Defensive emotional reactions and stress
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CHAPTER 1

GENERAL INTRODUCTION
Stress defines a state in which the physiological or psychological equilibrium of an individual is threatened (Chrousos and Gold, 1992; Selye, 1976). In presence of a threat, individuals of all species possess defense systems that allow them to survive. Clinical and ethological studies have established that the defense pattern of a given species is complex and its expression determined by the physical distance of the threat and the controllability of the situation (Blanchard et al., 1993; Fanselow, 1986). Among the defensive emotional reactions that many species possess, including humans, fear and anxiety are the most studied. Fear is the emotional response elicited by a proximal threat; it engages active behavioral reactions, such as fight or flight, depending on the nature of the threat. In contrast, anxiety is a state induced by distant and therefore potential threat; it engages protective reactions (Davis et al., 2010). Although fear and anxiety can have similar manifestation and are caused by threat, they differ in onset, duration and decay (de Jongh et al., 2003). They also have different neurochemical and neuroanatomical substrates (Davis et al., 2010). This thesis is divided into two independent parts. The first part will focus on the dorsolateral periaqueductal gray (dlPAG), which control innate response to imminent threat, and the origin of its dopaminergic innervation. The second part will examine the anatomical, neurochemical and behavioral aspects of defensive emotions, focusing on the basolateral amygdala (BLA) which is involved in anxiety behavior.
Panic is a reaction in the presence of a life threatening danger and is characterized by intense fear, strong autonomic arousal and escape behavior. In animal, this would typically be the defensive reaction elicited by the encounter with a predator (Hamm et al., 2014). In human, a panic attack is a brief and intense manifestation of this behavior in absence of real threat and constitutes the core symptoms of panic disorder (Hamm et al., 2014). Extensive evidence indicates that the dorsolateral column of the periaqueductal gray (dPAG) is critically involved in the expression of panic-like defensive behavior as shown in human and animal studies (Bandler & Shipley, 1994; Behbehani, 1995). In patients suffering from chronic pain, electrical stimulation of the midbrain tectum, that includes the dPAG, has been reported to cause intense fear accompanied by physiological manifestations such as palpitation, sweating and dizziness similarly to what is observed during a panic attack (Nashold et al., 1969; Young, 1989). Besides, the relative volume of the dorsal midbrain is larger in patients suffering from panic disorder compared to healthy controls (Fujiwara et al., 2011). Neuroimaging studies in healthy human subjects have shown an activation of the PAG during lactate-induced panic attacks or the presentation of proximal threats (Mobs et al., 2007; Reiman et al., 1989). In rat and cat, stimulation of the dPAG elicits intense motor and autonomic responses resembling the manifestations displayed during a panic attack in humans (Adams, 2006; Bandler, et al. 1985). Therefore the stimulation of the dPAG has become a valid and well-accepted model for provoking panic attacks or defensive behaviors (Deakin and Graeff, 1991; Jenck et al., 1995). Several types of neurotransmitters are involved in complex and subtle interactions in order to mediate the dPAG-induced defensive response. Glutamate and GABA, respectively the main excitatory and inhibitory transmitters, play a major role. Activation of glutamate receptors induces defensive and fear-related reactions while GABAergic transmission inhibits them (Bandler and Carrive, 1988; Bertoglio and Zangrossi, 2006; Carobrez et al, 2001; Fogaça et al., 2012). Glutamatergic and GABAergic transmissions in the dPAG are tuned by many neuromodulators, among which serotonin and cholecystokinin (CCK) are the most studied. Antidepressant drugs acting through 5-HT receptors are used to treat patients suffering from panic disorder, and in rat models serotonin has an impairing effect on panic-like behavior (de Oliveira Sergio et al, 20011; Graeff, 2004; Graeff and Zangrossi, 2010). On the other hand, CCK seems to exert a facilitation effect (Netto and Guimaraes, 2004). Recent evidence has shown the implication of other neurotransmitters such as endocannabinoids, nitric oxide or endovanilloids in dPAG-related defensive behavior (Fogaça et al., 2012). However dopamine is still missing in the picture, despite its presence in the dPAG and its role in the hypothalamus defense system (Kitahama et al., 2000 and 2007; Maeda et al., 1976 and 1985; Mansour et al., 1990). Anatomical and behavioral studies need to determine its role in dPAG-mediated defensive responses.
Stress is defined as a state in which the homeostasis of an organism is threatened by a physiological and/or psychological stimulus (stressor) (Selye, 1976; Chrousos and Gold, 1992). In response to stress, the organism engages physiological, behavioral and cognitive mechanisms to reestablish homeostasis and cope with the stressful situation (McEwen, 2007). The brain is the organ that orchestrates and coordinates those mechanisms, via the recruitment of different brain structures and neurotransmitters to allow the organism to cope with the stress (Joëls and Baram, 2009).

