opposite effects on anxiety; AVP is anxiogenic, while oxytocin has anxiolytic effects (for a review, see [154]). AVP-containing neurons have also been found in the rat's medial amygdala, innervating limbic structures such as the lateral septum and the vHPC [155]. AVP released within the brain in general has been proposed to modulate stress-induced anxiety [156] and AVP receptor V_{1b} receptor antagonists produce anxiolytic effects on mice [157]. The third neuropeptide released by the PvN is CRF, which was discussed earlier in this chapter.

6.4. Bed nucleus of the stria terminalis (BNST)

The BNST is a component of the "extended amygdala," which plays a critical role in the integration of autonomic and behavioral responses to stress [7, 9, 158]. Earlier studies suggested that the CeA may be involved in processing explicit cue information associated with fear, while less explicit information associated with anxiety may activate the BNST [7, 8]. As stated earlier, given the vast evidence suggesting a role for both areas in anxiety, this view has begun to be contested [10].

The BNST has numerous subregions that differ markedly in their contributions to stress integration. Anteroventral subregions are important in HPA axis excitation, as lesions there

reduce HPA axis responses and inhibit acute activation of PvN neurons following restraint stress [159, 160]. The anterolateral BNST contains CRF neurons that project to the PvN [161, 162], suggesting a mechanism for central excitatory actions of CRF on the HPA axis. Lesions of the posteromedial BNST increase ACTH and corticosterone secretion, PvN c-fos mRNA and PvN CRF mRNA expression [159, 160]. Tracing studies indicate that PvN-projecting neurons in the BNST are predominantly GABAergic [163], suggesting that, in contrast to the anterolateral PvN, posterior regions inhibit HPA responses to stress. Thus, it appears that different regions within the BNST may have opposing roles in anxiety. Further evidence to support this idea comes from a study showing that optogenetic stimulation of the oval BNST has anxiogenic effects, while activation of the anterodorsal BNST has anxiolytic effects [164].

Several studies have tested the effects of overall BNST modulation of different neurotransmitters in anxiety and in anxiety induced by acute or chronic stress. Changes in anxiety levels can be induced by intra-BNST manipulation of CRF [165, 166], glutamatergic AMPA receptors [167], serotonin 1A [168], calcium channel blockers [169], GABA synthesis [170, 171], calcitonin gene-related peptide [172], orexin A and B [173], and noradrenergic activity [174–176].

In awake animals, pharmacological activation of BNST elicits a rapid pressor response followed by bradycardia [177, 178], whereas inactivation exacerbates restraint-induced increases in heart rate [179]. This indicates that BNST signaling is necessary for inhibiting cardiovascular responses to stress. Modulation of heart rate by BNST stimulation or inhibition seems to be mediated by the parasympathetic nervous system [177, 178].

Chronic stress and chronic corticosterone both increase BNST volume [31] and modulation of cholinergic activity and galanin-mediated signaling in the BNST can block the anxiogenic effects of restraint stress [180, 181].

Finally, there are large inter-individual variations in fear responces of clinically anxious humans, who exhibit a tendency to generalize learned fear to safe stimuli. A study using lesions of the BNST in rodents showed that inter-individual variations in fear generalization and anxiety are determined by BNST influencing the amygdala and other limbic areas [182].

6.5. Septum

The septum is usually subdivided into the medial and lateral septum. Blockade of glutamatergic activity at the overall septum by microinjection of AMPA receptor antagonist CNQX induces anxiolytic effects [183]. Cholinergic antagonists microinjected into the medial septum have anxiolytic effects [184], while intra-septal histamine has anxiogenic effects [185].

CRF receptor type 2 agonist urocortin, when injected into the lateral septum, increases anxiety [53], while its antagonist has anxiolytic effects on mice exposed to stress and EPM [186]. Intraseptal microinjections of AVP has anxiolytic effects [187], while injection of an AVP receptor antagonist has anxiogenic effects on rats subjected to EPM [188].

6.6. Insula

The insula or insular cortex is located deep within the temporal lobe in humans and surrounds the rhinal fissure in rodents. The rat insula or insular cortex is reciprocally connected to several anxiety-related regions, including the paraventricular thalamic nucleus [189], infralimbic cortex [190], the BLA and CeA [191–194], BNST [195, 196], the lateral hypothalamic area (LHA) [192], and visceromotor regions in the brainstem including the vago-solitary complex [196–198]. There are massive reciprocal connections between the insular cortex and the amygdala [191, 192, 199, 200] to all amygdalar subdivisions [201].

Several studies have shown increased insular activity in patients suffering from different anxiety-related disorders including GAD [202], panic disorders [203], phobias [204, 205], OCD [204, 206], and PTSD [204, 207]. In all the above disorders, insular activity has been reported to decrease in response to effective treatments [202, 206, 208].

Despite the numerous studies demonstrating a relationship between insula activity and anxiety-related disorders, evidence regarding the role the insula in anxiety per se is still limited. Evidence in humans supports the notion that the insula may have a role in anxiety and a close relationship with the amygdala, as the severity of anxiety is positively correlated with CeA-insula functional connectivity [209], and the anxiolytic effects of lorazepam induce a dose-dependent decrease of activation in both the amygdala and insula during emotion processing [210].

The Insula receives interoceptive information, including pain, itch, muscular, and visceral sensations, as well as hunger and thirst [211]. Given its interoceptive inputs, it has been proposed that the insula may be crucial in determining the difference between the interoceptive sensation expected from a stimulus and the prediction of its outcome, represented as a prediction signal in the anterior insular cortex. Altered interoception would be the primary process underlying the initiation of an anxiety state, and the affective, cognitive, and behavioral components that characterize anxiety would be a consequence of this altered prediction signal, for which the insula would be pivotal [210]. There is presently not sufficient evidence to support this hypothesis.

Studies in rodents support the notion that the insula is involved in anxiety. Muscarinic cholinergic manipulation in the insular cortex in rats modulates anxiety in the EPM [212], while intrainsular modulation of adrenergic activity modulates arousal-induced increases in neophobia (reluctance to novelty), also known as hyponeophagia, which is used to measure of anxiety in rodents [213]. Direct studies to determine the areas of the insula involved in anxiety and its position relative to other brain regions associated with anxiety are a fertile ground for advancement.

6.7. Lateral hypothalamic area

The lateral hypothalamic area (LHA) has a critical role in sleep-wake states, feeding, energy balance, and motivated behavior. Cell populations in the LHA are typically defined

by neurochemical markers such as neuropeptides hypocretin/orexin, and melanin-concentrating hormone (for a review see [214]). Current evidence suggests that hypocretin/orexin neurons in the LHA integrate stress-related central and peripheral information [215–220] and produce hypocretin/orexin that is released at synapses in anxiety-related brain regions, including the amygdala, which shows reciprocal connections with the LHA [196]. Hypocretin/orexin neurons increase their firing rates *in vivo* during exposure to novel environments or other arousing situations [221]. Furthermore, *in vivo* optogenetic stimulation of hypocretin/orexin neurons results in hypercorticosteronemia [214]. Food deprivation enhances hypocretin/orexin-dependent HPA axis activation, while local infusion of leptin (a satiation signal produced by fat) into the LHA blunts hypocretin/orexin neuronal activity. Optogenetic activation of LHA leptin responsive neurons reduces both corticosterone release and suppressed hypocretin/orexin neuron activation in response to stress [214]. Further support for a role of hypocretin/orexin neurons in anxiety comes from studies showing that anxiolytic drug treatment with BZDs decreases c-fos activation of orexin neurons in the LHA [222]. Moreover, injection of orexin-A or orexin-B into the paraventricular nucleus of the thalamus increases anxiety, effect that can be blocked by an orexin 2 receptor antagonist [223]. For a review of the orexin system's role in neuropsychiatry see [224].

