

## Title page

### Neuroanatomy of Anxiety

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### Abstract

The evolutionary approach to human anxiety is based on the defensive responses that non-human animals show to fear-provoking stimuli. Studies performed mostly in rodents have related areas such as the medial prefrontal cortex, amygdaloid and hypothalamic nuclei, hippocampal formation and midbrain central gray to these responses. It is clear, however, that animals show different and sometimes opposite responses according to the threatening stimulus. These responses include immediate reactions such as freezing or flight, behavioral inhibition or avoidance, which are organized by at least partially distinct brain systems. As discussed in the present chapter, several pieces of evidence indicate that these brain systems are similar in rodents and primates. In addition, recent neuroimaging studies also suggest dysfunctions in these systems are probably related to anxiety disorders in humans.

## 1. Introduction

A key work in the study of emotion was Charles Darwin's book 'The expression of the emotions in man and animals.' (1872). From there came the key concept that the biological origin of human anxiety is based on the defensive responses that non-humans show to threatening stimuli. These responses, therefore, can be seen as normal emotional states with adaptive function aimed at promoting survival across species (Rauch et al. 2006).

For obvious technical and ethical issues the study of the neural basis of anxiety has been made to a great extent in non-humans. Therefore, in the present chapter we will discuss data originated mostly from studies made in rodents. It was written, however, on grounds that a translational approach that correlate these data with the neural substrate responsible for anxiety in humans is possible. As will be briefly discussed at the end of the chapter, this conviction has received great support from recent neuroimaging studies in humans. Together they confirm that human anxiety is accompanied by changes in neural systems that coordinate distinct defensive responses to threats. They include areas such as the medial prefrontal cortex, amygdaloid and hypothalamic nuclei, hippocampal formation and midbrain central gray.

It is generally accepted that animals have a set of several, genetically determined, prepackaged behaviors to solve certain functional problems. This idea has been used in the species-specific defensive reaction (SSDR) theory, suggesting that the same innately determined defensive behavior (such as freezing and flight) should occur during the response to either a natural predator or an artificial harmful stimulus (Bolles 1970). This seemingly limited way of reacting to a dangerous stimulus has led to a misleading assumption that animals process predators' cues in the same way they do an artificial threat. However, animals are naturally selected to protect themselves from dangers associated with predators, differently from what they do in relation to an artificial harm, in a way that just the predator presence should be able to evoke a fear sensation and the associated behavioral responses.

With this caveat in mind, we shall discuss two main experimental approaches that have been used to investigate the question of the brain systems underlying fear and anxiety (i.e., Pavlovian fear conditioning to a context or to a neutral stimulus, and exposure to a real predator or its odor).

## 2. Pavlovian Fear conditioning Studies

Pavlovian conditioning is by far the more commonly used approach. The phenomenon of Pavlovian fear conditioning is highly reproducible and generates clearly measurable responses. Moreover, the main responses measured in this context, freezing and startle, are both seen in the repertory of animals confronting unconditioned threats, and have been thought to provide a good validation to both sets of models (Fanselow 1994). Studies on the neural basis of Pavlovian conditioned fear indicate the amygdala as a major player in learning, storage, and expression of fear conditioning. Among the amygdalar regions, three nuclei have been particularly focused on the fear conditioning research, namely, the lateral nucleus, the central nucleus, and the basolateral nucleus (LeDoux 2000). As shown in Fig. 1, associative learning between the conditioned and unconditioned stimuli is likely to occur in the lateral nucleus. In fact, both acquisition and

retention of fear conditioning occur in the lateral nucleus, where electrolytic and excitotoxic lesions, as well as pharmacological blockade, prevent acquisition and expression of fear conditioning (Campeau and Davis 1995, Muller et al. 1997, LeDoux 2000, Gale et al. 2004). The lateral nucleus presents clear synapse plasticity during fear conditioning, and changes the way a conditioned stimulus is processed after the shock pairing. The lateral nucleus, in turn, projects to the central nucleus both directly and indirectly via projections to the basolateral nucleus (Pitkänen et al. 1997; Pare and Smith 1998). The central nucleus, via projections to the hypothalamus and brainstem (Fig. 1), is critical for the expression of fear conditioning. In fact, lesions of the central nucleus disrupt freezing, along with the autonomic reactions observed during fear conditioning (LeDoux 2000). More recent studies, however, have also suggested a role for the central nucleus in learning and storage of fear conditioning (Wilensky et al. 2006). The role of the basolateral nucleus in fear conditioning is more controversial, and only post-training, but not pre-training, lesions appear to interfere with the expression of conditioned fear (Anglada-Figueroa and Quirk 2005).

This well-developed neural circuitry model for the processing of fear conditioning provides an excellent framework for comparison to the neural processing for innate fear responses, such as those that occur during exposure to a predator. Some differences are immediately apparent, as lesions of the central nucleus, a key region for the expression of fear conditioning, produce only marginal deficits, at best, on defensive responses to a predator (De Oca and Fanselow 2004) or its odor (Li et al. 2004). Also, latent *Toxoplasma* infection resulting in entry into rodent brains of parasites that presumably affect the circuits involved in responding to predator threats, inducing loss of innate defensive responses toward cat odor, has recently been reported to have no effect on fear responses to a conditioned stimulus previously paired to a shock (Berdoy et al. 1995; Vyas et al. 2007). Taken together, the evidence suggests that, at least in rats, innate defensive behavior to a predator or its odor is likely to be processed by neural circuits somewhat different from those for fear conditioning.