The stress response in the central nervous system

The stress response is engaged by two complementary systems, the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenocortical (HPA) axis (Fig. 1). Activated shortly after stress, the ANS enables the release of catecholamines (epinephrine from the adrenal medulla and norepinephrine from the nerve terminals), which will act throughout the body and brain to produce rapid and efficient physiological and behavioral responses to the presence of stressors (Chrousos and Gold, 1992). The HPA axis has a slower onset. Upon stress, the parvocellular neurons of the paraventricular nucleus of the hypothalamus (PVN) produce and release corticotropin-releasing hormone (CRH) and vasopressin (AVP). These peptides stimulate the anterior pituitary, leading to the release of adrenocorticotropic hormone (ACTH) into the blood circulation. Finally, ACTH stimulation of the adrenal glands cortex provokes the synthesis and release of glucocorticoids (cortisol in human, and corticosterone in rodents) which reach a peak in the plasma at 15-30 min after the initiation of stress and reach the brain about 30 min after beginning of the stressful event (Herman et al., 2003; Ulrich-Lai and Herman, 2009). Glucocorticoids promote the mobilization of stored energy and potentiate the mechanisms induced by the ANS (Ulrich-Lai and Herman, 2009). Importantly, glucocorticoids also regulate the termination of the stress response by containing the stress-induced mechanisms and exerting a negative feedback on the HPA axis (Herman and Spencer, 1998; Wang et al., 2013).
Noradrenergic system in the brain
The central noradrenergic system is composed of norepinephrine-containing cells principally located in the locus coeruleus (LC) (also called A6 noradrenergic cell group) in the brain stem, but also in other minor cell groups (A1, A2, A4, A5 and A7) located in the pons and medulla (Moore and Bloom, 1979; Morilak et al., 2005; Samuels and Szabadi, 2008a; Tully and Bolshakov, 2010). These discrete cell groups provide widespread noradrenergic projections throughout the entire brain and spinal cord, at the exception of the basal ganglia (Fuxe et al., 1970; Jones et al., 1977a and b; Ungerstedt, 1971). The activation of the central noradrenergic system by diverse stressors leads to the release of norepinephrine in the targeted regions, such as the hypothalamus and the limbic system (Abercrombie and Jacobs, 1987; Amaral and Sinnamon, 1977; Audet et al., 1988; Cecchi et al., 2002a; Morilak et al., 2005; Moore and Bloom, 1979). Similarly to other neuromodulators, norepinephrine is principally characterized by a volume transmission mode (Beaudet and Descarries, 1978; Fuxe et al., 2010; Sara, 2009). Once released in the extracellular space, it diffuses to its various targets where it binds on the appropriate receptors. The adrenergic receptors (adrenoceptors) are G-protein-coupled receptors that can be divided in two classes of receptors, the α- and β-adrenoceptors, each of them includes different subtypes: α1 and α2 within the class of α-adrenoceptors; β1, β2 and β3 for the class of β-adrenoceptors (Ahlquist, 1967; Marzo et al., 2009). The α1-adrenoceptors are located postsynaptically while α2- and β-adrenoceptors are found both in pre- and postsynaptic compartments (Marzo et al., 2009). The α1 subtype, coupled to a Gq protein, causes an increase of intracellular calcium concentration, through activation of the phospholipase C/inositol triphosphate pathway. The α2 subtype, coupled to a Gi protein, inhibits the...
production of cyclic adenosine monophosphate (cAMP), while the β subtypes enhance this production, resulting in the activation of protein kinase A (PKA), which in turn phosphorylates diverse proteins such as CREB (Marzo et al., 2009). In summary, α1- and the β-adrenoceptors exert excitatory effects on neurons, whereas α2-adrenoceptors generally have inhibitory effects (Marzo et al. 2009). Each of the pathways activated by norepinephrine modulates neuronal activity of the circuitry involved in generating adaptive stress response at the physiological, behavioral and cognitive levels (Gorman and Dunn, 1993; Stone and Platt, 1982; Morilak et al., 2005; Pacáck and Palkovits, 2001; Sved et al., 2002).

