

# Stress and the Neuroendocrinology of Anxiety Disorders

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**Abstract** Stress is a risk factor for depressive and anxiety disorders. Changes in lifestyle patterns that are associated with increased stress therefore place a greater burden on mental health. Stress challenges the organism's homeostatic mechanisms, triggering a cascade of events that should, normally, maintain or allow a return to equilibrium. Stressful events are perceived by sensory systems in the brain, facilitating evaluation and comparison of the existing and previous stimuli as well as the activation of hormones responsible for energy mobilization. The limbic system coordinates the release of corticosteroids, the primary stress hormones, by modulating activation of the hypothalamic paraventricular nucleus (PVN). The amygdala, a limbic structure related to emotional behavior, has a putative role in the evaluation of emotional events and formation of fearful memories; it is also a

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target of the neurochemical and hormonal mediators of stress. Clinical and experimental data have correlated changes in the structure/function of the amygdala with emotional disorders such as anxiety. In this chapter we review the neuroendocrinology of the stress response, focusing on the role of the limbic system in its establishment and supplementing that information with new experimental data that demonstrates the relationship between stress and anxiety disorders; we also discuss the structural changes that occur in the amygdala after stress.

**Keywords** Anxiety · Bed Nucleus of the Stria Terminalis · Rat · Stress · Animal Models · Amygdala · Corticosteroids

## 1 Stress and Emotional Behavior

Stress is implicated in the etiology of several emotional disorders, including depression (McEwen 2004; Hammen 2005), phobia (Risbrough and Stein 2006) and anxiety (Charney et al. 1993). Stress-related disorders affect a substantial portion of the work force and therefore represent an important burden on health providers, caregivers and society at large. World Health Organization statistics indicate emotional/mood disorders as a leading cause of disability worldwide and predict that by 2020 these disorders will be the second leading global burden of illness, only surpassed by cardiovascular disease.

Stress may be defined as a challenge to homeostasis, imposed by physical or psychological events. Exposure to stress triggers a cascade of hormonal and behavioral changes that, under normal conditions, facilitate the organism's adjustment to, and control over, the threatening events. Adaptation to stress involves activation of neural, neuroendocrine and neuroimmune mechanisms. Effective coping implies that a stress response is triggered when needed and terminated thereafter. However, if the stress response is exaggerated or protracted, a series of pathophysiological changes occur in the brain and immune system (and viscera) that may ultimately result in disease (Mayer and Fanselow 2003; Musselman and Nemeroff 2000; Sorrells and Sapolsky 2007).

The hypothalamo-pituitary-adrenal (HPA) axis plays a central role in the stress response. It is activated early after the arrival of stressful stimuli, resulting in the secretion of the adrenocorticotrophic hormone (ACTH) secretagogues, corticotrophin-releasing factor (CRF) and arginine-vasopressin (AVP), by neurosecretory neurons in the parvocellular component of the PVN; in turn ACTH stimulates the synthesis and secretion of corticosteroids by the adrenal glands. Therefore, by integrating the output of different stress-sensitive brain circuitries (Herman and Cullinan 1997), the PVN plays a key role in regulating the HPA stress response (Whitnall 1993).

The PVN is subject to differential activation by distinct neuronal pathways, depending on the quality and/or immediacy of the demand for an appropriate

response (Herman et al. 1997, 2003): stressors such as hemorrhage, respiratory distress or systemic inflammation, which represent an immediate threat to homeostasis, directly activate the PVN, bypassing cortical and limbic areas, by activation of somatic, visceral or circumventricular sensory pathways (Chan et al. 1993; Cole and Sawchenko 2002). More precisely, excitatory ascending pathways originating in the brainstem nuclei that convey noradrenergic inputs from the nucleus of tractus solitarius (Abercrombie and Jacobs 1987; Cullinan et al. 1995; Gann et al. 1977; Smith et al. 1991), serotonergic inputs from the raphe nuclei (Feldman et al. 1987; Sawchenko et al. 1983) or inputs from adjacent hypothalamic nuclei (Herman et al. 2003) are well positioned to receive visceral and autonomic inputs so as to evoke rapid neuroendocrine responses. Other stressors result in the central generation of responses, allowing mobilization of energy and immune reserves in anticipation of homeostatic disruption. The activation of these complex pathways seems to occur by contextual, conditioned (memory) or species-specific cues that predict adversity. The mnemonic aspects of this response are important determinants of the magnitude of the HPA response as, per definition, stimuli are only stressful by comparison with previous experience(s). The limbic system plays a central role in the coordination of this “anticipatory” stress response (Herman et al. 2005) and characterizes stress-induced anxiety behavior, as will be addressed later.