### **3. Anti-predatory defensive systems**

#### ***3.1. Amygdalar systems involved in predator detection***

Over the last years, a great deal has been learned about the neural system involved in processing innate defensive behaviors to a predator or its odor. Similarly to what has been discussed for fear conditioning, the amygdala also occupies a central role in integrating the sensory clues related to the predator. A recent finding is that predator odors may in fact be processed by prey species in the accessory olfactory bulb, rather than the main olfactory bulb (McGregor et al. 2004). This suggests that cat odor is processed by rats more as a pheromone than a conventional odor, and the authors suggested that cat odor may be an example of a “kairomone” – a semiochemical released by one species that has a favorable adaptive effect on a different “receiving” species (McGregor et al. 2004). The accessory olfactory bulb projects principally to the medial amygdala, and rats exposed to cat odor also show substantial activation in this nucleus, particularly in its posteroventral part (Dielenberg et al. 2001, McGregor et al. 2004). In line with this view, rats with cytotoxic lesions in the medial nucleus, but not in the central nucleus, exhibited a significant reduction in unconditioned fear responses to cat odor (Li et al. 2004). During exposure to a live predator, in addition to activation of

the posteroventral part of the medial amygdalar nucleus, we have also observed a distinct Fos increase in two other amygdalar sites, namely, the posterior basomedial amygdalar nucleus and caudal levels of the lateral amygdalar nucleus (Canteras et al. 2001). Importantly, these amygdalar nuclei receive inputs from visual and auditory association areas, and are likely to integrate predator-derived sensory clues, other than olfactory ones (McDonald 1998). As shown in Fig. 1, the amygdalar sites related to predator detection project either directly or indirectly, via the transverse nucleus of the bed nucleus of the stria terminalis (BST), to the ventromedial nucleus of the hypothalamus, where its dorsomedial part receives most of the direct projections from the amygdala and is particularly mobilized during exposure to a live predator or its odor. Therefore, the studies using rats exposed to a live cat or its fur odor suggest an amygdalar-BST-hypothalamic path to detect a live predator or its cues (Canteras et al. 2001). Recent work testing mice exposed to rats substantiate the idea of this particular amygdalar-hypothalamic path for predator detection in other prey species (Martinez et al. 2008).

### ***3.2 The Hippocampal Formation and the contextual analysis for predatory environment.***

Apart from the well-known hippocampal functions related to mnemonic processing, spatial learning, and navigation, the hippocampus also works as a context analyzer. In this regard, it is relevant to point out that the hippocampal formation receives inputs from the amygdalar sites involved in detecting predator-related cues and, therefore, are likely to be involved in associating predator threats to a given environment (Petrovich et al. 2001).

The septo-hippocampal system is directly related to the medial hypothalamic defensive system, and, therefore, is in a position to control anti-predatory defensive responses. In fact, recent findings suggest that the ventral hippocampus is related to anxiety functions (Bannerman et al. 2004, Bertoglio et al. 2006, Pentkowski et al. 2006, Nascimento Hackl and Carobrez 2008). This match was first pointed out by Gray (1970), based on data confined mainly to barbiturates, and later proven to be valid for other classical (benzodiazepines) and novel (buspirone) clinically effective, centrally acting, anxiolytic drugs. In line with this view, the ventral half of field CA1 and subiculum targets distinct septal regions that project to the anterior hypothalamic nucleus, which is part of the medial hypothalamic defensive system (Risold and Swanson 1996, 1997).

### ***3.3 The medial hypothalamic defensive system***

Exposure to a predator or its odor induces in the medial hypothalamic zone a distinct Fos up-regulation in the anterior hypothalamic nucleus, the dorsomedial part of the ventromedial hypothalamic nucleus, and the dorsal premammillary (Canteras et al. 1997, Dielenberg et al. 2001, Martinez et al. 2008).

Both the anterior hypothalamic nucleus and the dorsomedial part of the ventromedial nucleus project to the dorsal premammillary nucleus, which, by far, represents the most sensitive brain region responding to a predator or its clues, and where lesions have been most effective in reducing anti-predator defensive responses (Canteras et al. 1997, Blanchard et al. 2003). In fact, the anterior hypothalamic nucleus, the dorsomedial part of the ventromedial hypothalamic nucleus, and the dorsal premammillary nucleus are particularly interconnected, forming a partially segregated circuit in the medial zone of

the hypothalamus, the so-called medial hypothalamic defensive circuit (Canteras 2002). Notably, the dorsal premammillary nucleus appears to work as an amplifier for the neural processing in the medial hypothalamic defensive circuit. This would explain why this region is so responsive to predator threats, and why lesions therein are able to reduce defensive responses so drastically (Canteras et al. 1997, Blanchard et al. 2003, 2005, Markham et al. 2004).

The hypothalamic systems are well known for integrating a number of behaviors critical for the survival of the individual or the species (Swanson 1987), and it comes as no surprise that the hypothalamus, and not the amygdala (as previously suggested by fear conditioning studies), should occupy this central role in integrating antipredator defensive responses. In fact, fear responses to predators are comparable to other forms of goal-oriented behavior, such as drinking, feeding and mating, in a way that they are underlain by strong motivational factors leading to behavioral actions critical for the maintenance of the species. The hypothalamus is classically known to have a central role in the organization of a number of goal-oriented behaviors (Swanson 1987).

### ***3.4 The Periaqueductal Gray and the integration of anti-predatory responses***

The periaqueductal gray (PAG) represents the main brainstem targets of the medial hypothalamic defensive system, and is critical for the expression of defensive responses. Of special relevance, the pattern of projection from the medial hypothalamic defensive system to the PAG largely overlaps the pattern of PAG activation in animals exposed to a predator or its odor, where Fos expression was mostly seen in the rostral two thirds of the PAG in the dorsomedial and dorsolateral regions, whereas in the caudal PAG, a less intense, but more widespread activation, was observed (Canteras and Goto 1999, Dielenberg et al. 2001). A similar pattern of PAG activation was also described after administration of drugs known to induce panic in humans (Singewald and Sharp 2000). In fact, particularly the dorsolateral PAG appears to play a critical role for integrating forebrain limbic information related to “psychological stressors” such as the presence of a natural predator (Canteras and Goto 1999, Dielenberg et al. 2001). Fear-conditioning-induced freezing is also known to depend on the PAG. However, in contrast to what was found in animals exposed to a predator or its odor, the ventrolateral PAG, but not the dorsolateral PAG, is mostly mobilized during fear conditioning to a context previously associated with footshocks (Carrive et al. 1997).