Glucocorticoids in the brain
Glucocorticoids are able to cross the blood-brain-barrier and enter the brain where they bind to two types of receptors of the steroid nuclear receptor family of transcription factors, the high affinity mineralocorticoid receptor (MR) and the low affinity glucocorticoid receptor (GR) (Groeneweg et al., 2012; Reul and de Kloet, 1985). While the GR is expressed ubiquitously throughout the brain with the highest expression in the PVN and hippocampus (Srinivasan et al., 2013), MR localization is restricted to the structures of the limbic system, principally the hippocampus, but also the prefrontal cortex and the amygdala (Reul and de Kloet, 1985). As a consequence of the difference in affinity for the hormones (MR has a 10-fold higher affinity for corticosteroids than GR), MR is occupied already at basal levels of corticosteroids, whereas GR is activated when the concentration of the hormone rises, during stress and ultradian peaks (de Kloet and Reul, 1987; Groeneweg et al., 2011). Thus, MR is believed to principally set up the threshold for the stress response while GR is directly implicated in the regulation of the HPA axis and recovery from stress (de Kloet et al., 1998; Groeneweg et al., 2012; Joels et al., 2008). In absence of their ligands, the receptors reside in the cytoplasm where they are associated with chaperone and cellular scaffolding proteins (Prager and Johnson, 2009; Pratt and Dittmar, 1998). Upon binding with the hormone, they are liberated from the molecular complex, and translocate as homodimers, heterodimers or monomers to the nucleus where they modulate gene expression by binding to hormone-response element (HRE) on the DNA or transcription factors (Beato and Sanchez-Pacheco, 1996; Datson et al., 2008; Derijk and de Kloet, 2008). The transcriptional activity of corticosteroids is regulated by the phosphorylation of the glucocorticoid and mineralocorticoid receptors by serine/threonine kinases (Chrousos and Kino, 2009; Ismaili and Garabedian, 2004; Krstic et al., 1997). In addition to its genomic effects that can require hours or even days to take place, corticosteroids also exert rapid effects (de Kloet et al., 2008). These effects, independent from protein synthesis, are involved in a large range of phenomena (from the suppression of the HPA-axis, regulation of synaptic neurotransmission to behavior) (Evanson et al., 2010; Groeneweg et al., 2011; Hinz et al., 2000; Srinivasan, 2013; Wong and Moss, 1994). Several studies suggested that those rapid actions are exerted through membrane-associated receptors (Groeneweg et al., 2012; Karst et al., 2010; Wang and Wang, 2009). There is now ultrastructural evidence showing that both GR
and MR are localized in dendritic spines and at post-synaptic densities (Johnson et al., 2005; Ooishi et al., 2012; Prager et al., 2010), indicating that membrane-associated GR and MR may participate to the rapid glucocorticoids-induced effects in the brain.