### ***1.1 Mediators of the Stress Response***

Circulating corticosteroids play a central role in the adaptative response to stress, primarily by influencing energy metabolism and dampening the immune and inflammatory responses; thus, corticosteroids act in the short term to prevent overshooting of the innate response (de Kloet et al. 2005; Sapolsky et al. 2000). However, prolonged exposure to elevated levels of corticosteroids leads to immune dysfunction (Sapolsky 2000; Sorrells and Sapolsky 2007), endocrine dysregulation (Sapolsky 2000; Sousa et al. 2008) and, ultimately, to behavioral and neuropathology (Cerqueira et al. 2008; Sapolsky 1999; Sousa and Almeida 2002; Sousa et al. 2007).

Corticosteroids actions are mediated by two biochemically distinct receptors which bind the same ligand (cortisol in humans, corticosterone in rodents), albeit with differing affinities. While glucocorticoid (GR) receptors are ubiquitously distributed, mineralocorticoid receptors (MR) are more discretely distributed; however, both receptors are expressed at particularly high levels in limbic areas that are responsible for the modulation of the stress response (Reul and de Kloet 1986). As compared to GR, MR have a higher affinity for cortisol/corticosterone and are, therefore, highly occupied even under basal (stress-free) conditions (Reul and de Kloet 1985). In contrast, GR become increasingly occupied as circulating corticosteroid levels rise, e.g., during stress. MR have been implicated in the appraisal process and onset of the stress response, while GR have been ascribed a role in the mobilization of energy substrates and most stress-induced changes in behavior.

The latter includes anxious-like behavior and facilitated learning and memory (in particular, consolidation of memories). On the other hand, long-term GR activation is associated with deleterious effects on several cognitive functions (Cerqueira et al. 2005a, 2007a, b; McEwen 2005; Sapolsky et al. 1986; Sousa et al. 2000, 2007). Interestingly, these effects have been correlated with neuroarchitectural changes in several brain regions, including the hippocampal formation, prefrontal cortex (PFC) and amygdala (McEwen 2007; Sousa et al. 2007). It is worth noting here that the hippocampal formation and PFC are involved in mediating corticosteroid negative feedback on the HPA axis and in curtailing the endocrine response to stress (Mizoguchi et al. 2003). Not surprisingly, these areas also undergo structural and functional remodeling by stress. These changes, thought to have a putative role in the perpetuation of the hypercortisolism state that characterizes chronic stress, are thought to make a pivotal contribution to the maladaptation by some individuals to stress (Cerqueira et al. 2008; Sousa et al. 2007).

While elevated corticosteroid levels characterize stress, it is important to note that high levels of corticosteroids do not mimic stress, but rather only one aspect of it. A plethora of other processes are altered by stressors, among them, the previously mentioned hypothalamic neuropeptide CRF (Lau et al. 1983) and the related peptides: Urocortin (Ucn), UcnII and UcnIII. The Ucn family of peptides act through the G-protein-coupled CRF receptors, CRFR<sub>1</sub> and CRFR<sub>2</sub>. While CRF and Ucn bind both CRFR<sub>1</sub> and CRFR<sub>2</sub> the more recently discovered UcnII and UcnIII show high selectivity for CRFR<sub>2</sub>. In the brain, there is a variable expression and distribution of CRF related peptides (Dautzenberg and Hauger 2002; Reul and Holsboer 2002). CRF is expressed in neurons of the PVN, cerebral cortex, cerebellum, amygdala and hippocampus; Ucn expression is limited to the Edinger-Westphal, lateral olivary and supraoptic nuclei; UcnII has a distinct subcortical expression in regions related to stress response, such as the PVN, locus coeruleus, hypothalamic supraoptic and arcuate nuclei, and several motor nuclei of the brainstem and spinal cord; UcnIII is expressed in the rostral perifornical area, posterior part of the bed nucleus of the stria terminalis (BNST), medial nucleus of the amygdala and lateral septum.