## **4. The septum-hippocampus and the behavioral inhibition system**

In addition to its role in context analysis in threatening situations, the septo-hippocampal system is also proposed to play a more widespread role in anxiety. This suggestion was originally based on the observation that the behavioral effects of anxiolytics parallel those of either permanent or temporary lesions to the septum and/or the hippocampus (Gray and McNaughton 2000, Degroot and Treit 2004). According to this view, the septo-hippocampus system generates anxiety in response to conflict situations by interrupting ongoing behavior, and increasing the level of arousal and attention to enhance gathering information (Fig. 2). These conflict situations occur when the animal has to choose between competing available goals. For instance, novelty is a stimulus that can elicit

approach and avoidance tendencies, creating conflict. In turn, this conflict will generate an immediate inhibition of motor programs (apparently at the level of its planning) that are in course of execution (Gray and McNaughton 2000). A reduction in the capacity for behavioral inhibition of pre-threat behaviors after either septal or hippocampal damage is consistent with this proposition. In fact, as noted above, the hippocampus, working as a context analyzer, can modulate adaptive motor activity in response to particular sensory events in the environment (Oddie and Bland 1998). Animals with hippocampal or fimbria-fornix lesions, but not with entorhinal cortex lesions, present increased motor activity, which can be blocked by lesions of the nucleus accumbens. This suggests that a subiculo-accumbal pathway is particularly important for hippocampal formation control of locomotor behavior (Oddie and Bland 1998, Countureau et al. 2000). Actually, it has been shown that hyperpolarization of subicular neurons is associated with an increase in accumbal dopamine transmission (Mitchell et al. 2000).

Here is noteworthy that the mediodorsal thalamic nucleus is thought to represent a key target for the neural processing in the accumbal circuit, and known to play a critical role in modulating locomotor activity (Swanson and Kalivas 2000). Lesions of the mediodorsal nucleus blunt psychomotor stimulant drug-induced behavioral activity (Kalivas et al. 1999), and microinjections in the mediodorsal nucleus of drugs mimicking GABAergic neurotransmission produce dose-dependent increases in locomotion (Churchill et al. 1996). Notably, the mediodorsal nucleus is known to project to the prefrontal cortex, which at the top of the perception-action cycle, plays a critical role in mediation of contingencies of action across time, thus influencing the temporal organization of behavior (Fuster 2000a and b).

## **5. The Prefrontal Cortex**

The prefrontal cortex (PFC) comprises the anterior pole of the mammalian brain which predominantly receives projections from the mediodorsal thalamic nucleus (Rose and Woolsey 1948). A major problem in studying the functional role of the PFC is the controversy regarding the equivalence between regions in different species (Dalley et al. 2004). In primates the PFC consists of three major divisions: orbital, medial and lateral parts (Vertes 2006). The lateral/dorsolateral PFC (DLPFC) receives information from cortical sensory processing and sends projections to somatomotor structures of the cortex, striatum and brainstem. The DLPFC has been related to executive functions, defined as a set of cognitive control processes necessary for optimal scheduling of complex sequences of behavior (Dalley et al. 2004). It receives few direct inputs from limbic structures such as the amygdala and hippocampus but has access to emotional information through significant connections with the orbito-medial prefrontal cortex (OMPFC). These inputs are probably important for an integrated response to environmental events (see Vertes 2006 for review). The OMPFC includes the orbital and medial networks. The orbital network consists of most of the areas on the orbital surface while the medial network includes areas on the medial wall and two areas in the posterolateral orbital cortex. Some areas are represented in both networks. The orbital network receives sensorial inputs from different modalities and connects with multisensorial areas in the ventrolateral prefrontal cortex and perirhinal cortex. The medial network is reciprocally connected to limbic structures and is also connects to a cortical circuit that includes the rostral part of the superior temporal gyrus and dorsal bank of the superior temporal sulcus, the cingulate

and retrosplenial cortex, the enthorhinal and posterior parahippocampal cortex and the dorsomedial prefrontal cortex (Price 2007).

The rat PFC has been divided into three distinct regions, medial (mPFC), lateral and ventral, based on functional and hodological criteria. The mPFC constitutes the major portion of the medial wall of the hemisphere anterior and dorsal to the genu of the corpus callosum (Leonard 1972, Groenewegen 1988), and can be further divided into a dorsal region that includes the precentral area, anterior cingulate (ACg) and dorsal part of the prelimbic (PL) area, and in a ventral region (vmPFC) comprised by the ventral part of the (PL), infralimbic (IL) and medial orbital (MO) cortices (Heidbreder and Groenewegen 2003). The lateral region includes the dorsal and ventral agranular insular (AID, AIV, respectively) and lateral orbital (LO) cortices. In addition, there is also a ventral region that includes the ventral orbital (VO) and ventral lateral orbital (VLO) cortices (Fig. 3, Dalley et al. 2004).

Although the PFC is much smaller in rodents and there are considerable variations across species, it is becoming clear, based in similarities of position and connections, that the caudal OMPFC is relatively comparable across species (Price 2007). For example, the IL, PL and ACg areas of the rat PFC seems to correspond to areas 25, 32 and 24 of primates, respectively (Price 2007). As in primates, a growing body of evidence indicates the presence of a functional heterogeneity in different regions of the rat PFC (Heidbreder and Groenewegen 2003).