**Interconnections between the central noradrenergic system and glucocorticoids**

Although the central noradrenergic system and the HPA axis have different temporal dynamics and different locations, they are not independent from each other and there is extensive evidence demonstrating their strong anatomical and functional interconnections. Norepinephrine, the end point of the ANS in the brain, influences the HPA axis directly through the dense projections from the different noradrenergic cell groups to the PVN (Cunningham and Sawchenko, 1988; Petrov et al., 1993). These noradrenergic terminals make direct synaptic contacts on CRH-containing neurons (Liposits et al., 1986). Norepinephrine depletion provoked by brainstem hemisection has been shown to reduce CRH mRNA expression in those cells (Pacáck, 2000). Additionally, infusion of norepinephrine or β-adrenoceptor agonist into the PVN causes an increase in plasma corticosterone levels (Berkenbosch et al., 1981; Leibowitz et al., 1989). The brain noradrenergic cell groups also control HPA axis function via relay regions that they innervate, such as the areas of the limbic system or the bed nuclei of the stria terminalis (Cecchi et al., 2002b; Herman et al., 2005; Radley et al., 2008; Radley, 2012). On the other hand, the different noradrenergic cell groups, in particular the locus coeruleus, contain numerous GR through which glucocorticoids can restrain the noradrenergic response (Aronsson et al., 1988; Härfstrand et al., 1986; Kvetnansky et al., 1995; Markey et al., 1982; Pavcovich and Valentino, 1997). In addition to the inter-regulation of the ANS and the HPA axis, the end products of these systems, noradrenaline and glucocorticoids, work in concert within neurons and modulate their activity in order to generate an optimal stress response (Zhou et al., 2011; Krugers et al., 2011 and 2012).

**Stress response and anxiety**

**Anxiety**

Anxiety is part of the integrated response to stress and includes physiological, affective, behavioral and cognitive changes (Davis et al., 2010; Morilak et al., 2005). This defensive emotion, which arises in response to unpredictable threat, is adaptive and has been well conserved across species during evolution (Hovatta and Barlow, 2008). Anxiety increases the vigilance of individuals in unfamiliar and uncertain environments, and allows them to detect and avoid danger (Kalin and Shelton, 1989; Robinson et al., 2013). However in normal conditions excess of anxiety is maladaptive for it distracts rational thinking of the individual who will be strongly focused on his/her negative thoughts. In the most severe case, excessive levels of anxiety turn into long lasting debilitating state that can affect cognitive performance and produce inadequate emotional responses. There are different types of anxiety disorders (general
anxiety disorder, panic disorder, phobias, posttraumatic stress disorder), but there is often a co-occurrence of these different anxiety types in one individual (Robinson et al., 2013). These pathologies, which are the most common psychiatric disorders, are caused by multiple factors, including genetics, environment and early life experiences (Barlow, 2000; Hartley and Casey, 2013). Because of the high prevalence of anxiety disorders in the general population and its considerable impact on individuals and on the society, there is considerable interest from research to better understand normal anxiety as well as its pathological version.

Stress hormones and anxiety
In normal conditions, acute stress can induce or exacerbate anxiety response; on the other hand, severe or chronic stress exposure can lead to anxiety disorders (Grillon et al., 2007). Consequently, stress hormones, especially glucocorticoids and norepinephrine, are thought to be important players in mediating normal and pathological anxiety. In healthy subjects, stress-induced norepinephrine release in the brain via stimulation of the locus coeruleus, or pharmacological elevation of noradrenergic transmission facilitates anxiety-like behavioral and physiological responses (Bremner et al., 1996a and 1996b; Morilak, 2005; Tanaka et al., 2000). The link between glucocorticoids and anxiety is more complex. While the effect of chronic corticosterone exposure is clearly anxiogenic (Ardayfio and Kim, 2006; Corodimas et al., 1994; Korte et al., 1996), there are conflicting findings concerning acute corticosterone administration, which can be either anxiolytic or anxiogenic depending on the level of arousal or stress of the subjects (File et al., 1979; Grillon et al., 2011; Mitra and Sapolsky, 2008; Putman et al., 2007). Nevertheless, genetic studies have shown that dysregulation of GR often results in an impaired anxiety response or even anxiety disorders while overexpression of the gene increases anxiety (Gass et al., 2001; Rochford et al., 1997; Tronche et al., 1999; Wei et al., 2004 and 2012). Pharmacological experiments confirmed the facilitating role of GR and MR activation on anxiety-like behavior (Brinks et al., 2007; Calvo and Volosi, 2001; Kabbaj et al., 2000; Smythe et al., 1997). At the level of the noradrenergic system, α1-AR and β-AR mediate norepinephrine anxiogenic effects (Cecchi et al., 2002; Flavin and Winder, 2013; Schank et al., 2008). The α2A-AR autoreceptors exert tonic inhibitory control on noradrenergic transmission and their pharmacological activation produce anxiolytic effects (Millan et al., 2000; Schramm et al., 2001). In anxiety disorders, dysfunctions in the glucocorticoids and noradrenergic systems, such as abnormal levels of circulating hormones or reactivity of the locus coeruleus or the HPA axis, are often observed (Brunello et al., 2003; Goddard et al., 2012; Vreeburg et al., 2010; Yehuda, 2009).