Each of the CRF receptors displays a different expression pattern and has distinct physiological functions (Chalmers et al. 1995; Chen et al. 1993; Dautzenberg and Hauger 2002; Lovenberg et al. 1995; Reul and Holsboer 2002; Steckler and Holsboer 1999). The CRFR<sub>1</sub>, which is mainly expressed in the anterior pituitary, cerebral cortex, cerebellum, amygdala, hippocampus and olfactory bulbs (Dautzenberg and Hauger 2002; Reul and Holsboer 2002), has been consistently implicated in stress-related behavior (Heinrichs and Koob 2004; Heinrichs et al. 1997; Liebsch et al. 1995, 1999; Skutella et al. 1998), and in particular the mediation of anxiety-like responses in various test paradigms such as the elevated-plus maze (File et al. 1988), social interaction test (Dunn and File 1987) and acoustic startle response test (Swerdlow et al. 1986). Additionally this receptor plays a role in hormonal (Dunn 1987) and autonomic activation (Nakamori et al. 1993) by stress. While the CRFR<sub>1</sub> appears to be crucial to the initiation of the stress response, it does not seem to be responsible for baseline drive of the HPA axis

(Reul Holsboes 2002). CRFR<sub>2</sub> are mainly expressed in subcortical areas, including the PVN, lateral septum, amygdala, hippocampus and BNST. A specific role has still to be ascribed to CRFR<sub>2</sub>: while some studies implicate it in anxiogenesis (Radulovic et al. 1999), others suggest that it may mediate anxiolytic effects (Valdez et al. 2003; Zhao et al. 2007); these opposing reports may reflect differences in regional activation of the receptor or treatment paradigms (e.g., duration and intensity of stress). Importantly however, there is consensus that CRFR<sub>2</sub> are critical for the extinction of HPA activation (Bale et al. 2000; Coste et al. 2000).

## 2 Anxiety Disorders and Stress

Anxiety and fear are two closely related aspects of emotional behavior. However, although they have “similar” phenotypic expressions, each is governed by specific brain circuitries that are activated by distinct types of stimuli. These differences will be given special consideration in order to clarify the characteristics that typify anxiety.

Anxiety disorders are highly prevalent (Alonso and Lepine 2007; Kessler et al. 2007b; Merikangas and Kalaydjian 2007; Moussavi et al. 2007) and represent a heterogeneous group of disorders that range from phobias to generalized anxiety disorders (American Psychiatric Association 1994). Anxiety is characterized by a number of mental and physical symptoms with no apparent explanation. Mental symptoms include a general sensation of discomfort and apprehension in response to unconditioned diffuse cues (Koch 1999). Healthy individuals commonly experience anxiety upon experience of unspecific frightening cues which, nevertheless, do not have clear threatening outcomes (e.g., loud noises, fast approaching objects, etc.). One might assume that the state of anxiety/fear has a biological advantage insofar that it helps the individual prepare a defense against potentially harmful encounters; however, apart from keeping the individual attentive for recurrences of the adverse situation, persistence of anxiety/fear after the “flight or fight” response has achieved its goals is likely to be disadvantageous. The symptomatic response to anxiogenic stimuli includes increased heart rate, muscle tension, dizziness, light headedness, nausea, and chest/abdominal pain, eventually focusing the individual’s attention to determine the source of threat, which translates into persistent activation of the autonomic nervous system. Frequently, the latter outlasts exposure to the anxiogenic stimulus, indicating long-term activation of the neural substrates and processes that regulate anxiety (Lee and Davis 1997a, b; Lee et al. 1994).

As already noted, anxiety disorders develop when symptoms occur without any recognizable stimulus or when the stimulus does not warrant such a reaction. In contrast, fear involves the conditional learning that a specific cue, or a more complex context, predicts imminent adversity (Brown et al. 1951; Davis 1986, 1992). Fear is also found in lower organisms (Davies et al. 2002; Eisenberg and Dudai 2004; Portavella et al. 2004), suggesting that it may have advantages related to the survival of the individual and continuity of the species. By learning which

situations are threatening, the organism can actively avoid those specific objects or contexts in the future. The fear response is characterized by a set of symptoms relayed by neuroendocrine and autonomic centers that comprise largely the same pathways activated in the anxiety response (Walker et al. 2003). However, these common pathways are activated in a particular set of conditions that result in the activation of distinct initiating centers. Fear responses are elicited by explicit, adversity-predicting, short-lasting cues, in which the period of danger is finite. In general, fear responses are self-limiting in time and magnitude, the symptoms resulting from its activation being abolished upon termination of the adverse stimulus. In summary, the closely related states of fear and anxiety are mediated by common pathways that are activated, however, over distinctly different time-frames and in response to particular conditioning stimuli.