Several findings indicate that the OMPFC, particularly its medial part (mPFC), is involved in the co-ordination of the behavioral responses related to fear and anxiety (Bishop 2007). For example, exposure to different stimuli such as a predator, elevated plus-maze, novel environment or swim and immobilization stress increases c-fos expression in the PL (Sewards and Sewards 2003). Activation of the mPFC in normal volunteers is changed during exposure to fearful stimuli, and abnormal mPFC activity has been associated with a number of anxiety disorders. Corroborating these findings, the mPFC has direct connections with subcortical, diencephalic and brainstem structures closely related to anxiety, such as the amygdala, hypothalamus, solitary nucleus, periaqueductal gray, dorsal raphe, and ventral tegmental area (Gabbott et al. 2005, Vertes 2006).

### ***5.1 mPFC and conditioned fear***

As discussed above, a large body of evidence from rodent studies indicates that the amygdala plays a critical role in the acquisition and expression of conditioned fear.

The mPFC, on the other hand, has been particularly related to extinction, an important form of emotional regulation characterized by a decrease in a conditioned response to a stimulus when the reinforcer is omitted (for review, see Quirk and Mueller 2008). The vmPFC is proposed to have a dual role in fear extinction, retaining information that fearful events are no longer fearful (retention of fear extinction), and acting on visceral structures to reduce fear (expression of fear extinction). Several pieces of evidence indicate that projections from the mPFC to the amygdala facilitate extinction of conditioned fear responses (Milad et al. 2006). For example, lesions of the vmPFC that have no effect on acquisition impaired extinction of freezing responses to an aversively conditioned stimulus (Milad et al. 2006). This observation was reminiscent of the perseverative behavior seen in monkeys with prefrontal lesions and was described as

“emotional perseveration”. Paralleling these lesion findings, single neurons in the IL exhibited no responses to tones during the conditioning or extinction phases of training, but showed robust responses to tones in the following day, when rats were recalling the extinction process. These findings support the hypothesis of extinction as new learning and are consistent with recent observations that extinction involves protein synthesis and gene expression in the mPFC.

The mechanism of PFC interference on extinction seems to involve projections from the vmPFC to the amygdala. Neurons from the IL project strongly to the capsular division of the central nucleus of the amygdala (CeA), which contains GABAergic intercalated cells that inhibit CeA output neurons (Fig. 3). IL stimulation, therefore, would prevent CeA neurons from being activated by BLA inputs. The IL could also modulate the expression of conditioned fear via direct projections to the hypothalamus, periaqueductal gray and other brain stem regions critical for generating conditioned responses (see Fig. 1). In addition to the IL, however, the medial, dorsomedial, and dorsolateral PFC areas have also been recently implicated in extinction. Finally, there is also evidence that the IL and PL play a distinct role in fear conditioning by producing opposing effects in the amygdala. Whereas conditioned stimuli (tones) paired with IL stimulation decreases fear (strengthened fear extinction) those paired with PL stimulation increased fear (impaired fear extinction). These opposite effects could be mediated by distinct neural pathways. Instead of projecting to GABAergic intercalated neurons in the amygdala, as does the IL (see above), the PL projects to the BLA which, in turn, exerts excitatory effects on the CeA to enhance fear (Fig. 3).

### ***5.2 mPFC and innate fear***

Although several studies indicate that lesions or inactivation of the mPFC affect anxiety responses in animals submitted to animal models based on innate fear, the results are still controversial, with reports of increased, decreased, or unchanged anxiety (Bums et al. 1996, Maaswinkel et al. 1996, Jinks and McGregor 1997, Lacroix et al. 1998, Heidbreder and Groenewegen 2003, Shah and Treit 2003, Wall et al. 2004). As discussed above, functional heterogeneity between subregions of the PFC could be partially responsible for these contradictory results (Jinks and McGregor 1997).

### ***5.3 mPFC and visceral reactions to threat***

Stimulation of the vmPFC induces changes in physiological correlates of anxiety, such as respiration, heart rate, piloerection and blood pressure. The vmPFC controls the increases in mean arterial pressure and heart rate associated with fear conditioning (Resstel and Correa 2006, Resstel et al. 2006) and stimulation of this region inhibits cardiovascular responses evoked by electrical stimulation of the hypothalamus or CeA. Although these effects were originally thought to be mediated by indirect projections (Verberne and Owens 1998), recent results indicate that the vmPFC influences these autonomic responses by means of its extensive connections with hypothalamic areas such as the region immediately ventral to the paraventricular nucleus (sub-PVN), the dorsomedial hypothalamic nucleus (DMH) and the lateral hypothalamus (LH) (Van Eden and Buijs 2000). The mPFC can also participate in endocrine responses to aversive stimuli, modulating hypothalamus-pituitary-adrenal (HPA) activity through its connections to the bed nucleus of the amygdala, stria terminalis (BNST) or the peri-PVN region. Finally, in



the midbrain the mPFC projects to the PAG in a topological manner where it can modulate the behavioral and autonomic responses to emotional inputs integrated by this structure (Van Eden and Buijs 2000).

#### ***5.4 PFC responses to stress***

Stress exposure has been related to the development of several anxiety disorders, particularly PTSD. Recent pieces of evidence indicate that the mPFC could be involved in this effect. Brief exposure to uncontrollable stress impairs fear extinction and induces retraction of terminal branches of apical dendrites of IL neurons (Izquierdo et al. 2006). Similarly, chronic restraint stress for 20 days decreases dendritic branching in the PFC (PL region) and hippocampus. It increases, however, dendritic branching in the basolateral amygdala (see Quirck and Mueller 2007, for review).