Role of the amygdala
The amygdala or amygdaloid complex is an ensemble of interconnected nuclei located in the temporal lobe. It is involved in detection of threat and danger, attribution of emotional values to afferent sensory information from all modalities (auditory, visual, somatosensory, nociceptive...) and generation of adaptive physiological and
behavioral responses (Sah et al., 2003; Wang et al., 2013). Logically, this region is critically implicated for anxious states and behaviors (Davis et al., 2000; Ledoux, 2000; Maren and Quirk, 2004).Amygdala hyperactivity has been observed in patients suffering from different types of anxiety disorders (Davidson, 2002; Etkin and Wager, 2007). There is growing evidence from animal studies that specific circuitries in the BLA are involved in generating anxiety behavior (Hale et al., 2006; Sajdyk and Shekhar, 1997; Truitt et al., 2009; Tye et al., 2011; Wang et al., 2011). Anxiety levels strongly correlate with the complexity of BLA principal neurons dendrites (Adamec et al., 2012). Extensive work form Chattarji and colleagues has shown that chronic and acute stress induce changes in the morphology of BLA principal neurons by increasing the complexity of their dendritic arbors, and that these changes are associated with elevation of anxiety levels (Mitra et al., 2005; Vyas et al., 2002; Vyas et al. 2004). Glucocorticoids, at least partly, mediate these effects of stress on the amygdala, indeed chronic or acute corticosterone administration to male rats can also induce changes in BLA morphology and anxiety-like behavior (Mitra and Sapolsky, 2008). It was further shown that treatment preventing the alterations of BLA neurons morphology would also prevent the increase of anxiety levels (Mitra et al., 2009; Mitra and Sapolsky, 2010). Pharmacological studies also demonstrated that glucocorticoids signaling or noradrenergic activity in the amygdala was implicated in anxiety behavior (Shepard et al., 2000; Tanaka et al., 2000). However, more studies are needed to determine exactly how these stress mediators in the amygdala participate to the elaboration of anxious state.

Figure 2. Stress and glucocorticoids effects on BLA neurons morphology (from Roozendaal et al., 2011)
Stress-induced elevation of corticosterone levels decreases GABAergic transmission in the BLA, while the glutamatergic transmission is increased. These mechanisms lead to the formation of new spines that continues after the termination of the stress episode, and eventually to the increased of anxiety levels. Chronic or repeated stress results in enhanced remodeling of BLA neurons including synaptogenesis and dendritic growth.
Stress response and memory

Events that cause stress or are associated with punishment are better remembered. For instance, many people remember what they were doing on September 11 in 2001. This consequence of stress is highly adaptive, for it endows individuals with the capacity to remember important information (Roozendaal et al., 2009).