Animal (Anisman and Matheson 2005; Arborelius et al. 1999; File 1996; Pêgo et al. 2008; Vyas et al. 2002) and clinical (Chrousos and Kino 2007; Dranovsky and Hen 2006; Greaves-Lord et al. 2007) studies have consistently shown a correlation between chronic stress and altered emotional behavior. Most importantly, anxiety disorders have been correlated with abnormalities in the HPA axis although the nature of these changes are different from those observed in mood disorders such as depression (Mathew et al. 2008; Risbrough and Stein 2006). For example, while many depressed patients display signs of a hyperactive HPA axis (Shelton 2007), patients with anxiety disorders show a wide spectrum of patterns of HPA activity, possibly because such disorders represent a very heterogeneous group of pathologies. Although particular subgroups of anxiety disorders (Mathew et al. 2008; Risbrough and Stein 2006), best exemplified by patients suffering from post-traumatic stress disorder (PTSD), show signs of increased CRF production (Baker et al. 1999; Bremner et al. 1997), they are characterized by abnormalities of the HPA which are more subtle and less consistent than those found in depression. In fact, PTSD patients tend to display an exaggerated inhibition of cortisol secretion after the administration of exogenous glucocorticoids such as dexamethasone (Shekhar et al. 2001). This suggests that the HPA axis of PTSD patients is sensitized, rather than blunted (as in depression); these findings in humans have been reproduced in rodent models of PTSD (Liberzon et al. 1999).

Independently of the triggering mechanisms, the impact of the hormonal mediators of stress mediators on anxiety behavior is notable (Arborelius et al. 1999). Glucocorticoids have been shown to enhance the excitability of amygdalar neurons and to influence the acquisition of fear behavior (Duvarci and Pare 2007; Skorzevska et al. 2006; Yang et al. 2007) and stress has been repeatedly shown to induce anxiety-like behavior (McEwen 2003, 2004; Pêgo et al. 2008; Vyas and Chattarji 2004; Vyas et al. 2002) while a role for GR in the modulation of anxiety-associated behavior has also been demonstrated (Boyle et al. 2006). The amygdala and extended amygdala (including the BNST), both of which are richly populated with corticosteroid and CRF receptors that are activated during stress (Cullinan et al. 1995; Figueiredo et al. 2003; Ju et al. 1989b; Mathew et al. 2008), have a putative role in the integration of polysensory information that culminates in the

expression of emotional behavior. Therefore, these brain areas have been targets of recent research in the field of stress-related anxiety behavior.

### 3 Animal Models of Stress and Anxiety

Animal models of emotional disorders attempt to reproduce various characteristics of human psychiatric disorders, from behavioral and physiological changes associated with a particular emotional state (face validity) to the etiology (construct validity) and responses to therapeutic interventions (predictive validity). These models have become invaluable tools in the analysis of various causes – genetic, environmental (e.g., stress) or pharmacological – of these disorders. Additionally, they have served as screening tools for identifying potential anxiolytic drugs as well as the neuroanatomical circuits responsible for mediating these behaviors. Despite evident flaws in the reproduction of certain aspects of human behavior, these models have been an essential first step towards understanding the neurobiology of psychiatric diseases (Bourin et al. 2007; Fuchs and Flugge 2006; Shekhar et al. 2001).

Several animal models of anxiety have been used since the 1960s. Most of these models were validated using behavioral tests that involved challenging the animal with a stressful/painful stimulus before assessment of the changes in response to by benzodiazepines (BZD) – at that time, the only approved class of drug with anxiolytic properties (Shekhar et al. 2001). However, results showing that new non-BZD anxiolytics were inactive in the classical laboratory tests of anxiety, led to the realization that anxiety disorders represent a heterogeneous group of disorders in which a variety of neurochemical systems may be involved and which have distinct etiological origins (Bourin et al. 2007). Those findings also highlighted the importance of using more than one test to examine the different aspects of anxiety behavior (Sousa et al. 2006).

It should also be mentioned that it is inappropriate to assume that a single animal model will necessarily display all aspects of a behavior as complex as anxiety. It is now consensual that animal models may represent “normal” or “state” anxiety on the one hand, and “pathological” or “trait” anxiety on the other hand (see Lister 1990). In fact, most experimental procedures involve exposure to external (e.g., pain, a predator) or internal (e.g., drugs) stimuli that induce anxiety. Behavioral responses in these paradigms may correlate to the level of stress induced by the stimuli (and method of presentation) but, nevertheless, only represent a normal response – “state” anxiety. In contrast, “trait” anxiety does not vary between occasions and is considered to be an enduring feature of an individual and to have a distinct neurobiological basis (Lister 1990).