### **6. Clinical findings and anxiety disorders**

Although methodological problems still persist, the difficulties of disclosing the human neural network involved in anxiety responses have been partially overcome with the use of recent neuroimaging techniques. These studies have implicated most of the neural substrates unveiled by animal studies. For example, there is increased activity in the amygdala of healthy volunteers during acquisition and extinction of the conditioned stimulus. As predicted by animal studies, during the latter process there is also enhanced activation of the mPFC and hippocampus (for review see Bishop 2007). In addition, success in interpreting negative stimuli as less threatening correlates with an increase in PFC and a decrease in amygdala activity (Bishop 2007, see Fig. 3). It seems, therefore, that the amygdala-prefrontal circuitry mediates basic mechanisms involved in human anxiety such as selective attention to threat, interpretation of stimuli and acquisition and extinction of conditioned fear. An increased and decreased activity in the amygdala and PFC, respectively, would lead to increased representation of negative stimuli and failure to activate alternate non-threatening related representations (Bishop 2007).

The role of these structures, however, is probably more complex. For example, stimulation of area 32 of the OMPFC (suggested to be analogous to the PL cortex of rodents) or the rostral cingulum below the anterior cingulate cortex in humans caused reports of fear and anxiety (for review see Swards and Swards 2003). In addition, a recent study by Butler et al. (2007) found increased activity of the amygdala only at the earlier portion of an experimentally-induced state of conscious fear. Interestingly, regions usually associated with motor behavior show robust responses in the same paradigm, with increased activation of the dorsal basal ganglia and de-activation of the primary motor cortex. Other regions with sustained activity were the insula, thalamus and brain stem. The amygdala, therefore, was proposed to work as a threat and novelty detector whose initial, brief and phasic activity is accompanied by sustained tonic activity of other brain regions responsible for maintaining the behavioral, anatomic and metabolic responses for danger stimuli (Butler et al. 2007).

In a recent neuroimaging study Mobbs et al. (2007) showed that subjects faced with an active avoidance paradigm where they are pursued through a maze by a virtual predator presented changes in brain activity according to the proximity of the threat stimulus.

When the predator was remote there was increased activity in the vmPFC and lateral amygdala. As the virtual predator grew closer there was a shift in activity from these areas to the PAG and central amygdala. Moreover, PAG activation was directly correlated with the degree of dread and inversely correlated with confidence to escape (Mobbs et al. 2007). These results suggest that vmPFC and lateral amygdala coordinate avoidance behavior triggered by distal threat whereas the central amygdala and PAG coordinate behavioral responses when threat is imminent (Maren 2007). They also agree with the proposal that activation of forebrain areas such as the PFC and amygdala by distal or potential threats upholds anxiety whereas activation of the PAG by proximal aversive stimuli promotes panic (Deakin and Graeff 1991, Maren 2007).

These neural mechanisms, involved in human normal anxiety, are probably more akin to the defensive responses in rodents discussed so far. The study of the neuroanatomical basis of pathological anxiety in animals, on the other hand, is complicated by the well documented cognitive bias towards cues signaling danger shown by patients with anxiety disorders. This difficult to mimic the capacity to worry excessively about the future, a key feature in most anxiety disorders, is a limitation of animal models (Bishop 2007). Since this capacity has been linked to the greater development of the PFC in primates, this brain region has been proposed to play a more important role in human anxiety (Berkowitz et al. 2007). A hypoactive OMPFC has been related to a failure to inhibit inappropriate fear or anxiety responses whereas conditions such as obsessive-compulsive disorder (OCD) would involve hyperactivity of the lateral orbital PFC (Milad and Rauch 2007). In agreement with this possibility, several neuroimaging studies have shown abnormalities in the PFC in patients with anxiety disorders, with decreased neuronal activity in disorders characterized by intense fear, such as panic disorder, post-traumatic stress disorders (PTSD) and phobias, and increased activity in disorders involving worry and rumination (generalized anxiety disorder and OCD) (Milad and Rauch 2007). OCD is proposed to arise from dysfunctions in a cortico-striato-thalamo-cortical circuitry, in a way that changes in the striatum lead to ineffective gating in the thalamus (Milad and Rauch, 2007) and hyperactivity of the orbital PFC and anterior cingulate cortex. Successful pharmacological or behavior therapy has been able to decrease activity in these areas (see Milad and Rauch 2007, for review).

PTSD, on the other hand, has also been related to changes in a circuit that involves the OMPFC plus the hippocampus and amygdala. Patients with PTSD have smaller vmPFC and hippocampus volumes, and increased activity in the amygdala (Quirk and Mueller 2008). These changes would translate in a failure to consolidate and retrieve extinction of aversive events (see Fig. 3).

Another human brain structure that shows hyperactivity in anxiety disorders such as PTSD, social anxiety disorder and specific phobias, is the insular cortex. It is activated by negative emotions and regulates the autonomic nervous system activity. The insula has been proposed, together with the amygdala, hypothalamus, periaqueductal gray, parabrachial nucleus and nucleus tractus solitarius, to be part of an internal regulation system that controls the visceromotor, neuroendocrine and pulmonary system as well as pain sensations (Nagai et al. 2007, for review see Etkin and Wager 2007). It has been implicated in the recognition and experience of disgust, sadness and fear (for review see Morris 2007).

Concerning panic disorder (PD), neuroimaging studies in patients or volunteers submitted to panic symptoms-inducing drugs have shown increase activation of parahippocampal gyrus, the superior temporal lobe, the anterior cingulate, cerebellar vermis, insula, temporal poles and the hypothalamus, and PAG (Javanmard et al. 1999, Boshuisen et al. 2002). Both before and after a drug-induced panic attack, however, PD patients showed hypoactivity in the precentral gyrus, OMPFC, the right amygdala, and the anterior insula (Boshuisen et al. 2002). PD patients that improve after cognitive-behavior therapy showed decreased activation of the right hippocampus, left anterior cingulate, left cerebellum and pons, and increased activity in the OMPFC. Further strengthening the proposal of PAG involvement, a significant correlation between activity in the midbrain and the number of panic attacks has been described (Sakay et al. 2006).