The amygdaloid complex mediates stress influence on memory

Because of its neurochemical feature and its unique connectivity, the amygdala can modulate emotional memories formation by processing stress signaling and interacting with other brain structures such as hippocampus and the prefrontal cortex (McGaugh, 2004; McIntyre et al., 2012). All the amygdaloid nuclei are richly innervated by noradrenergic fibers, principally originating from the locus coeruleus, and contain a high density of adrenergic receptors and glucocorticoid receptors (Asan, 1998; Fallon et al., 1978; Johnson et al., 2005; Pickel et al., 1974; Prager et al., 2010). A considerable amount of findings from pharmacological studies have established that the activation of the adrenergic receptors, especially the β-adrenoceptors subtypes, and glucocorticoids receptors are the means by which the amygdala, and particularly the BLA, integrates stress influence on memory processes (Ferry et al., 1999; Galvez et al., 1996; Liang et al., 1986; Roozendaal et al., 2009). Norepinephrine levels in the amygdala after inhibitory avoidance training correlate with subsequent memory of the task (McIntyre et al., 2002). Post-training infusion of norepinephrine or β-adrenoceptors agonist into the BLA enhances memory consolidation of several types of learning and memory tasks whereas blockade by β-adrenergic antagonist impairs it (Hatfield and McGaugh, 1999; LaLumiere et al., 2003; McGaugh et al., 1988). The cAMP-PKA pathway is involved in the β-AR actions on memory consolidation (Ferry et al., 1999). Similarly to norepinephrine, glucocorticoids facilitate memory formation in a dose dependent manner through the GR present in the amygdala, and specific lesion of the BLA blocks the memory enhancing effect of glucocorticoids (Abercrombie et al., 2006; Oitzl et al., 1998; Roozendaal et al., 2006a and 2009; Roozendaal and McGaugh, 1997). These effects on memory are mediated by genomic mechanisms and modification of gene transcription (Oitzl et al., 2001). However, a recent study, in which corticosterone conjugated to a membrane-impermeable bovine serum albumin molecule was used, suggests that GR non-genomic mechanisms also contribute to the modulation of memory consolidation via a membrane-associated GR (Roozendaal et al., 2010). Interestingly, this non-genomic pathway is also necessary for the several steps of the classical genomic pathways, such as CREB phosphorylation and chromatin modification (Roozendaal et al., 2010). Although, most of the findings concerning the action of stress mediators on memory have been discovered in animal models, there is now direct evidence from clinical studies of the importance of glucocorticoids, norepinephrine and β-adrenoceptor in integrating stress influence on memory functions (Segal and Cahill, 2009; Smeets et al., 2009; van Stegeren et al., 2005, 2006 and 2010).
Interaction between noradrenergic and glucocorticoids signaling in memory process

Norepinephrine and glucocorticoids work in concert in the amygdala to modulate memory functions. Evidence from animal and human studies have demonstrated that glucocorticoids effects on memory consolidation require emotional arousal-induced norepinephrine activity (Bryant et al., 2013; Buchanan and Lovallo, 2001; Okuda et al., 2004; Roozendaal et al., 2006b). A recent imaging study has shown that elevation of norepinephrine and corticosterone in healthy subjects modified the amygdala response to emotional stimuli (Kukolja et al., 2008). In rats, glucocorticoids-induced enhancement of memory consolidation is blocked by application of β-AR antagonist in the BLA (Quirarte et al., 1997; Roozendaal et al., 1999 and 2002). Glucocorticoids-induced impairment of memory retrieval and working memory also requires noradrenergic activity (Barsegyan et al., 2010; Kuhlmann et al., 2005; Roozendaal and McGaugh, 2010). The exact molecular mechanisms underlying the interaction between glucocorticoids and the noradrenergic system are not known, but evidence suggests that glucocorticoids might potentiate noradrenergic transmission and signaling (Fig. 2) (McGaugh and Roozendaal, 2002). In line with this idea, it has been demonstrated that a higher dose of clenbuterol, a β-adrenergic agonist, is necessary to enhance memory consolidation when a GR antagonist is infused into the BLA; and that systemic glucocorticoids administration increases norepinephrine levels in the BLA (McReynolds et al., 2010; Roozendaal et al., 2002). Recent findings indicate that endocannabinoids are necessary for the glucocorticoids action on noradrenergic activity (Atsak et al., 2012; Campolongo et al., 2009; Hill et al., 2009 and 2010). However these studies also showed that endocannabinoids effects also require arousal-induced noradrenergic activity. This indicates that the interaction between the two main stress mediators is complex and requires the participation of other neurotransmitter systems.