Anxiety tests in rodents are usually based on the assessment of fear/anxiety behavior by creating a conflict between the animal’s innate exploratory activity and an aversive condition. In most instances, animals are submitted to an acute stressful or traumatic event and their response to these experiences is expressed as an index

of exploratory/avoidance behavior. Although these approaches are very useful to test behavioral responses in acute settings and how they are modulated by pharmacological agents they do not necessarily reflect the etiological aspects of anxiety disorders. Anxiety like many other psychiatric disorders is thought to result from the interaction between constitutive factors and exposure to disruptive environmental events, often in a chronic fashion. Human studies (Hettema et al. 2001, 2005) assessing the role of the family inheritance in the genesis of anxiety disorders have shown a strong influence of the genetic background. Both specific phobias and unspecific anxiety disorders showed a strong familial aggregation which clustered into families. Although a strong genetic contribution was identified part of the variability could be explained by family and nonshared environmental factors. These observations highlight the relevance of recognizing predisposing factors for anxiety disorders and understand their interaction with individual constitution in order to identify potential therapeutic targets. Recently, an effort has been made to develop models which emulate predisposing/causative environmental events and which take genetic background, gender and age into account (Shekhar et al. 2001).

Genetic models have used either knock-out mice that exhibit phenotypic behaviors considered to be suggestive of anxiety with the aim of identifying the involvement of specific genes in such behavior, or the selective breeding of rodent lines that exhibit high and low levels of emotionality. Mutant mice have shown involvement of the serotonergic system in the genesis of anxiety behavior (Sibille et al. 2000), consistent with the therapeutic efficiency of antidepressants and non-BZD anxiolytics (e.g., budipirone) that interact with serotonin receptors in the treatment of specific types of anxiety. In fact, serotonin receptor (1A) receptor was shown to be important to the anxiolytic effect of BZD and that the lack of 5-HT<sub>1A</sub> receptor elicits the down regulation of BZD-sensitive GABA<sub>A</sub> receptors predominantly in the amygdala and to a lesser extent in the hippocampus and cortex. The authors postulate that the expression of certain GABA<sub>A</sub> subunits are under serotonergic control exerted by 5-HT<sub>1A</sub> receptors in areas of the brain that are relevant to the expression of anxious-like behavior. Strains of rats (Liebsch et al. 1998b) and mice (Kessler et al. 2007a) exhibiting *high-anxiety related behavior* (HAB) have pointed to the contribution of genetic background and associated disruption of the HPA axis to anxiety-like behaviors (Liebsch et al. 1998a; Wigger et al. 2001; Neumann et al. 1998). Actually, cross-fostering did not influence the expression of behavioral phenotype after establishment of the behavioral trait of the strain (Wigger et al. 2001), reinforcing the major contribution of the genetic background. Moreover, although on basal conditions HAB rats show little differences in the activation of the HPA axis, there is increased secretion of either ACTH or corticosterone in response to anxiogenic stimuli and during pregnancy (Liebsch et al. 1998a; Wigger et al. 2001; Neumann et al. 1998). This latter finding raises the concern that the inheritance of anxious-like behavior may reflect a hormonal imprinting of the HPA axis during prenatal/early life development. Additionally, other studies (Hermann et al. 2000; Keck et al. 2005; Lancel et al. 2002) have also shown that these behavioral traits can be reversed with traditional anxiolytic drugs, much like the observations in humans disorders.



Early life events are thought to constitute a developmental risk factor for the expression of anxiety/fear behavior in adulthood; i.e., adverse events occurring early in life seem to program behavior in adulthood. This relationship is particularly well established in anxiety disorders both in humans and animal studies. Longitudinal studies have shown that anxiety disorders associated to adverse events occurring during early childhood (Carpenter et al. 2007; Espejo et al. 2007; Moffitt et al. 2007) are correlated with changes in function of the HPA axis. On the other hand, animal models have shown that early life-stress, like *prenatal stress* (Weinstock 2001) or the *maternal separation stress* (Mesquita et al. 2007) paradigm result in profound dysregulation of the HPA axis in tandem with an increased incidence of anxiety-like behavior. Importantly, these changes appear to be established during restricted windows of susceptibility at a time when the HPA axis seems to be particularly vulnerable to stress. Such observations accord with clinical studies showing that children exposed to traumatic events or emotional stressors are at a greater risk of developing psychopathologies (Shekhar et al. 2001).