## 7. Conclusions

Similar to other complex functions, anxiety does not appear to depend on specific areas performing unique functions but should be rather seen as an emerging property of interacting brain regions (Morgane et al. 2005). Even so, it is clear that animals show different and sometimes opposite behavioral responses to specific stimuli such as distal or proximal threats and that these responses are organized by at least partially distinct brain systems (Deakin and Graeff 1991, McNaughton and Corr 2004, Maren 2007).

The several pieces of evidence reviewed here indicate that these brain systems are similar in rodents and humans. They would include a defense system, aimed at making immediate responses to threatening stimuli, and a behavioral inhibition system, responsible for the suppression of behaviors that could enhance danger. Other partially distinct networks could be responsible for responses such as avoidance. These systems would work in concert and engage distinct hierarchic circuits depending on increasing demand for cognitive processing (McNaughton and Corr 2004, Sandford et al. 2000). Dysfunctions in these circuits are likely to generate chronic anxiety disorders (Maren 2007). In addition, these circuits are under modulatory influence of several other neural systems such as, for example, serotonergic, noradrenergic and dopaminergic inputs from the raphe nuclei, locus coeruleus and ventral tegmental area, respectively. A complete review of these modulatory circuits would be beyond the aims of this chapter. They are however, particularly important for the anxiolytic effects of drugs such as antidepressants and benzodiazepines and will be reviewed elsewhere in this book.

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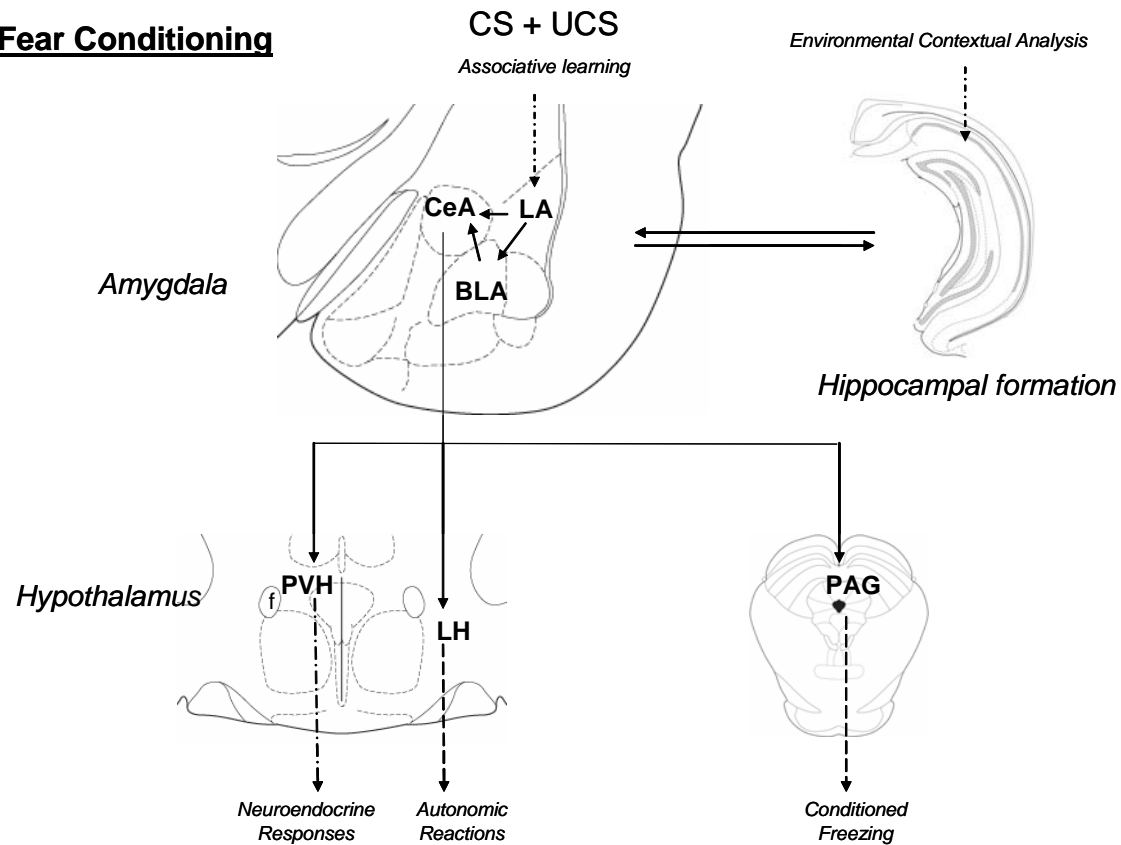
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## Fear Conditioning