Given the critical role of hypocretin/orexins in increasing appetite, it is tempting to associate anxiety and appetite and proposes that the LHA is involved in eating disorders. Whether it is anorexia nervosa, obesity due to anxious eating or binge eating, eating disorders are characterized by changes in feeding behavior in response to anxiety.

There are other areas believed to play a role in anxiety, which include the paraventricular thalamic nucleus, periaqueductal grey, reward-related areas like nucleus accumbens and VTA (reviewed in Ref. [1, 225]). For a scheme of brain regions associated with anxiety, see **Figure 1**.

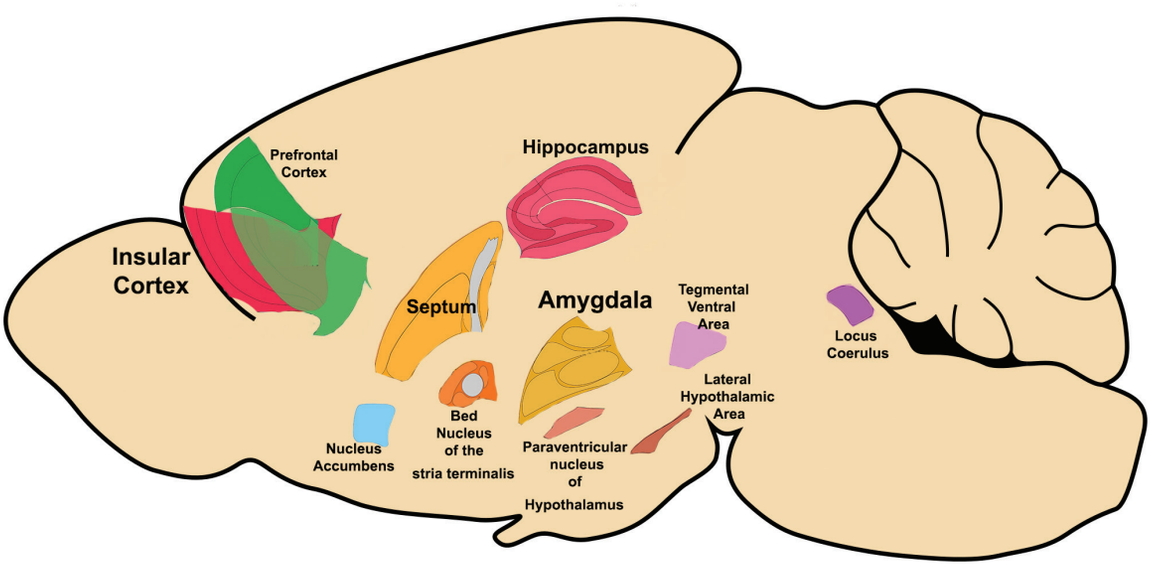

Figure 1. Scheme of main brain areas believed to be involved in anxiety.

7. Connections between the amygdala and other brain regions associated with anxiety

The recent development of optogenetic approaches allowing the activation or inhibition of specific cell types or neuronal projections using the inducible expression of channel rhodopsins has begun to allow a much greater understanding of the circuitries associated with anxiety. In a recent study, it was shown that inhibition of vHPC input to the mPFC and bilateral, but not unilateral inhibition of the input to the BLA disrupts anxiety [146]. In the same line, the activation of BLA inputs to the mPFC produces anxiogenic effects in the EPM and openfield tests, whereas inhibition of the structure produces anxiolytic effects [226]. Furthermore, systemic activation of Kapa opiod receptors was shown to inhibit glutamate release from BLA projections to the BNST and prevent the anxiolytic effects induced by optogenetic activation of BLA-BNST projections, while deletion Kapa opiod receptors from amygdala neurons induces anxiolytic effects [227]. In yet another study, it was reported that the stimulation of VTA-projecting BNST glutamatergic neurons has anxiogenic effects, while the activation of VTA-projecting BNST GABAergic neurons has anxiolytic effects, similar to the effects of direct inhibition of VTA GABAergic neurons [228].

In an elegant study by Kim et al., it was reported that the stimulation of the efferent projections from the anterodorsal BNST to the LHA reduced risk-avoidance, while the stimulation of the projections to the parabrachial nucleus reduced respiratory rate, and to the VTA, increased positive valence, all features associated with anxiolysis [164]. **Figure 2** shows a simplified scheme of the effects of stimulating the different brain regions associated with anxiety, and their

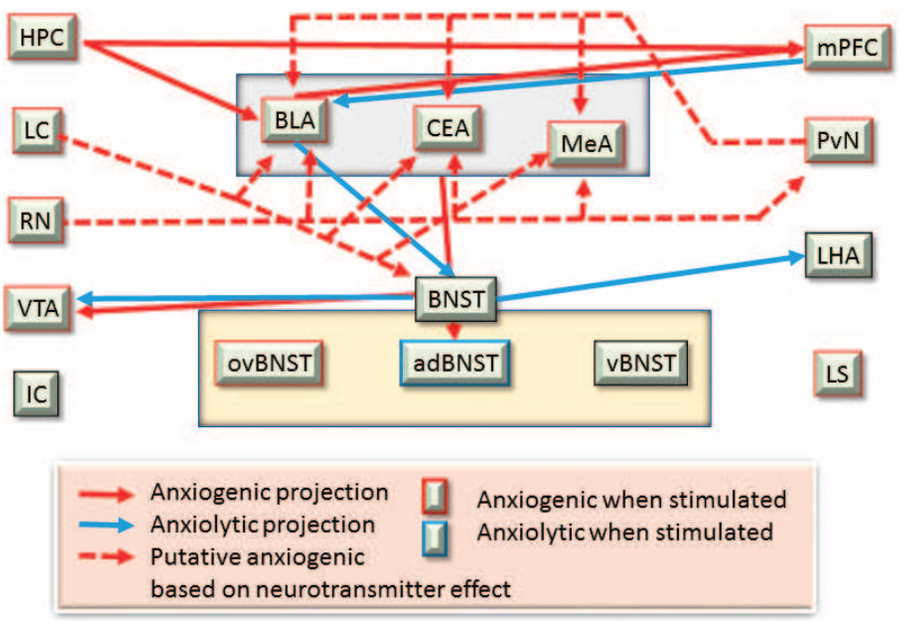

Figure 2. Effects in anxiety of the pharmacological activation of the different brain regions involved in anxiety and their projections. HPC, hippocampus; LC, locus coeruleus; RN, Raffe nucleus; VTA, ventral tegmental area; BLA, basolateral amygdala; CEA, central amygdala; MeA, medial amygdala; mPFC, medial prefrontal cortex; PvN, paraventricular nucleus of the hypothalamus; LHA, lateral hypothalamic area; IC, insula (insular cortex); LS, lateral septum; BNST, bed nucleus of the stria terminalis; ovBNST, oval BNST; adBNST, anterodorsal BNST; vBNST, ventral BNST.

projections, including those projections known to have specific effects on anxiety, and those that may have a role based on the effects of the neurotransmitters microinjected into those regions.