Surprisingly, few animal studies have addressed gender and age as constitutionally relevant risk factors (Bessa et al. 2005; Imhof et al. 1993; Pego et al. 2006; Shekhar et al. 2001). This observation contrasts with clinical experience showing that women have a greater incidence of anxiety disorders, as compared to men (Bekker and van Mens-Verhulst 2007) and that anxiety disorders are highly prevalent among aged human subjects (Gum and Cheavens 2008). When compared to men women show an almost 2 times higher incidence of anxiety disorders (Vesga-López et al. 2008) and had significantly higher rates of co morbid mood disorders (except bipolar disorder) and anxiety disorders (except social anxiety disorder). Most importantly, coping strategies were different among genders, men showing lower rates for treatment seeking. Interestingly, animal studies report sex differences in exploratory and defensive responses to threatening stimuli (e.g., Shekhar et al. 2001), reflecting different coping strategies between males and females. These findings are apparently the result of exposure to sex-specific gonadal steroids that also exert an important influence on anxiety levels (Mitev et al. 2003). Anxiety disorders are highly prevalent in elderly persons, and they are associated with functional impairment, poorer quality of life, and adverse long-term consequences such as cognitive decline (Lenze and Wetherell 2009). Although these observations emphasize the importance of daily-life stressors in the shaping of anxiety disorders little is known about specific aspects of unhealthy ageing. This fact is paralleled by the paucity of morphological changes in animal models of aging that display an anxious phenotype (Pêgo et al. 2006).

Stress models have been widely used for studying anxiety and testing the efficacy of potential anxiolytic substances. The various models used differ in two important respects, namely duration of exposure to stress (acute vs. chronic) and the nature (quality) of stress imposed. The pitfall of the many anxiety tests in which acute stressors have been applied to induce an anxiety “state” is that the deleterious effects of stress on anxiety-related behavior are only really expressed when stress is applied chronically. Moreover, the suitability of such models has been questioned since testing anxiety responses in otherwise “normal” animals does not reproduce

the indolent course of the onset, or the nature of, anxiety disorders. In fact, the inadequate response to stressful challenges that characterizes chronic stress is the end-result of the exhaustion of several immediate stress-responsive systems and is time-dependent (Cerqueira et al. 2007a; McLaughlin et al. 2007). Our experimental observations (Cerqueira et al. 2005b; Pego et al. 2008) have shown that most biological markers associated with chronic stress (e.g., poor coat quality, occurrence of gastric ulcers, impaired weight gain, etc.) only develop overtly after 2–3 weeks of exposure to stress; a similar time-lag is observed with respect to the manifestation of the stress-induced anxious phenotype. Thus, other authors (e.g., Vyas et al. 2002) who used stress paradigms similar to those used by us (Cerqueira et al. 2005b; Pego et al. 2008), albeit over shorter periods, failed to observe behavioral traits. Interestingly, clinical cases of anxiety disorders do not appear until well after the presumed causal stressor, being dependent on a prolonged period of sensitization (e.g., generalized anxiety disorder) (American Psychiatric Association 1994). Also, PTSD and several specific phobias often have a particularly late onset (American Psychiatric Association 1994).

With respect to the nature of the stressor(s) applied, one should keep in mind that stressors can be physical (e.g., exposure to cold or hot temperatures, food deprivation, etc.) or psychological (e.g., restraint, overcrowding, social dominance, etc.), and certain paradigms may combine elements of both; further, the stressors may be given in a predictable (order of multiple stressors, time of day) or unpredictable fashion. Psychological stressors, in particular social stressors, recapitulate “real life” situations and are thought to more closely mimic the situation in clinical cases. The (un)predictable presentation of stressful insults is considered a useful amplification factor, i.e., reinforces disruption of stress-coping mechanisms; it is known that predictable events, even if unavoidable and inescapable are more aversive both, in terms of behavior and incidence of gastric ulcers (Gliner 1972). The anticipation of the arrival of random and unpredictable stimuli induces a state of permanent alertness and preparedness to mount a prompt and appropriate behavioral and physiological response. The latter responses are perpetuated by the persistently active HPA axis which, as mentioned before, results in deleterious effects on the physical and behavioral well-being of the individual. To once again draw parallels with the clinical setting: in PTSD and obsessive-compulsive disorders, where anxiety prevails as an underlying condition, patients report feelings of anxiety in response to ordinary daily life events, suggesting a diminished threshold of alertness.

#### **4 The Neurocircuitry of Stress – Implications for Anxiety**

Stimuli such as stress and fear conditioning that require the assembly and integration of information from multiple sensory input-modalities are processed by limbic centers that regulate emotional behavior (amygdala and extended amygdala related to fear and anxiety) (LeDoux 2000, 2007), memory and learning (hippocampus)

and executive and cognitive functions (PFC) (Maier et al. 2006). Acting in a coordinated way, the intricate neuronal circuitries that characterize these networks establish a temporal and contextual framework of interpretation that determines the aversive/rewarding value of the particular stimulus. Characteristically, the modulatory action that limbic structures exert on the HPA is not conveyed by direct excitatory pathways; rather, information from the different limbic inputs are integrated through the modulatory action of neurons located in the hypothalamus or in the BNST (Herman et al. 1994, 2003); the latter, in turn, convey signals to the neurosecretory neurons of the PVN. More specifically, GABAergic neurons projecting from the BNST exert an inhibitory tone over the PVN, under the control of excitatory glutamatergic input originating in the PFC and hippocampus (Cullinan et al. 1993) and of an inhibitory GABAergic/CRF input from the central and medial nuclei of the amygdala (Herman et al. 2005; Prewitt and Herman 1998).