**Figure 3. Model of interaction between norepinephrine and glucocorticoids in the BLA (from McGaugh and Roozendaal, 2002).** Norepinephrine, released in the BLA as a result of stress-induced activation of the locus coeruleus (LC) and the nucleus of the solitary tract (NTS), activates adrenoceptors on BLA neurons. This in turn activates cAMP signaling. By bindings to their receptors in these neurons, glucocorticoids potentiate the adrenoceptors/cAMP cascade, leading to a change of BLA activity that will influence memory processing in other brain regions.
**Post-traumatic stress disorder**

Post-traumatic stress disorder (PTSD) is a psychiatric disorder that develops after exposure to an extreme trauma that provokes fear, helplessness or horror (American Psychiatric Association, 2000). It is characterized by three types of symptoms, which consist of re-experiencing of the traumatic event, avoidance and hyper-arousal. Re-experiencing corresponds to the revival of the traumatic event through intrusive thoughts, nightmares or flashbacks. Avoidance is characterized by the attempt of individuals to avoid everything that could remind them the traumatic experience. Finally, hyper-arousal is reflected by hyper-vigilance and exacerbated physiological response. Strong fear and enhanced autonomic responses are observed in many traumatized people, but in most of the cases, the stress response is terminated after some times and people recover. However in a small portion of individuals, the stress response becomes chronic and the symptoms persist over months and years (Parsons and Ressler; 2013; Yehuda 2004). The fact that only a minority of traumatized people develops PTSD highlights the importance of individual vulnerability and resilience in the development of this condition. Besides, this incapacity to terminate the stress response and to reinstate homeostasis suggests a critical role of the neuroendocrine stress system in the pathophysiology of PTSD. Accordingly, many studies have showed dysfunctions of the ANS and HPA axis and their endpoints, norepinephrine and glucocorticoids. Clinical studies have reported elevated baseline central norepinephrine concentration and higher noradrenergic activity in response to stimuli, mediated by the α2-adrenergic receptors (Geracioti et al., 2001; McFall et al., 1992; Southwick et al., 1999; Strawn and Geracioti, 2008). At the periphery, measure of circulating norepinephrine levels have yielded to conflicting results, with some studies reporting increased norepinephrine concentration while others showed no increase or even a decrease (Southwick et al., 1993; Strawn and Geracioti, 2008; Yehuda et al., 1998). PTSD patients also display higher responsiveness of GR and enhanced suppression of the HPA axis to dexamethasone challenge (Yehuda, 2009). The measures of circulating cortisol levels in PTSD patients show variable results (Yehuda, 2009). However, there is strong evidence that individuals who have low levels of cortisol at the time of the trauma have more risk to develop PTSD, suggesting that the alteration in the HPA axis is preexisting and therefore may be an actual risk factor (Delahanty et al., 2003; McFarlane et al., 1997; Yehuda, 2009; Zohar et al., 2011). Consequently, increasing the concentration of cortisol has been shown to reduce the incidence of PTSD in patients of intensive care units who are normally at high risk (Schelling et al., 2001 and 2004; Weis et al., 2006). These dysregulations of neuroendocrine systems are strongly associated with the re-experiencing symptoms. At the moment of the trauma, low levels of glucocorticoids associated with high noradrenergic activity are believed to considerably enhance the consolidation of the memory of the traumatic event, which produces re-experiencing symptoms. These abnormal hormones levels also contribute to the retrieval and reconsolidation of the traumatic memory and therefore prevent its extinction (Goswami et al., 2013; Milad et al., 2006). Consistent with this view, a recent study showed that elevation of cortisol levels in PTSD patients could reduce the
re-experiencing symptoms, providing further evidence that glucocorticoids are able to
weaken traumatic memories (Aerni et al., 2004; De Quervain et al., 2009). Cognitive-
behavioral therapies, and in particular exposure therapy which consists in reactivating
the dramatic memories and aim at inducing fear extinction, are particularly efficient
for treating PTSD patients (Foa, 2000 and 2006; Taylor et al., 2003). There is some
interest in the association of cortisol and exposure therapy, this is supported by recent
findings suggesting that administration of hydrocortisone could enhance the effect of
exposure therapy (de Klein, 2013; Yehuda et al., 2010). Given that the amygdala is a
target of stress hormones, a critical site for the modulation of emotional memories and
anxiety-like responses, this structure has been examined in PTSD patients. Several
clinical studies reported exaggerated amygdala activity in response to cues related
to the trauma (Shin and Liberzon, 2010). Enhanced activity of the amygdala has also
been observed in neutral conditions or even at rest (Bryant et al, 2005; Semple et al,
2000; Simmons et al., 2011). Interestingly, the intensity of amygdala activity before
trauma seems to positively correlate with the severity of the symptoms (Admon et al.,
2009; Pissiota et al., 2002; Protopopescu et al., 2005). Consistent with these findings,
a recent study examining Vietnam veterans, showed lower occurrence of PTSD in
individuals with amygdala damage (Koenigs et al., 2008).
PTSD is a complex disorder, in which pathological anxiety and pathology of memory
are interconnected. Multiple neurochemical mechanisms and neuronal circuitries are
altered in this disease, and among them, norepinephrine and glucocorticoids activity
in the amygdala seem to play a central role. Extensive evidence, as cited above,
demonstrated their implication in the pathophysiology of PTSD. And because of their
critical role in regulating the HPA axis and modulating emotional memory and anxiety
behavior, studying their interaction would bring considerable insight regarding the
etiology of PTSD but also allow developing efficient therapeutical strategies.
Outline of the thesis