## Cat Exposure

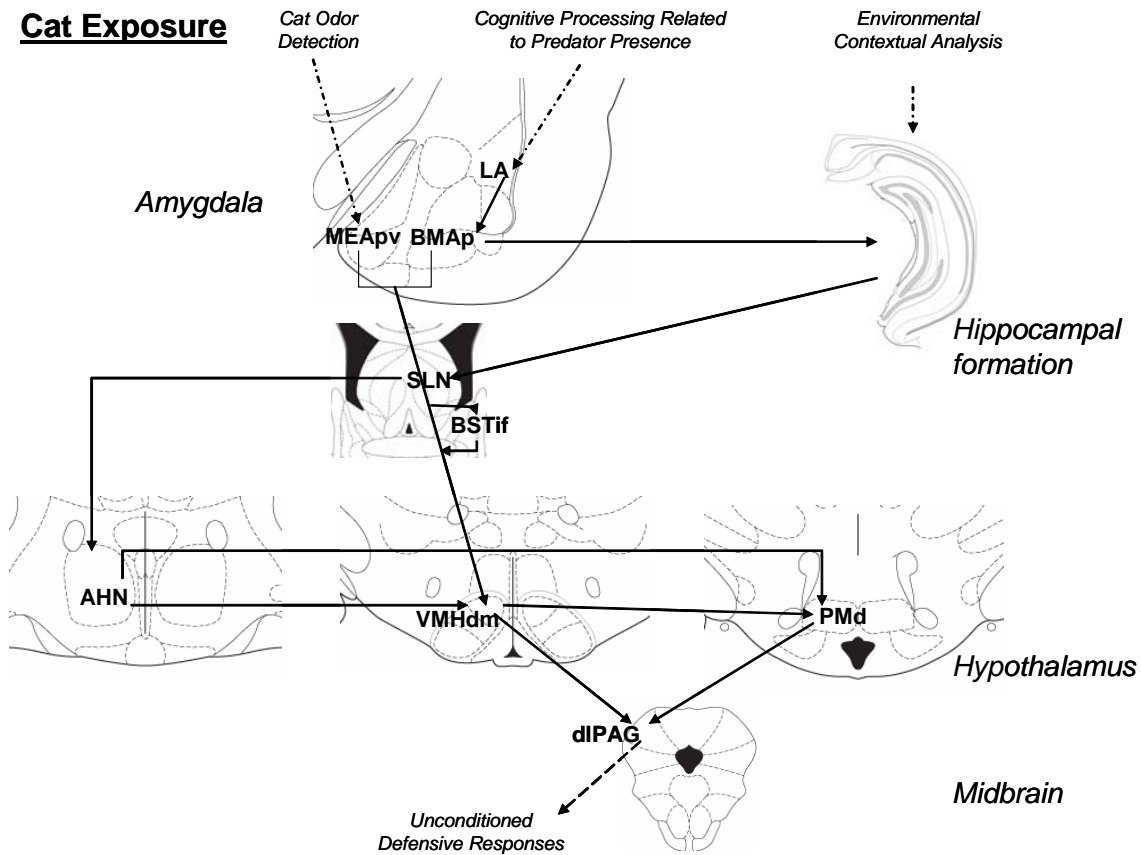


Fig. 1. Upper part. Neural substrate proposed to be involved in Pavlovian conditioned fear. Associative learning between the conditioned and unconditioned stimuli is likely to occur in the lateral nucleus of the amygdala (LA), which, in turn, projects to the central nucleus (CeA) both directly and indirectly via projections to the basolateral nucleus (BLA). The central nucleus, via projections to the hypothalamus and brainstem, is critical for the expression of fear conditioning.

Lower part. Neural substrate involved in odor-elicited antipredator defensive responses. Predator odors seem to be processed in the accessory olfactory bulb, which projects principally to the medial amygdala. The amygdalar sites related to predator detection project either directly or indirectly, via the transverse nucleus of the BST, mostly to the dorsomedial part of the ventromedial nucleus of the hypothalamus (VMHdm). Together with the anterior and the dorsal premammillary hypothalamic nuclei, the VMHdm is part of a medial hypothalamic defensive system. The main brainstem target of this system is the periaqueductal gray (PAG). The septo-hippocampal system, via its direct connections to the medial hypothalamic defensive system, and, therefore, is in a position to control anti-predatory defensive responses by providing contextual analysis.

ANH: anterior hypothalamic nucleus, BMAp: posterior basomedial amygdala, BSTif: transverse nucleus of the bed nucleus of the stria terminalis, dlPAG: dorsolateral periaqueductal gray, LH: lateral hypothalamus, MEApv: posteroventral medial amygdala, PMd: dorsal premammillary hypothalamic nucleus, PVH: paraventricular nucleus of the hypothalamus, SLN: lateral septal nucleus (Drawings based on Paxinos and Watson, 2005).