#### ***4.1 Structural and Functional Organization of the Amygdala***

The amygdala (Mandelkern or almond-shaped nucleus; Burdach 1819–1822), a deep temporal structure with rich interconnections with the hippocampus and PFC, has long been known to be involved in emotional behavior (Goddard 1964; Robinson 1963; Weiskrantz 1956). Lesions in the amygdala induce disturbances in emotional and social behavior, attention, and memory consolidation/extinction (Bucy and Kluver 1955; Weiskrantz 1956). The lesions in the original studies were later recognized to include the BNST, and recent work using more selective lesion and pharmacological approaches have pointed to the differential role of the amygdala and BNST in the control of emotional behavior (Davis 1992, 1998, 2006; Davis et al. 1997; Lee and Davis 2007a).

The early view that the amygdala is a relatively homogeneous structure has been challenged by more recent studies (De Olmos et al. 2004; Swanson and Petrovich 1998), that have proposed a new subdivision that takes into consideration connective, neurochemical and detailed anatomical data. Briefly, the following subdivisions can be recognized (Swanson and Petrovich 1998): (1) the caudal olfactory system (nucleus of the lateral olfactory tract, cortical nucleus and postpiriform and piriform-amygdalar areas), which displays the typical laminar arrangement of cortical areas and forms the caudal part of the piriform lobe and receive projections that originate in the main and accessory olfactory lobes (Alheid and Heimer 1988); (2) the frontotemporal system (lateral, basal and posterior nuclei), a ventral extension of the claustrum that forms part of the deepest cortical layers of the temporal, endopiriform and frontal lobes (Swanson and Petrovich 1998), that receives privileged information from several sensory modalities (somato-sensorial, auditory, visual) (LeDoux 2007), and is intricately connected to the hippocampus and the PFC (Akirav and Maroun 2007; Canteras and Swanson 1992; Petrovich et al. 2001) establishing a reciprocal modulatory influence over executive and mnemonic (Bishop 2007; McEwen 2007)

functions and emotional behavior (Akirav and Maroun 2007; Phillips and LeDoux 1992); iii) a specialized ventromedial expansion of the striatum (central [CeA] and medial [MeA] amygdaloid nuclei, and anterior amygdaloid area) that exerts an inhibitory output modulating basal activity of several brainstem and basal forebrain nuclei through the CeA nucleus, the main output of the amygdala, relaying processed information from the amygdaloid nuclei.

#### ***4.2 Structural and Functional Organization of the BNST***

Johnston (1923) initially described the BNST as a ventral extension of the pallidum, that forms a continuum extending from the olfactory tubercle and nucleus accumbens anteriorly and the amygdala posteriorly. It receives massive projections from adjacent areas including the amygdala through a bundle of fibers that forms the stria terminalis. In fact, the lateral and medial parts of the BNST form two corridors of sublenticular neurons that are contiguous with the CeA and MeA and have, therefore, led to their being designated as the central and medial extended amygdala (Alheid 2003). Extensive studies by Swanson and collaborators (Dong et al. 2001; Ju and Swanson, 1989a; Ju et al. 1989b; Swanson 1998) have resulted in different proposals about the anatomical organization of the BNST which is still under debate. The BNST are parceled into the major anterior and posterior divisions (relative to stria terminalis main fiber bundles); the former can be further parceled into medial and lateral groups. The medial division, which includes the anterodorsal and anteroventral areas (Dong and Swanson 2006a, b, c), is characterized by dense projections to hypothalamic regions that are implicated in neuroendocrine regulation; the lateral group of the anterior division (which includes the anterolateral area) is characterized by projections to hypothalamic areas concerned with autonomic and energy homeostasis as well as feeding behavior (Dong and Swanson, 2004).

The importance of the BNST in the activation of the HPA axis (Choi et al. 2007; Herman et al. 2003) may be appreciated when its anatomical connections with the neuroendocrine hypothalamus (Dong and Swanson 2006a; Dunn 1987) as well as with other brain regions such as the brainstem and ventral striatopallidal – all areas that regulate defensive, sexual, ingestive, and exploratory behaviors – are considered. Additionally, the BNST, along with the nucleus of tractus solitarius, preoptic area and dorsal hypothalamus, is one of the relay stations where inputs from stress-sensitive areas of the cortex and limbic systems are conveyed and integrated to elicit adequate activation of the HPA axis (Herman et al. 1994, 2003, 2005; Herman et al., 1997).