8. Conclusive remarks

The amygdala appears to be a pivotal orchestrator of anxiety, particularly through its subregions CeA, MeA, and BLA. Many of the other brain regions involved in anxiety have been identified, and it is quite clear that the interactions between these areas through a large number of different neurotransmitters and neuropeptides fine tune anxiety levels in response to diverse stressful situations (for a scheme of some of the known projections involved in anxiety see **Figure 2**). Recent optogenetic approaches allow a more detailed description for the role of the different connections among anxiety-subserving brain regions. Further research using more specialized tools, enabling the activation or inhibition of more specific cell populations, will allow us to understand with greater detail how different cell populations within and between brain areas interact to orchestrate behavior and anxiety in particular. There are many difficulties ahead. Distinguishing, for example, anxiety from stress responses—being anxiety a part of the stress response—is difficult in animal models where anxiety is attained through arousal or stress. From what we know so far, there are many stress mediators and brain areas that may trigger anxiety in response to different stressors, whether they are feeding-related, pain-related, acute- or chronic stress-related. The dysfunction of the brain circuitry subserving anxiety and stress, and the neurotransmitter systems involved, may be critical for the development of anxiety and stress-related pathologies. It is interesting to note that pharmacological activation of most anxiety-related areas has anxiogenic effects (see **Figure 2**), and their inhibition, anxiolytic. Which of those areas are downstream or upstream from each other within the circuitry of anxiety still remains elusive. It is easy to speculate that visceromotor areas should be downstream. Yet emotions require constant sensory feedback. Most visceromotor areas are also viscerosensory and as explained in the chapter, most areas involved in anxiety, including the amygdala, when activated, can elicit direct activation of the HPA axis. So, the pieces of the puzzle are all there, but it may still require some time to put them all together.

Funding and acknowledgements

This chapter was funded by Grant FONDECYT 1160986. We wish to thank the Book Editor, Barbara Ferry, for her useful comments.

Author details

Sergio Linsambarth, Rodrigo Moraga-Amaro, Daisy Quintana-Donoso, Sebastian Rojas and Jimmy Stehberg*

*Address all correspondence to: jstehberg@unab.cl

Laboratorio de Neurobiologia, Centro de Investigaciones Biomedicas, Universidad Andres Bello, Santiago, Chile

References

[1] Tovote P, Fadok JP, Luthi A. Neuronal circuits for fear and anxiety. Nature Reviews Neuroscience. 2015;**16**(6):317-331

[2] American Psychiatric Association Publishing. Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Washington, DC; 2013

[3] Sylvers P, Lilienfeld SO, LaPrairie JL. Differences between trait fear and trait anxiety: Implications for psychopathology. Clinical Psychology Review. 2011;**31**(1):122-137

[4] Sah P, Lopez De Armentia M. Excitatory synaptic transmission in the lateral and central amygdala. Annals of the New York Academy of Sciences. 2003;**985**:67-77

[5] McDonald AJ. Cytoarchitecture of the central amygdaloid nucleus of the rat. Journal of Comparative Neurology. 1982;**208**(4):401-418

[6] Davis M, Whalen PJ. The amygdala: Vigilance and emotion. Journal of Molecular Psychiatry. 2001;**6**(1):13-34

[7] Davis M, Shi C. The extended amygdala: Are the central nucleus of the amygdala and the bed nucleus of the stria terminalis differentially involved in fear versus anxiety? Annals of the New York Academy of Sciences. 1999;**877**:281-291

[8] Walker DL, Miles LA, Davis M. Selective participation of the bed nucleus of the stria terminalis and CRF in sustained anxiety-like versus phasic fear-like responses. Progress in Neuropsychopharmacology & Biological Psychiatry. 2009;**33**(8):1291-1308

[9] Herman JP, Cullinan WE. Neurocircuitry of stress: Central control of the hypothalamo-pituitary-adrenocortical axis. Trends in Neurosciences. 1997;**20**(2):78-84

[10] Shackman AJ, Fox AS. Contributions of the central extended amygdala to fear and anxiety. Journal of Neuroscience. 2016;**36**(31):8050-8063

[11] LeDoux JE. Emotion circuits in the brain. Annual Review of Neuroscience. 2000;**23**:155-184

[12] Davis M, Rainnie D, Cassell M. Neurotransmission in the rat amygdala related to fear and anxiety. Trends of Neurosciences. 1994;**17**(5):208-214

[13] Kalin NH, Shelton SE, Davidson RJ. The role of the central nucleus of the amygdala in mediating fear and anxiety in the primate. Journal of Neuroscience, 2004;**24**(24):5506-5515

[14] Ventura-Silva AP, et al. Excitotoxic lesions in the central nucleus of the amygdala attenuate stress-induced anxiety behavior. Frontiers in Behavioral Neuroscience. 2013;**7**:32

[15] Pesold C, Treit D. The central and basolateral amygdala differentially mediate the anxiolytic effects of benzodiazepines. Brain Research. 1995;**671**(2):213-221

[16] Sanders SK, Shekhar A. Regulation of anxiety by GABAA receptors in the rat amygdala. Pharmacology Biochemistry and Behavior. 1995;**52**(4):701-706

[17] Truitt WA, et al. Anxiety-like behavior is modulated by a discrete subpopulation of interneurons in the basolateral amygdala. Neuroscience. 2009;**160**(2):284-294

[18] Davis M. The role of the amygdala in fear and anxiety. Annual Review of Neuroscience. 1992;**15**:353-375

[19] Flores-Gracia C, et al. GABA(A) rho receptor mechanisms in the rat amygdala and its role in the modulation of fear and anxiety. Psychopharmacology (Berl). 2010;**212**(4):475-484

[20] Bishop SJ, Duncan J, Lawrence AD. State anxiety modulation of the amygdala response to unattended threat-related stimuli. Journal of Neuroscience. 2004;**24**(46):10364-10368

[21] Stein MB, et al. Increased amygdala and insula activation during emotion processing in anxiety-prone subjects. American Journal of Psychiatry. 2007;**164**(2):318-327

[22] Somerville LH, et al. Human amygdala responses during presentation of happy and neutral faces: Correlations with state anxiety. Biological Psychiatry. 2004;**55**(9):897-903

[23] Kim MJ, et al. The structural and functional connectivity of the amygdala: From normal emotion to pathological anxiety. Behavioural Brain Research. 2011;**223**(2):403-410

[24] Cullinan WE, et al. Pattern and time course of immediate early gene expression in rat brain following acute stress. Neuroscience. 1995;**64**(2):477-505

[25] Dayas CV, et al. Stressor categorization: Acute physical and psychological stressors elicit distinctive recruitment patterns in the amygdala and in medullary noradrenergic cell groups. European Journal of Neuroscience. 2001;**14**(7):1143-1152

[26] Sawchenko PE, et al. The paraventricular nucleus of the hypothalamus and the functional neuroanatomy of visceromotor responses to stress. Progress in Brain Research. 1996;**107**:201-222

[27] Dayas CV, Buller KM, Day TA. Neuroendocrine responses to an emotional stressor: Evidence for involvement of the medial but not the central amygdala. European Journal of Neuroscience. 1999;**11**(7):2312-2322

[28] Bhatnagar S, Vining C, Denski K. Regulation of chronic stress-induced changes in hypothalamic-pituitary-adrenal activity by the basolateral amygdala. Annals of the New York Academy of Sciences. 2004;**1032**:315-319

[29] Prewitt CM, Herman JP. Anatomical interactions between the central amygdaloid nucleus and the hypothalamic paraventricular nucleus of the rat: A dual tract-tracing analysis. Journal of Chemical Neuroanatomy. 1998;**15**(3):173-185

[30] Vallee M, et al. Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: Correlation with stress-induced corticosterone secretion. Journal of Neuroscience. 1997;**17**(7):2626-2636

[31] Pego JM, et al. Dissociation of the morphological correlates of stress-induced anxiety and fear. European Journal of Neuroscience. 2008;**27**(6):1503-1516

[32] Rainnie DG, et al. Corticotrophin releasing factor-induced synaptic plasticity in the amygdala translates stress into emotional disorders. Journal of Neuroscience. 2004;**24**(14):3471-3479

[33] Vyas A, et al. Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. Journal of Neuroscience. 2002;**22**(15):6810-6818

[34] Vyas A, Jadhav S, Chattarji S. Prolonged behavioral stress enhances synaptic connectivity in the basolateral amygdala. Neuroscience. 2006;**143**(2):387-393

[35] Mitra R, et al. Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. Proceedings of the National Academy of Sciences of the United States. 2005;**102**(26):9371-9376

[36] Vyas A, Pillai AG, Chattarji S. Recovery after chronic stress fails to reverse amygdaloid neuronal hypertrophy and enhanced anxiety-like behavior. Neuroscience. 2004;**128**(4):667-673.