The first part of the thesis consists of a single chapter (chapter 1) and will focus on the dorsolateral periaqueductal gray, which is a core structure in the brain system for generating defensive reactions to immediate and acute psychological or physiological threat. In this chapter, we will examine the distribution of dopaminergic afferent projections to this region and discuss a possible role of this neurotransmitter in the panic-like defensive behavior circuitry.

The second part of this thesis will investigate the anatomical and molecular basis of the interaction between norepinephrine and glucocorticoids in the BLA, and the long term effect of this interaction for anxiety and memory.

Norepinephrine activity in the BLA in interaction with glucocorticoids signaling modulates the consolidation of emotionally arousing memories. These effects on memory are mediated by β-adrenoceptors (β-AR) and glucocorticoids receptors (GR). Although the exact nature of this interaction remains unclear, there is evidence indicating that glucocorticoids might amplify the effects of norepinephrine on synaptic transmission within BLA neurons, suggesting that GRs and β-ARs are located within the same synapses. However, there is no anatomical evidence supporting the colocalization of these receptors within the BLA. In addition all the glucocorticoids effects on memory have been shown to require arousal-induced noradrenergic activity, suggesting that glucocorticoid signaling is first influenced by norepinephrine. In chapter 3, we examine the anatomical and molecular basis of the interaction between the noradrenergic system and glucocorticoids in the BLA. Using immunofluorescence and immunolabeling for electron microscopy, we investigate the ultrastructural distribution of β2-AR and GR in the BLA. We also investigate the consequences of β2-AR activation on GR phosphorylation status.

There is strong evidence that cortisol has beneficial effects in preventing PTSD, but also in reducing traumatic memories. These therapeutic effects are thought to be due to the role of glucocorticoids in the modulation of memory consolidation and memory retrieval. However little is known about the direct role of cortisol on anxiety levels in PTSD patients, although it is well established that glucocorticoid hormones influence anxious responses. Chapter 4 investigates the mechanisms of corticosterone influence on anxiety behavior, in respect to the temporal dynamics and the implication of the noradrenergic system and the HPA axis.

Learning and memory processes share common neurochemical and neuroanatomical substrates with anxiety. There is strong evidence that glucocorticoids modulate these two brain functions. However it is not clear whether the same corticosteroid-induced mechanisms affect memory processing and anxiety behavior simultaneously. In chapter 5, we investigate whether the corticosterone effects on anxiety observed in chapter 4 co-occur with effects on learning and memory performance. Further we examine whether those effects depend on the level of arousal of the memory task.

In chapter 6, the principal findings and conclusions of the studies are summarized and discussed. This chapter provides also perspectives for future research.
REFERENCES


