21 Stress Hormones in Psychophysiological Research: Emotional, Behavioral, and Cognitive Implications

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SCOPE AND PURPOSE

This chapter describes regulation of the stress hormones and their measurement and interpretation in psychophysiological research. A hormone is a signaling molecule, secreted by a gland that travels via the bloodstream to a distant target tissue and exerts an action on that target. A stressor, by its very nature, is an event that poses a real or potential threat to well-being. The resulting stress response engages numerous protective systems, including communication by hormonal messengers, to reduce the threat (Lovallo, 2016). Choosing a list of stress hormones is necessarily arbitrary since no hormone is active only during times of stress. For example, the primary stress hormone cortisol (CORT), serves normal homeostatic functions and it regulates the stress response (Munck, Guyre, & Holbrook, 1984). Similarly, the catecholamines, epinephrine (EPI) and norepinephrine (NE), are elevated during states of stress, but NE in particular is employed in normal physiological regulation. Other hormones, such as the sex steroids, may be affected by stress, but are not typically classified as stress hormones(Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004). Since physiology does not show us a clear boundary, we will confine this discussion to the core stress hormones, CORT, and the catecholamines.

A good example of this boundary problem is the case of the hormone oxytocin. Oxytocin was first identified as a posterior pituitary hormone that caused uterine contractions during the birth process and stimulated milk production afterward (Dale, 1909). Interestingly for stress and behavior, it was later found to act as a neuropeptide in the brain, where its actions reinforce parenting and social-affiliative behaviors in most species (Insel, 1992). In turn, parenting and social behaviors are responsive to stress (Weaver et al., 2004) and so are the actions of oxytocin (Grimm et al., 2014; Starr-Phillips & Beery, 2014). Oxytocin may buffer some acute and chronic effects of stress due to actions in the brain (Montag & Reuter, 2014; Smith & Wang, 2014; Tops, Koole, IJzerman, & Buisman-Pijlman, 2014; Uvnas-Moberg, Handlin, & Petersson, 2014), and peripheral oxytocin may be responsive to stress in girls exposed to early life adversity (Seltzer, Ziegler, Connolly, Prososki, & Pollak, 2014). This illustrates how oxytocin affects nurturing behaviors in the short term, with consequences for stress reactivity, and also affects subsequent parenting behaviors in offspring exposed to stress during development (Preston, 2013). However, for present purposes, changes in oxytocin actions during and after stress, and their programming of the stress axis, are properly seen as secondary to the core of the acute stress response. Moreover, the social effects of oxytocin are due to its peptide actions and not to its classic hormonal functions.

We will discuss CORT as central to the stress response and the catecholamines as secondary. CORT acts on all peripheral tissues, but it also crosses the blood–brain barrier, allowing it to regulate brain function during acute stress episodes and to influence behavior during future episodes. The CORT literature has grown rapidly over the past 35 years because of its impact on behavior and health. In contrast, the catecholamines do not cross the blood–brain barrier, although EPI influences the central nervous system indirectly by way of the vagus nerve (McGaugh & Roozendaal, 2002). Although we refer to the catecholamines as hormones, this designation is correct only for EPI, as discussed below. In leaving some topics out, we note that the stress literature is too large to allow a full review of endocrine effects during stress, even when confined to CORT and the catecholamines. Some topics will not be discussed in detail, such as stress response differences due to: sex (Kirschbaum, Wust, & Hellhammer, 1992; Turner & Weaver, 1985), age (Ehlert, 2013; Kudielka, Hellhammer, & Wust, 2009; Kudielka et al., 1998), obesity (Epel et al., 2000; Jessop, Dallman, Fleming, & Lightman, 2001; Nieuwenhuizen & Rutters, 2008; Steptoe, Kunz-Ebrecht, Brydon, & Wardle, 2004), diurnal cycles (Kudielka, Federenko, Hellhammer, & Wust, 2006), immune system function (Bauer, 2008; Cacioppo et al., 1995; McEwen et al., 1997), post-traumatic stress disorder (Schelling et al., 2006; Yehuda et al., 2010; Zoladz & Diamond, 2013), or depression (Herbert, 2013; Pariante & Miller, 2001). Similarly,

although the endogenous opioid beta-endorphin is secreted during stress and has both hormonal and neuropeptide actions, the interactions between opioids and glucocorticoids will not be discussed despite the increasing importance of the topic (Bodnar, 2014; Ducat et al., 2013; Lovallo et al., 2012b; McCubbin, Kaplan, Manuck, & Adams, 1993; Snyder, 1977; Van Bockstaele & Valentino, 2013).

HISTORICAL CONTEXT

The founding physiologist Claude Bernard noted that living things require a stable and nurturing environment to survive and remain healthy (Bernard, 1865/1927). Complex organisms have achieved this stability by evolving a protective skin to keep the external environment at bay while maintaining the internal environment, including the blood and interstitial fluid, within limits favorable to individual cells. Bernard also noted that stability of the internal environment calls for regulatory mechanisms to compensate for changes in the external environment. From this perspective, physiology is the study of how the nervous and endocrine systems regulate the organs of the body to mitigate changes in the external and internal environments.