[37] Di S, et al. Acute stress suppresses synaptic inhibition and increases anxiety via endocannabinoid release in the basolateral amygdala. Journal of Neuroscience. 2016;**36**(32): 8461-8470

[38] Graeff FG, Netto CF, Zangrossi Jr H. The elevated T-maze as an experimental model of anxiety. Neuroscience & Biobehavioral Reviews. 1998;**23**(2):237-246

[39] Moller C. et al. Decreased experimental anxiety and voluntary ethanol consumption in rats following central but not basolateral amygdala lesions. Brain Research. 1997;**760**(1-2):94-101

[40] Kopchia KL, Altman HJ, Commissaris RL. Effects of lesions of the central nucleus of the amygdala on anxiety-like behaviors in the rat. Pharmacology Biochemistry and Behavior. 1992;**43**(2):453-461

[41] Tye KM, et al. Amygdala circuitry mediating reversible and bidirectional control of anxiety. Nature. 2011;**471**(7338):358-362

[42] Pawlak R, et al. Tissue plasminogen activator in the amygdala is critical for stress-induced anxiety-like behavior. Nature Neuroscience. 2003;**6**(2):168-174

[43] Liu ZP, et al. Chronic stress impairs GABAergic control of amygdala through suppressing the tonic GABAA receptor currents. Molecular Brain. 2014;**7**:32

[44] Koe AS, Ashokan A, Mitra R. Short environmental enrichment in adulthood reverses anxiety and basolateral amygdala hypertrophy induced by maternal separation. Translational Psychiatry. 2016;**6**:e729

[45] Sandi C, et al. Chronic stress-induced alterations in amygdala responsiveness and behavior—modulation by trait anxiety and corticotropin-releasing factor systems. European Journal of Neuroscience. 2008;**28**(9):1836-1848

[46] Mah L, Szabuniewicz C, Fiocco AJ. Can anxiety damage the brain? Current Opinion of Psychiatry. 2016;**29**(1):56-63

[47] McEwen BS. Glucocorticoids, depression, and mood disorders: Structural remodeling in the brain. Metabolism. 2005;**54**(5 Suppl 1):20-23

[48] Vale W, et al. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science. 1981;**213**(4514):1394-1397

[49] Takahashi LK, et al. Corticotropin-releasing factor modulates defensive-withdrawal and exploratory behavior in rats. Behavioral Neuroscience. 1989;**103**(3):648-654

[50] Smith GW, et al. Corticotropin releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. Neuron. 1998;**20**(6):1093-1102

[51] Zorrilla EP, et al. Effects of antalarmin, a CRF type 1 receptor antagonist, on anxiety-like behavior and motor activation in the rat. Brain Research. 2002;**952**(2):188-199

[52] Cipriano AC, Gomes KS, Nunes-de-Souza RL. CRF receptor type 1 (but not type 2) located within the amygdala plays a role in the modulation of anxiety in mice exposed to the elevated plus maze. Hormones and Behavior. 2016;**81**:59-67

[53] Henry B, Vale W, Markou A. The effect of lateral septum corticotropin-releasing factor receptor 2 activation on anxiety is modulated by stress. Journal of Neuroscience. 2006;**26**(36):9142-9152

[54] Alves SW, et al. The activation and blockage of CRF type 2 receptors of the medial amygdala alter elevated T-maze inhibitory avoidance, an anxiety-related response. Behavioural Brain Research. 2016;**305**:191-197

[55] Vicentini JE, et al. CRF type 1 receptors of the medial amygdala modulate inhibitory avoidance responses in the elevated T-maze. Hormones and Behavior. 2014;**65**(3):195-202

[56] Callahan LB, Tschetter KE, Ronan PJ. Inhibition of corticotropin releasing factor expression in the central nucleus of the amygdala attenuates stress-induced behavioral and endocrine responses. Frontiers in Neuroscience. 2013;**7**:195

[57] Kalin NH, et al. Overexpressing corticotropin-releasing factor in the primate amygdala increases anxious temperament and alters its neural circuit. Biological Psychiatry, 2016;**80**(5):345-355

[58] Owens MJ, Bissette G, Nemeroff CB. Acute effects of alprazolam and adinazolam on the concentrations of corticotropin-releasing factor in the rat brain. Synapse. 1989;**4**(3): 196-202

[59] Sarnyai Z, Biro E, Telegdy G. Cocaine-induced elevation of plasma corticosterone is mediated by different neurotransmitter systems in rats. Pharmacology Biochemistry and Behavior. 1993;**45**(1):209-214

[60] Yang XM, et al. Anxiogenic effects of acute and chronic cocaine administration: Neurochemical and behavioral studies. Pharmacology Biochemistry and Behavior. 1992;**41**(3): 643-650

[61] Zhang R, et al. Loss of hypothalamic corticotropin-releasing hormone markedly reduces anxiety behaviors in mice. Mol Psychiatry. 2017 May;**22**(5):733-744. doi: 10.1038/mp.2016.136

[62] Mitra R, Sapolsky RM. Acute corticosterone treatment is sufficient to induce anxiety and amygdaloid dendritic hypertrophy. Proceedings of the National Academy of Sciences of the United States. 2008;**105**(14):5573-5578

[63] Bruchas MR, et al. CRF1-R activation of the dynorphin/kappa opioid system in the mouse basolateral amygdala mediates anxiety-like behavior. PLoS One. 2009;**4**(12): e8528

[64] Smith RJ, Aston-Jones G. Noradrenergic transmission in the extended amygdala: Role in increased drug-seeking and relapse during protracted drug abstinence. Brain Structure and Function. 2008;**213**(1-2):43-61

[65] Tran L, Greenwood-Van Meerveld B. Altered expression of glucocorticoid receptor and corticotropin-releasing factor in the central amygdala in response to elevated corticosterone. Behavioural Brain Research. 2012;**234**(2):380-385

[66] Kim H, et al. Regional differences in acute corticosterone-induced dendritic remodeling in the rat brain and their behavioral consequences. BMC Neuroscience. 2014;**15**: 65

[67] Lim H, et al. Enhancement of anxiety and modulation of TH and pERK expressions in amygdala by repeated injections of corticosterone. Biomolecules & Therapeutics (Seoul). 2012;**20**(4):418-424

[68] Morilak DA, et al. Role of brain norepinephrine in the behavioral response to stress. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2005;**29**(8):1214-1224

[69] Cecchi M, Khoshbouei H, Morilak DA. Modulatory effects of norepinephrine, acting on alpha 1 receptors in the central nucleus of the amygdala, on behavioral and neuroendocrine responses to acute immobilization stress. Neuropharmacology. 2002; **43**(7):1139-1147

[70] Marcinkiewcz CA, et al. Serotonin engages an anxiety and fear-promoting circuit in the extended amygdala. Nature. 2016;**537**(7618):97-101

[71] Lowry CA. Functional subsets of serotonergic neurones: Implications for control of the hypothalamic-pituitary-adrenal axis. Journal of Neuroendocrinology. 2002;**14**(11): 911-923

[72] Zhang Y, et al. Evidence that 5-HT2A receptors in the hypothalamic paraventricular nucleus mediate neuroendocrine responses to (-)DOI. Journal of Neuroscience. 2002;**22**(21):9635-9642

[73] Chegini HR, Nasehi M, Zarrindast MR. Differential role of the basolateral amygdala 5-HT3 and 5-HT4 serotonin receptors upon ACPA-induced anxiolytic-like behaviors and emotional memory deficit in mice. Behavioural Brain Research. 2014;**261**:114-426