The role of the BNST in emotional behavior has been extensively explored by Davis and collaborators (Davis 1986, 1992, 1998; Davis et al. 1997; Lee and Davis 2007a). Although the phenotypic expressions of BNST and amygdala activities resemble each other closely, differences that confer distinct roles to each of these structures in the control of emotional behavior can be recognized. Involvement of the BNST is evident in paradigms in which behavior is influenced by long-duration

stimuli (e.g., CRF- or light-enhanced startle) and in paradigms that assess the persistent behavioral effects of even a brief unconditioned stressor (e.g., long-term shock-dependent increases in baseline startle, conditioned defeat, the effects of inescapable shock in the learned helplessness model or on subsequent eye blink conditioning) (Davis et al. 1997; Walker et al. 2003). Despite behavioral outcomes that are often similar in form (i.e., increased startle), the BNST does not appear to mediate behaviors elicited by specific short-lasting threats in which the period of endangerment is finite (i.e., fear-potentiated startle or freezing to a discrete conditioned fear stimulus) (Walker et al. 2003). These characteristics of BNST-dependent behavior suggest its special role in anxiety, as opposed to fear, insofar that anxiety, unlike fear, is typically viewed as a sustained state of apprehension that is unrelated to immediate environmental threats (Walker et al. 2003).

In summary, whereas the amygdala is transiently activated in fear conditioning (an emotional state elicited by explicit neutral clues), anxiety (a similar emotional state thought to be elicited by diffuse contextual clues) seems to result from persistent activation of the BNST (Davis et al. 1997).

### ***4.3 Structural Remodeling of the Amygdala and BNST – Implications for Anxiety and the Stress Response***

Mounting evidence indicates that the amygdala and BNST are sensitive to the chemical/humoral mediators of stress (Pego et al. 2008; Rubinow et al. 2007; Vyas et al. 2002, 2003). Different models of stress which increase the level of anxiety have been found to induce structural reorganization of neurons in the basolateral nucleus of the amygdala and in the anteromedial division of the BNST (Pego et al. 2008; Vyas et al. 2002, 2003). One of the most surprising observations in our own studies was the relatively good preservation of the morphology of the amygdala after exposure to chronic unpredictable stress, contrasting with a significant level of plasticity in the BNST. The absence of structural changes in the amygdala after chronic stress is consistent with our observation that chronic stress does not produce changes in fear-acquisition (Pego et al. 2008). Although such observations may seem to be at odds with previous reports (Vyas et al. 2002), they highlight the stimulus-dependent specificity of the neuronal circuitry responsible for generating anxiety (Dagnino-Subiabre et al. 2005; Gewirtz et al. 1998; Miracle et al. 2006; Rosen et al. 1998). The inescapability associated with the chronic immobilization stress paradigm used by Vyas et al. (2002) may trigger an emotional phenotype that results in the expression of fear responses rather than anxiety, explaining the stress-induced morphological changes in the amygdala observed by those authors; in fact, the same laboratory failed to observe anxiety when a chronic unpredictable stress paradigm was applied (Mitra et al. 2005; Vyas et al. 2002).

In fact, by playing a key role in the learning and long-term storage of fearful memories, the amygdala appears to be essential for the establishment of strong associations between overtly threatening cues (or contexts) and an aversive condition that must be avoided. This process represents a specific form of learning that provides biological advantage, inasmuch as it diminishes the probability of encounters with harmful/threatening events. Importantly, however, if this process were to be triggered by unspecific stressors experienced in daily life (the so-called “allostatic load” – see McEwen, 2003), survival would be jeopardized by an overactive “all-or-nothing” system. Thus, the refractoriness of the amygdala to certain stressful situations makes evolutionary sense. The BNST, on the other hand, is known to be involved in specific aspects of anxiety behavior (Walker et al. 2003). Consequently, the structural changes in the BNST observed after chronic stress correlate with the behavioral responses to stress.

## 5 Final Remarks

We have reviewed the organization and structure of areas of the brain involved in the coordination of the endocrine response to stress. Stressful events are perceived by limbic structures which generate a contextual image and integrate polysensory information. The amygdala and BNST play important roles in the assessment of emotional values and in the formation of fearful memories. Supporting this, recent data suggest that the structural changes that occur in these areas after exposure to stress hormones are probably correlated with anxiety behavior. Future research should focus on identifying the molecular processes and mechanisms that occur in amygdala and BNST, therefore extending our understanding of the neurobiological basis of anxiety and improving the therapeutic options for anxiety disorders.

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