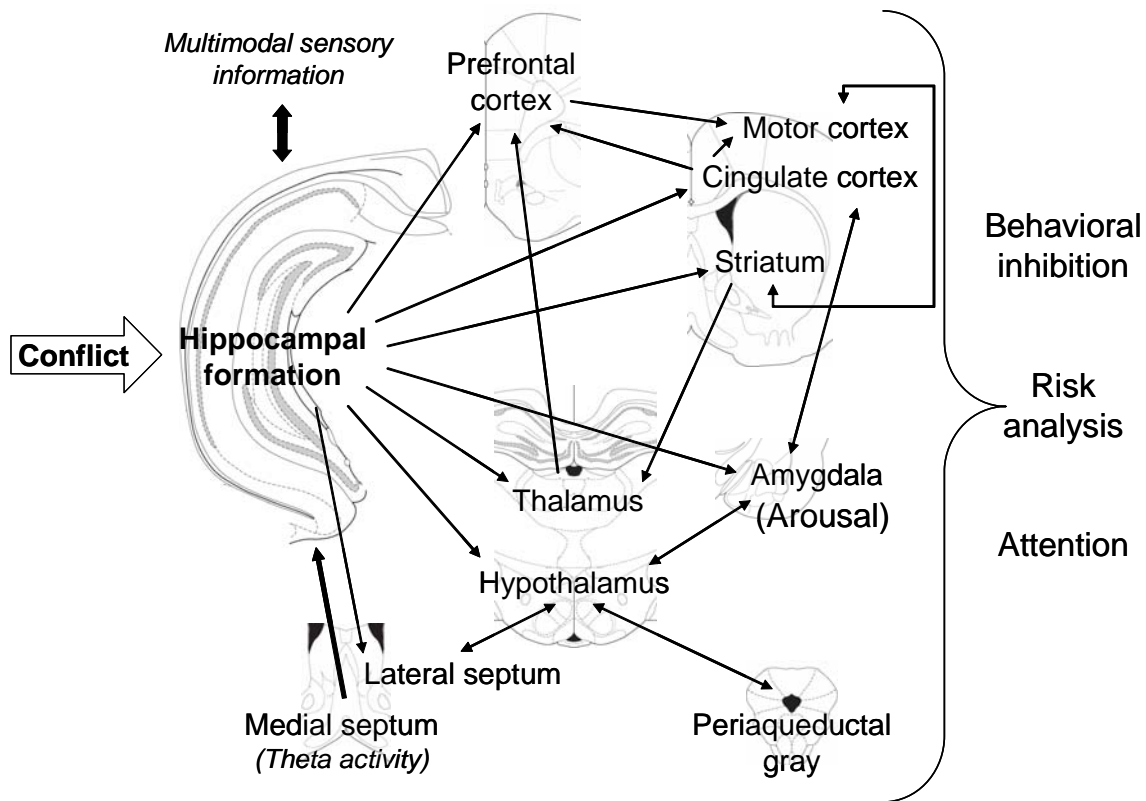


Fig. 2. Outline of the septo-hippocampal circuitry subserving the behavioral inhibition, risk analysis, and attentional outputs triggered by conflict. The arousal output of the behavioral inhibition system is held to be mediated independently by the amygdala and has been omitted. Gray and McNaughton (2000) see these modules as mainly concerned with the organization of goal-directed action. The hippocampal formation comprises the entorhinal cortex, the dentate gyrus, the subicular area, and the posterior cingulate cortex (Drawings based on Paxinos and Watson, 2005).

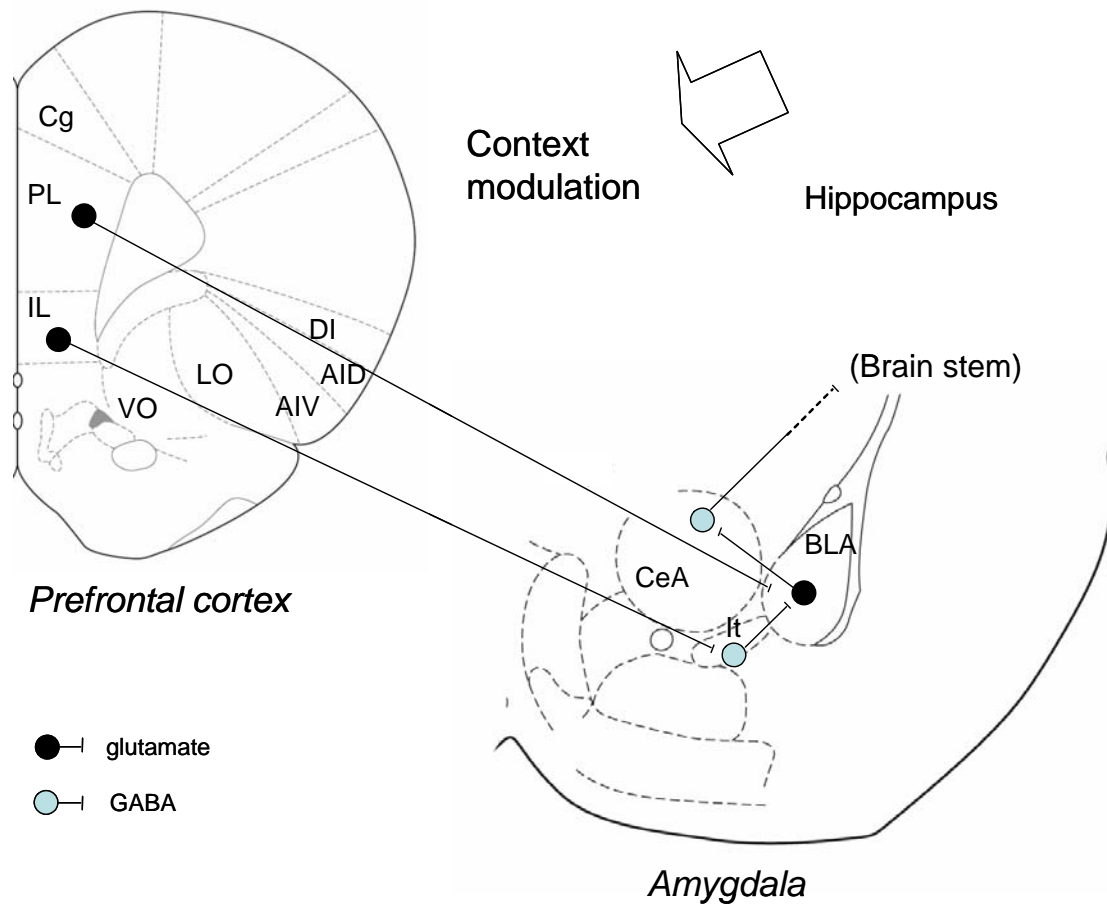


Fig. 3. Basic circuits describing a proposed interplay between the mPFC, amygdala and hippocampus in fear conditioning extinction. IL glutamatergic neurons project to GABAergic intercalated cells that inhibit CeA output neurons to promote extinction of aversively conditioned responses. On the other hand, PL neurons project to the BLA which, in turn, exerts excitatory effects on the CeA to enhance fear. The hippocampus, meanwhile, is thought to provide contextual modulation of the process. Cg: cingulate cortex, PL: prelimbic cortex, IL: infralimbic cortex, VO: ventral orbital cortex, LO: lateral orbital cortex, AIV: ventral agranular insular cortex, AID: dorsal agranular insular cortex, DI: dysgranular insular cortex, CeA: central amygdaloid nuclei, BLA: basolateral amygdala, It: intercalated nucleus (Dalley et al. 2004, Quirk and Mueller, 2008, Bishop 2007, Drawings based on Paxinos and Watson, 2005).