[74] Costall B, et al. Neuroanatomical sites of action of 5-HT3 receptor agonist and antagonists for alteration of aversive behaviour in the mouse. British Journal of Pharmacology. 1989;**96**(2):325-332

[75] Gonzalez LE, Andrews N, File SE. 5-HT1A and benzodiazepine receptors in the basolateral amygdala modulate anxiety in the social interaction test, but not in the elevated plus-maze. Brain Research. 1996;**732**(1-2):145-153

[76] Andersen SL, Teicher MH. Serotonin laterality in amygdala predicts performance in the elevated plus maze in rats. Neuroreport. 1999;**10**(17):3497-3500

[77] de la Mora MP, et al. Role of dopamine receptor mechanisms in the amygdaloid modulation of fear and anxiety: Structural and functional analysis. Progress in Neurobiology. 2010;**90**(2):198-216

[78] Zarrindast MR, Khakpai F. The modulatory role of dopamine in Anxiety-like behavior. Archives of Iranian Medicine. 2015;**18**(9):591-603

[79] Bananej M, et al. D1 and D2 dopaminergic systems in the rat basolateral amygdala are involved in anxiogenic-like effects induced by histamine. Journal of Psychopharmacology. 2012;**26**(4):564-574

[80] Zarrindast MR, et al. Involvement of the dopaminergic receptors of the rat basolateral amygdala in anxiolytic-like effects of the cholinergic system. European Journal of Pharmacology. 2011;**672**(1-3):106-112

[81] Rezayof A, Hosseini SS, Zarrindast MR. Effects of morphine on rat behaviour in the elevated plus maze: The role of central amygdala dopamine receptors. Behavioural Brain Research. 2009;**202**(2):171-178

[82] Hajizadeh Moghaddam A, et al. GABA and histamine interaction in the basolateral amygdala of rats in the plus-maze test of anxiety-like behaviors. Pharmacology. 2008;**82**(1):59-66

[83] Green S, Vale AL. Role of amygdaloid nuclei in the anxiolytic effects of benzodiazepines in rats. Behavioural Pharmacology. 1992;**3**(3):261-264

[84] Jimenez-Velazquez G, Lopez-Munoz FJ, Fernandez-Guasti A. Parallel anxiolytic-like and antinociceptive actions of diazepam in the anterior basolateral amygdala and dorsal periaqueductal gray. Brain Research. 2010;**1349**:11-20

[85] Alo R, et al. Excitatory/inhibitory equilibrium of the central amygdala nucleus gates anti-depressive and anxiolytic states in the hamster. Pharmacology Biochemistry and Behavior. 2014;**118**:79-86

[86] Zarrindast MR, et al. Effect of intra-amygdala injection of nicotine and GABA receptor agents on anxiety-like behaviour in rats. Pharmacology. 2008;**82**(4):276-284

[87] Woolf NJ, Butcher LL. Cholinergic systems in the rat brain: III. Projections from the pontomesencephalic tegmentum to the thalamus, tectum, basal ganglia, and basal forebrain. Brain Research Bulletin. 1986;**16**(5):603-637

[88] Hill MN, et al. Functional interactions between stress and the endocannabinoid system: From synaptic signaling to behavioral output. Journal of Neuroscience. 2010;**30**(45): 14980-14986

[89] Viveros MP, et al. The role of the hippocampus in mediating emotional responses to nicotine and cannabinoids: A possible neural substrate for functional interactions. Behavioural Pharmacology. 2007;**18**(5-6):375-389

[90] Rubino T, et al. CB1 receptor stimulation in specific brain areas differently modulate anxiety-related behaviour. Neuropharmacology. 2008;**54**(1):151-160

[91] Zarrindast MR, et al. Cannabinoid CB1 receptors of the rat central amygdala mediate anxiety-like behavior: Interaction with the opioid system. Behavioural Pharmacology. 2008;**19**(7):716-723

[92] Zarrindast MR, et al. Anxiety-like behavior induced by histaminergic agents can be prevented by cannabinoidergic WIN55,212-2 injected into the dorsal hippocampus in mice. Pharmacology Biochemistry and Behavior. 2010;**94**(3):387-396

[93] Haas DA, George SR. Neuropeptide Y-induced effects on hypothalamic corticotropin-releasing factor content and release are dependent on noradrenergic/adrenergic neurotransmission. Brain Research. 1989;**498**(2):333-338

[94] Heilig M, et al. Anxiolytic-like action of neuropeptide Y: Mediation by Y1 receptors in amygdala, and dissociation from food intake effects. Neuropsychopharmacology. 1993;**8**(4):357-363

[95] Broqua P, et al. Behavioral effects of neuropeptide Y receptor agonists in the elevated plus-maze and fear-potentiated startle procedures. Behavioural Pharmacology. 1995;**6**(3):215-222

[96] Sorensen G, et al. Differential roles for neuropeptide Y Y1 and Y5 receptors in anxiety and sedation. Journal of Neuroscience Research. 2004;**77**(5):723-729

[97] Primeaux SD, et al. Effects of altered amygdalar neuropeptide Y expression on anxiety-related behaviors. Neuropsychopharmacology. 2005;**30**(9):1589-1597

[98] Tasan RO, et al. The central and basolateral amygdala are critical sites of neuropeptide Y/Y2 receptor-mediated regulation of anxiety and depression. Journal of Neuroscience. 2010;**30**(18):6282-6290

[99] Stanley BG, Leibowitz SF. Neuropeptide Y: Stimulation of feeding and drinking by injection into the paraventricular nucleus. Life Sciences. 1984;**35**(26):2635-2642

[100] Kuo LE, et al. Neuropeptide Y acts directly in the periphery on fat tissue and mediates stress-induced obesity and metabolic syndrome. Nature Medicine. 2007;**13**(7): 803-811

[101] Moraga-Amaro R, et al. Role of astrocytes in memory and psychiatric disorders. Journal of Physiology Paris. 2014;**108**(4-6):240-251

[102] Donner NC, et al. Serotonergic systems in the balance: CRHR1 and CRHR2 differentially control stress-induced serotonin synthesis. Psychoneuroendocrinology. 2016;**63**:178-190

[103] Fan Y, et al. Corticosterone administration up-regulated expression of norepinephrine transporter and dopamine beta-hydroxylase in rat locus coeruleus and its terminal regions. Journal of Neurochemistry. 2014;**128**(3):445-458

[104] Reyes BA, et al. Localization of the delta opioid receptor and corticotropin-releasing factor in the amygdalar complex: Role in anxiety. Brain Struct Funct. 2017 Mar;**222**(2):1007-1026. doi: 10.1007/s00429-016-1261-6

[105] Smith JP, et al. Intensity of anxiety is modified via complex integrative stress circuitries. Psychoneuroendocrinology. 2016;**63**:351-361

[106] Bremner JD, et al. Noradrenergic mechanisms in stress and anxiety: I. Preclinical studies. Synapse. 1996;**23**(1):28-38

[107] Yeung M, et al. FG7142, yohimbine, and betaCCE produce anxiogenic-like effects in the elevated plus-maze but do not affect brainstem activated hippocampal theta. Neuropharmacology. 2013;**75**:47-52

[108] Gozzi A, et al. Differential effect of orexin-1 and CRF-1 antagonism on stress circuits: A fMRI study in the rat with the pharmacological stressor Yohimbine. Neuropsychopharmacology. 2013;**38**(11):2120-2130

[109] McDonald AJ, Mott DD. Functional neuroanatomy of amygdalohippocampal interconnections and their role in learning and memory. Journal of Neuroscience Research. 2017;**95**(3):797-820

[110] Liotti M, et al. Differential limbic-cortical correlates of sadness and anxiety in healthy subjects: Implications for affective disorders. Biological Psychiatry. 2000;**48**(1):30-42

[111] Felix-Ortiz AC, et al. BLA to vHPC inputs modulate anxiety-related behaviors. Neuron. 2013;**79**(4):658-664

[112] Desmedt A, et al. Adaptive emotional memory: The key hippocampal-amygdalar interaction. Stress. 2015;**18**(3):297-308

[113] Jacobson L, Sapolsky R. The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. Endocrine Reviews. 1991;**12**(2):118-134

[114] Herman JP, et al. Central mechanisms of stress integration: Hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. Frontiers in Neuroendocrinology. 2003;**24**(3):151-180

[115] Dunn JD, Orr SE. Differential plasma corticosterone responses to hippocampal stimulation. Experimental Brain Research. 1984;**54**(1):1-6

[116] Rubin RT, Mandell AJ, Crandall PH. Corticosteroid responses to limbic stimulation in man: Localization of stimulus sites. Science. 1966;**153**(3737):767-768

[117] Herman JP, Mueller NK. Role of the ventral subiculum in stress integration. Behavioural Brain Research. 2006;**174**(2):215-224

[118] Ruit KG, Neafsey EJ. Cardiovascular and respiratory responses to electrical and chemical stimulation of the hippocampus in anesthetized and awake rats. Behavioural Brain Research. 1988;**457**(2):310-321

[119] Cheeta S, Kenny PJ, File SE. Hippocampal and septal injections of nicotine and 8-OH-DPAT distinguish among different animal tests of anxiety. Progress in Neuro-Psychopharmacology & Biological Psychiatry. 2000;**24**(7):1053-1067

[120] Engin E, Treit D. Dissociation of the anxiolytic-like effects of Avpr1a and Avpr1b receptor antagonists in the dorsal and ventral hippocampus. Neuropeptides. 2008;**42**(4):411-421

[121] File SE, Gonzalez LE, Andrews N. Endogenous acetylcholine in the dorsal hippocampus reduces anxiety through actions on nicotinic and muscarinic1 receptors. Behavioral Neuroscience. 1998;**112**(2):352-359

[122] Plaza-Zabala A, et al. Hypocretins regulate the anxiogenic-like effects of nicotine and induce reinstatement of nicotine-seeking behavior. Journal of Neurosciences. 2010;**30**(6):2300-2310

[123] Besson M, et al. Alterations in alpha5* nicotinic acetylcholine receptors result in midbrain- and hippocampus-dependent behavioural and neural impairments. Psychopharmacology (Berl). 2016 Sep;**233**(18):3297-314. doi: 10.1007/s00213-016-4362-2.

[124] Greenough A, et al. Untangling the effects of hunger, anxiety, and nausea on energy intake during intravenous cholecystokinin octapeptide (CCK-8) infusion. Physiology & Behavior. 1998;**65**(2):303-310

[125] Rezayat M, et al. Cholecystokinin and GABA interaction in the dorsal hippocampus of rats in the elevated plus-maze test of anxiety. Physiology & Behavior. 2005;**84**(5):775-782

[126] Kennett GA, et al. Anxiogenic-like effects of mCPP and TFMPP in animal models are opposed by 5-HT1C receptor antagonists. European Journal of Pharmacology. 1989;**164**(3):445-454

[127] Whitton P, Curzon G. Anxiogenic-like effect of infusing 1-(3-chlorophenyl) piperazine (mCPP) into the hippocampus. Psychopharmacology (Berl). 1990;**100**(1):138-140

[128] Hasenohrl RU, et al. Substance P and its role in neural mechanisms governing learning, anxiety and functional recovery. Neuropeptides. 2000;**34**(5):272-280

[129] McLean S. Do substance P and the NK1 receptor have a role in depression and anxiety? Current Pharmaceutical Design. 2005;**11**(12):1529-1547

[130] Borhegyi Z, Leranth C. Distinct substance P- and calretinin-containing projections from the supramammillary area to the hippocampus in rats; a species difference between rats and monkeys. Experimental Brain Research. 1997;**115**(2):369-374

[131] Carvalho MC, et al. Anxiolytic-like effects of substance P administration into the dorsal, but not ventral, hippocampus and its influence on serotonin. Peptides. 2008;**29**(7):1191-1200

[132] Bremner JD. Brain imaging in anxiety disorders. Expert Review of Neurotherapeutics. 2004;**4**(2):275-284

[133] Lacroix L, et al. Differential role of the medial and lateral prefrontal cortices in fear and anxiety. Behavioral Neuroscience. 2000;**114**(6):1119-1130

[134] Euston DR, Gruber AJ, McNaughton BL. The role of medial prefrontal cortex in memory and decision making. Neuron. 2012;**76**(6):1057-1070

[135] Gonzalez LE, et al. Medial prefrontal transection enhances social interaction. I: Behavioral studies. Brain Research. 2000;**887**(1):7-15

[136] Shah AA, Treit D. Excitotoxic lesions of the medial prefrontal cortex attenuate fear responses in the elevated-plus maze, social interaction and shock probe burying tests. Brain Research. 2003;**969**(1-2):183-194

[137] Diorio D, Viau V, Meaney MJ. The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. Journal of Neuroscience. 1993;**13**(9):3839-3847

[138] Gerrits M, et al. Increased stress vulnerability after a prefrontal cortex lesion in female rats. Brain Research Bulletin. 2003;**61**(6):627-635

[139] Figueiredo HF, et al. The medial prefrontal cortex differentially regulates stress-induced c-fos expression in the forebrain depending on type of stressor. European Journal of Neuroscience. 2003;**18**(8):2357-2364

[140] Radley JJ, Arias CM, Sawchenko PE. Regional differentiation of the medial prefrontal cortex in regulating adaptive responses to acute emotional stress. Journal of Neuroscience. 2006;**26**(50):12967-12976

[141] Akana SF, et al. Corticosterone exerts site-specific and state-dependent effects in prefrontal cortex and amygdala on regulation of adrenocorticotropic hormone, insulin and fat depots. Journal of Neuroendocrinology. 2001;**13**(7):625-637

[142] Sullivan RM, Gratton A. Behavioral effects of excitotoxic lesions of ventral medial prefrontal cortex in the rat are hemisphere-dependent. Brain Research. 2002;**927**(1): 69-79

[143] Frysztak RJ, Neafsey EJ. The effect of medial frontal cortex lesions on cardiovascular conditioned emotional responses in the rat. Brain Research. 1994;**643**(1-2):181-193

[144] Resstel LB, et al. Involvement of medial prefrontal cortex neurons in behavioral and cardiovascular responses to contextual fear conditioning. Neuroscience. 2006;**143**(2):377-385

[145] Resstel LB, Fernandes KB, Correa FM. Medial prefrontal cortex modulation of the baroreflex parasympathetic component in the rat. Brain Research. 2004;**1015**(1-2):136-144

[146] Padilla-Coreano N, et al. Direct ventral Hippocampal-Prefrontal input is required for Anxiety-Related neural activity and behavior. Neuron. 2016;**89**(4):857-866

[147] Costa NS, et al. Functional lateralization of the medial prefrontal cortex in the modulation of anxiety in mice: Left or right? Neuropharmacology. 2016;**108**:82-90

[148] Vytal KE, et al. Sustained anxiety increases amygdala-dorsomedial prefrontal coupling: A mechanism for maintaining an anxious state in healthy adults. Journal of Psychiatry and Neuroscience. 2014;**39**(5):321-329

[149] Neumann ID, Slattery DA. Oxytocin in general anxiety and social fear: A translational approach. Biological Psychiatry. 2016;**79**(3):213-221

[150] Torner L, et al. Forced swimming-induced oxytocin release into blood and brain: Effects of adrenalectomy and corticosterone treatment. Psychoneuroendocrinology. 2016;**77**:165-174

[151] Argiolas A, Melis MR. Central control of penile erection: Role of the paraventricular nucleus of the hypothalamus. Progress in Neurobiology. 2005;**76**(1):1-21

[152] Bulbul M, et al. Opposite effects of central oxytocin and arginine vasopressin on changes in gastric motor function induced by chronic stress. Peptides. 2017;**87**:1-11

[153] Csikota P, et al. Vasopressinergic control of stress-related behavior: Studies in Brattleboro rats. Stress. 2016;**19**(4):349-361

[154] Neumann ID, Landgraf R. Balance of brain oxytocin and vasopressin: Implications for anxiety, depression, and social behaviors. Trends in Neurosciences. 2012;**35**(11):649-659

[155] De Vries GJ, Buijs RM. The origin of the vasopressinergic and oxytocinergic innervation of the rat brain with special reference to the lateral septum. Brain Research. 1983;**273**(2):307-317

[156] Zelena D. Vasopressin in health and disease with a focus on affective disorders. Central Nervous System Agents in Medicinal Chemistry. 2012;**12**(4):286-303

[157] Griebel G, et al. Anxiolytic- and antidepressant-like effects of the non-peptide vasopressin V1b receptor antagonist, SSR149415, suggest an innovative approach for the treatment of stress-related disorders. Proceedings of the National Academy of Sciences of the United States. 2002;**99**(9):6370-6375

[158] Davis M, Neurobiology of fear responses: The role of the amygdala. The Journal of Neuropsychiatry and Clinical Neurosciences. 1997;**9**(3):382-402

[159] Choi DC, et al. Bed nucleus of the stria terminalis subregions differentially regulate hypothalamic-pituitary-adrenal axis activity: Implications for the integration of limbic inputs. Journal of Neuroscience. 2007;**27**(8):2025-2034

[160] Gray TS, et al. Ibotenic acid lesions in the bed nucleus of the stria terminalis attenuate conditioned stress-induced increases in prolactin, ACTH and corticosterone. Neuroendocrinology. 1993;**57**(3):517-524

[161] Champagne D, Beaulieu J, Drolet G. CRFergic innervation of the paraventricular nucleus of the rat hypothalamus: A tract-tracing study. Journal of Neuroendocrinology. 1998;**10**(2):119-131

[162] Dong HW, Gan Q, Knuepfer MM. Central corticotropin releasing factor (CRF) and adrenergic receptors mediate hemodynamic responses to cocaine. Brain Research. 2001;**893**(1-2):1-10

[163] Cullinan WE, Herman JP, Watson SJ. Ventral subicular interaction with the hypothalamic paraventricular nucleus: Evidence for a relay in the bed nucleus of the stria terminalis. Journal of Comparative Neurology. 1993;**332**(1):1-20

[164] Kim SY, et al. Diverging neural pathways assemble a behavioural state from separable features in anxiety. Nature. 2013;**496**(7444):219-223

[165] Tran L, Schulkin J, Greenwood-Van Meerveld B. Importance of CRF receptor-mediated mechanisms of the bed nucleus of the stria terminalis in the processing of anxiety and pain. Neuropsychopharmacology. 2014;**39**(11):2633-2645

[166] Sahuque LL, et al. Anxiogenic and aversive effects of corticotropin-releasing factor (CRF) in the bed nucleus of the stria terminalis in the rat: Role of CRF receptor subtypes. Psychopharmacology (Berl). 2006;**186**(1):122-132

[167] Walker DL, Davis M. Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. Journal of Neuroscience. 1997;**17**(23):9375-9383

[168] Levita L, et al. 5-hydroxytryptamine1A-like receptor activation in the bed nucleus of the stria terminalis: Electrophysiological and behavioral studies. Neuroscience. 2004; **128**(3):583-596

[169] Resstel LB, et al. Anxiolytic-like effects induced by acute reversible inactivation of the bed nucleus of stria terminalis. Neuroscience. 2008;**154**(3):869-876

[170] Casada JH, Dafny N. Restraint and stimulation of bed nucleus of the stria terminalis produce similar stress-like behaviors. Brain Research Bulletin. 1991;**27**(2):207-212

[171] Sajdyk T, et al. Chronic inhibition of GABA synthesis in the bed nucleus of the stria terminalis elicits anxiety-like behavior. Journal of Psychopharmacology. 2008;**22**(6): 633-641

[172] Sink KS, et al. Calcitonin gene-related peptide in the bed nucleus of the stria terminalis produces an anxiety-like pattern of behavior and increases neural activation in anxiety-related structures. Journal of Neuroscience. 2011;**31**(5):1802-1810

[173] Lungwitz EA, et al. Orexin-A induces anxiety-like behavior through interactions with glutamatergic receptors in the bed nucleus of the stria terminalis of rats. Physiology & Behavior. 2012;**107**(5):726-732

[174] Fendt M, Siegl S, Steiniger-Brach B. Noradrenaline transmission within the ventral bed nucleus of the stria terminalis is critical for fear behavior induced by trimethylthiazoline, a component of fox odor. Journal of Neuroscience. 2005;**25**(25):5998-6004

[175] Morilak DA, Cecchi M, Khoshbouei H. Interactions of norepinephrine and galanin in the central amygdala and lateral bed nucleus of the stria terminalis modulate the behavioral response to acute stress. Life Sciences. 2003;**73**(6):715-726

[176] Naka T, et al. Activation of beta-adrenoceptors in the bed nucleus of the stria terminalis induces food intake reduction and anxiety-like behaviors. Neuropharmacology. 2013;**67**:326-330

[177] Alves FH, et al. Cardiovascular effects of carbachol microinjected into the bed nucleus of the stria terminalis of the rat brain. Brain Research. 2007;**1143**:161-168

[178] Crestani CC, et al. Bed nucleus of the stria terminalis alpha(1)-adrenoceptor modulates baroreflex cardiac component in unanesthetized rats. Brain Research. 2008;**1245**:108-115

[179] Crestani CC, et al. Role of the bed nucleus of the stria terminalis in the cardiovascular responses to acute restraint stress in rats. Stress. 2009;**12**(3):268-278

[180] Gouveia MK, et al. Dissociation in control of physiological and behavioral responses to emotional stress by cholinergic neurotransmission in the bed nucleus of the stria terminalis in rats. Neuropharmacology. 2016;**101**:379-388

[181] Khoshbouei H, Cecchi M, Morilak DA. Modulatory effects of galanin in the lateral bed nucleus of the stria terminalis on behavioral and neuroendocrine responses to acute stress. Neuropsychopharmacology. 2002;**27**(1):25-34

[182] Duvarci S, Bauer EP, Pare D. The bed nucleus of the stria terminalis mediates inter-individual variations in anxiety and fear. Journal of Neuroscience. 2009;**29**(33):10357-10361

[183] Menard J, Treit D. Intra-septal infusions of excitatory amino acid receptor antagonists have differential effects in two animal models of anxiety. Behavioural Pharmacology. 2000;**11**(2):99-108

[184] Zarrindast MR, et al. Role of the medial septum cholinoceptors in anxiogenic-like effects of nicotine. Physiology & Behavior. 2013;**119**:103-109

[185] Zarrindast MR, et al. Histaminergic system of the lateral septum in the modulation of anxiety-like behaviour in rats. European Journal of Pharmacology. 2008;**583**(1):108-114

[186] Radulovic J, et al. Modulation of learning and anxiety by corticotropin-releasing factor (CRF) and stress: Differential roles of CRF receptors 1 and 2. Journal of Neuroscience. 1999;**19**(12):5016-5025

[187] Appenrodt E, Schnabel R, Schwarzberg H. Vasopressin administration modulates anxiety-related behavior in rats. Physiology & Behavior. 1998;**64**(4):543-547

[188] Everts HG, Koolhaas JM. Differential modulation of lateral septal vasopressin receptor blockade in spatial learning, social recognition, and anxiety-related behaviors in rats. Behavioural Brain Research. 1999;**99**(1):7-16

[189] Moga MM, Weis RP, Moore RY. Efferent projections of the paraventricular thalamic nucleus in the rat. Journal of Comparative Neurology. 1995;**359**(2):221-238

[190] Hurley KM, et al. Efferent projections of the infralimbic cortex of the rat. Journal of Comparative Neurology. 1991;**308**(2):249-276

[191] Shi CJ, Cassell MD. Cascade projections from somatosensory cortex to the rat basolateral amygdala via the parietal insular cortex. Journal of Comparative Neurology. 1998;**399**(4):469-491

[192] Allen GV, et al. Organization of visceral and limbic connections in the insular cortex of the rat. Journal of Comparative Neurology. 1991;**311**(1):1-16

[193] Nakashima M, et al. An anterograde and retrograde tract-tracing study on the projections from the thalamic gustatory area in the rat: Distribution of neurons projecting to the insular cortex and amygdaloid complex. Neuroscience Research. 2000;**36**(4):297-309

[194] Yasui Y, et al. Autonomic responses and efferent pathways from the insular cortex in the rat. Journal of Comparative Neurology. 1991;**303**(3):355-374

[195] Cechetto DF, Saper CB. Role of the cerebral cortex in autonomic functions. In: Loewy ADS, Spyer KM, editors. Central Regulation of Autonomic Functions, Oxford University Press. New York: 1990. pp. 208-223

[196] Saper CB. Convergence of autonomic and limbic connections in the insular cortex of the rat. Journal of Comparative Neurology. 1982;**210**(2):163-173

[197] van der Kooy D, et al. Visceral cortex: A direct connection from prefrontal cortex to the solitary nucleus in rat. Neuroscience Letters. 1982;**33**(2):123-127

[198] Shipley MT. Insular cortex projection to the nucleus of the solitary tract and brainstem visceromotor regions in the mouse. Brain Research Bulletin. 1982;**8**(2):139-148

[199] Sripanidkulchai K, Sripanidkulchai B, Wyss JM. The cortical projection of the basolateral amygdaloid nucleus in the rat: A retrograde fluorescent dye study. Journal of Comparative Neurology. 1984;**229**(3):419-431

[200] McDonald AJ, and Jackson TR. Amygdaloid connections with posterior insular and temporal cortical areas in the rat. Journal of Comparative Neurology. 1987;**262**(1):59-77

[201] Ottersen OP. Connections of the amygdala of the rat. IV: Corticoamygdaloid and intraamygdaloid connections as studied with axonal transport of horseradish peroxidase. Journal of Comparative Neurology. 1982;**205**(1):30-48

[202] Hoehn-Saric R, Schlund MW, Wong SH. Effects of citalopram on worry and brain activation in patients with generalized anxiety disorder. Psychiatry Research. 2004;**131**(1):11-21

[203] Uchida RR, et al. Regional gray matter abnormalities in panic disorder: A voxel-based morphometry study. Psychiatry Research. 2008;**163**(1):21-29

[204] Rauch SL, et al. The functional neuroanatomy of anxiety: A study of three disorders using positron emission tomography and symptom provocation. Biological Psychiatry. 1997;**42**(6):446-452

[205] Wendt J, et al. Brain activation and defensive response mobilization during sustained exposure to phobia-related and other affective pictures in spider phobia. Psychophysiology. 2008;**45**(2):205-215

[206] Lazaro L, et al. Cerebral activation in children and adolescents with obsessive-compulsive disorder before and after treatment: A functional MRI study. Journal of Psychiatric Research. 2008;**42**(13):1051-1059

[207] Lindauer RJ, et al. Effects of psychotherapy on regional cerebral blood flow during trauma imagery in patients with post-traumatic stress disorder: A randomized clinical trial. Psychological Medicine. 2008;**38**(4):543-554

[208] Goossens L, et al. Amygdala hyperfunction in phobic fear normalizes after exposure. Biological Psychiatry. 2007;**62**(10):1119-1125

[209] Wang SM, et al. Music and preoperative anxiety: A randomized, controlled study. Anesthesia and Analgesia. 2002;**94**(6):1489-1494 (table of contents)

[210] Paulus MP, Stein MB. An insular view of anxiety. Biological Psychiatry. 2006;**60**(4):383-387

[211] Craig AD. How do you feel? Interoception: The sense of the physiological condition of the body. Nature Reviews Neuroscience. 2002;**3**(8):655-666

[212] Li H, et al. Insular muscarinic signaling regulates anxiety-like behaviors in rats on the elevated plus-maze. Behavioural Brain Research. 2014;**270**:256-260

[213] Rojas S, et al. The insula modulates arousal-induced reluctance to try novel tastes through adrenergic transmission in the rat. Frontiers in Behavioral Neuroscience. 2015;**9**:164

[214] Bonnavion P, et al. Hubs and spokes of the lateral hypothalamus: Cell types, circuits and behaviour. Journal of Psychology. 2016;**594**(22):6443-6462

[215] Winsky-Sommerer R, et al. Interaction between the corticotropin-releasing factor system and hypocretins (orexins): A novel circuit mediating stress response. Journal of Neuroscience. 2004;**24**(50):11439-11448

[216] Espana RA, Valentino RJ, Berridge CW. Fos immunoreactivity in hypocretin-synthesizing and hypocretin-1 receptor-expressing neurons: Effects of diurnal and nocturnal spontaneous waking, stress and hypocretin-1 administration. Neuroscience. 2003;**121**(1): 201-217

[217] Furlong TM, et al. Hypocretin/orexin contributes to the expression of some but not all forms of stress and arousal. European Journal of Neuroscience. 2009;**30**(8):1603-1614

[218] Berridge CW, Espana RA, Vittoz NM. Hypocretin/orexin in arousal and stress. Brain Research. 2010;**1314**:91-102

[219] Johnson GG, White MC, Grimaldi M. Stressed to death: Targeting endoplasmic reticulum stress response induced apoptosis in gliomas. Current Pharmaceutical Design. 2011;**17**(3):284-292

[220] Giardino WJ, de Lecea L. Hypocretin (orexin) neuromodulation of stress and reward pathways. Current Opinion in Neurobiology. 2014;**29**:103-108

[221] Mileykovskiy BY, Kiyashchenko LI, Siegel JM. Behavioral correlates of activity in identified hypocretin/orexin neurons. Neuron. 2005;**46**(5):787-798

[222] Panhelainen AE, Korpi ER. Evidence for a role of inhibition of orexinergic neurons in the anxiolytic and sedative effects of diazepam: A c-Fos study. Pharmacology Biochemistry and Behavior. 2012;**101**(1):115-124

[223] Li Y, et al. Orexins in the paraventricular nucleus of the thalamus mediate anxiety-like responses in rats. Psychopharmacology (Berl). 2010;**212**(2):251-265

[224] Chen Q, et al. The hypocretin/orexin system: An increasingly important role in neuropsychiatry. Medicinal Research Reviews. 2015;**35**(1):152-197

[225] Bremmer S, et al. Simultaneous defecography and peritoneography in defecation disorders. Diseases of the Colon & Rectum. 1995;**38**(9):969-973

[226] Felix-Ortiz AC, et al. Bidirectional modulation of anxiety-related and social behaviors by amygdala projections to the medial prefrontal cortex. Neuroscience. 2016;**321**:197-209

[227] Crowley NA, et al. Dynorphin controls the gain of an amygdalar anxiety circuit. Cell Reports. 2016;**14**(12):2774-2783

[228] Jennings JH, et al. Distinct extended amygdala circuits for divergent motivational states. Nature. 2013;**496**(7444):224-228

IntechOpen