Walter Cannon, the first professor of physiology at Harvard Medical School, studied these compensatory mechanisms and coined the term "homeostasis" to describe the physiological balance attained by their collective actions (Cannon, 1929, 1935). It naturally follows that if there is homeostasis, there are threats to that homeostasis, and also occasional failures to compensate for those threats. The founder of biological stress research, Hans Selye, explored the limits of these compensatory mechanisms by exposing animals to a wide range of severe insults, including extremes of heat and cold and exposure to toxic substances and pathogens (Selye, 1936). His most influential observation was that all severe challenges to homeostasis produced three unvarying signs: (a) swelling of the adrenal glands, (b) atrophy of the thymus gland and lymph nodes of the immune system, and (c) development of gastrointestinal ulcers. This three-armed response was associated with extremely high levels of corticosterone (the rat analogue of CORT), leading Selye to identify CORT elevation as the core component of the stress response.

The non-specific nature of the acute stress response is not confined to CORT. During times of severe threat the system moves into an emergency mode of action that Cannon called the "fight-or-flight" response that prepares us to fight an aggressor or flee to save ourselves (Cannon, 1929). The sympathetic nervous system (SNS) becomes highly activated during fight-or-flight episodes, and this leads to increased release of NE from sympathetic nerve endings and to secretion of EPI from the medulla of the adrenal gland. Because the fight-or-flight response engages the organism in such a deeply embedded and global fashion, researchers often study the integrated hormonal and SNS responses to physical and psychological stress as useful probes for the health and integrity of the individual (McEwen, 2007). By extension, individual differences in responses to stress are generally seen as useful markers of differences in risk for disease (Gianaros & Manuck, 2010; Kaplan et al., 1983).

Changing Perspectives on the Stress Response

Selye emphasized the fixed nature of the stress endocrine response (Selye, 1936). However, John Mason introduced a major shift in this thinking when he observed that CORT responses to a novel environment diminished with repeated exposures (Mason, 1968). This provided a background for the emerging concept of *psychological stress*, in which experience could modify the physiological response to an event. The stressfulness of an event was now seen as the result of the individual's history with the environment. This transactional idea set the stage for Richard Lazarus's influential model, in which stress reactions depended on how the person appraised (a) the potential threat value of an event followed by (b) an estimate of the available coping resources (Lazarus, Baker, Broverman, & Mayer, 1957; Lazarus & Folkman, 1984; Lovallo, 2016). This established the principle that events in daily life acquire their power as stressors in relation to the person's threat and coping appraisals.

The interaction of psychological processes and stress responses was amplified by the recognition that CORT itself could affect those same psychological processes. This insight dates from the key discovery by Bruce McEwen, Jay Weiss, and Leslie Schwartz that the hippocampus, amygdala, and cortex of the rat contain high densities of glucocorticoid receptors (McEwen, Weiss, & Schwartz, 1968). The same holds true for primates (Sanchez, Young, Plotsky, & Insel, 2000). Recent observations in humans show that circulating CORT, in the form of intravenously injected hydrocortisone, reaches the amygdala and hippocampus within 15 minutes and alters their activity (Lovallo, Robinson, Glahn, & Fox, 2010b). These effects of CORT on higher brain centers has opened up a vast territory for future psychophysiological research on the role of CORT on cognitive processes, temperament, and behavior (Buchanan & Lovallo, 2001; McGaugh & Roozendaal, 2002; van Stegeren, Roozendaal, Kindt, Wolf, & Joels, 2010).

PHYSICAL CONTEXT

Cortisol

CORT is the primary steroid hormone secreted by the cortex of the adrenal gland. Termed a *glucocorticoid* because it was first recognized as essential for glucose metabolism, CORT was later seen to influence all physiological processes and to be essential for general health

(Lupien, McEwen, Gunnar, & Heim, 2009; McEwen, 2015). CORT's actions are mediated by glucocorticoid receptors (GR) and mineralocorticoid receptors (MR), which are found in every cell type. Reul and de Kloet (1985) systematized CORT's respective actions via the MR vs. GR as *permissive* and *regulatory*. Accordingly, CORT is seen as permitting normal metabolic and diurnal functions to occur, via the MR, and also as regulating responses to stress, via the GR.

Metabolic and Diurnal Regulation of CORT

Daily secretory pattern. CORT secretion varies across the day and in relation to metabolic demands. CORT peaks shortly after awakening, declines gradually until sleep, and reaches a nadir in the early morning hours (Figure 21.1) (Czeisler & Klerman, 1999), with minor elevations occurring after the midday and evening meals (Van Cauter, Shapiro, Tillil, & Polonsky, 1992). This circadian cycle is driven by clock genes in the suprachiasmatic nucleus of the hypothalamus, which elevate CORT secretion during the waking period and diminish it during the sleep cycle (Leproult, Copinschi, Buxton, & Van Cauter, 1997). The changing level of basal CORT secretion throughout the day presents a challenge for the design of stress studies and interpretation of CORT data, as will be discussed below.

Transport, tissue compartments, and cellular actions. CORT is transported in the bloodstream, and it has access to all tissue compartments because it is both water and lipid soluble. It readily crosses the blood–brain barrier, allowing it to reach the neurons and glia of the CNS (Banks, 2012). It also diffuses across the choroid plexus into the cerebral ventricles, where it reaches the hypothalamus, hippocampus, amygdala, and medial prefrontal cortex (Mason, Pariante, Jamel, & Thomas, 2010). In exerting its physiological effects, CORT crosses the cell membrane where it binds to both GR and MR found in the intra-cellular fluid. After binding, the CORT–receptor complex is transported into the cell nucleus, where it regulates gene transcription, with effects on all cellular and systems function. CORT also has a rapid mode of action by which GR and MR in the cell membrane control neuronal excitability during states of stress (Groeneweg, Karst, de Kloet, & Joels, 2012). The interplay of rapid (membrane) and slow (nuclear) CORT effects is an emerging area with potentially important implications for prefrontal cortex and limbic system

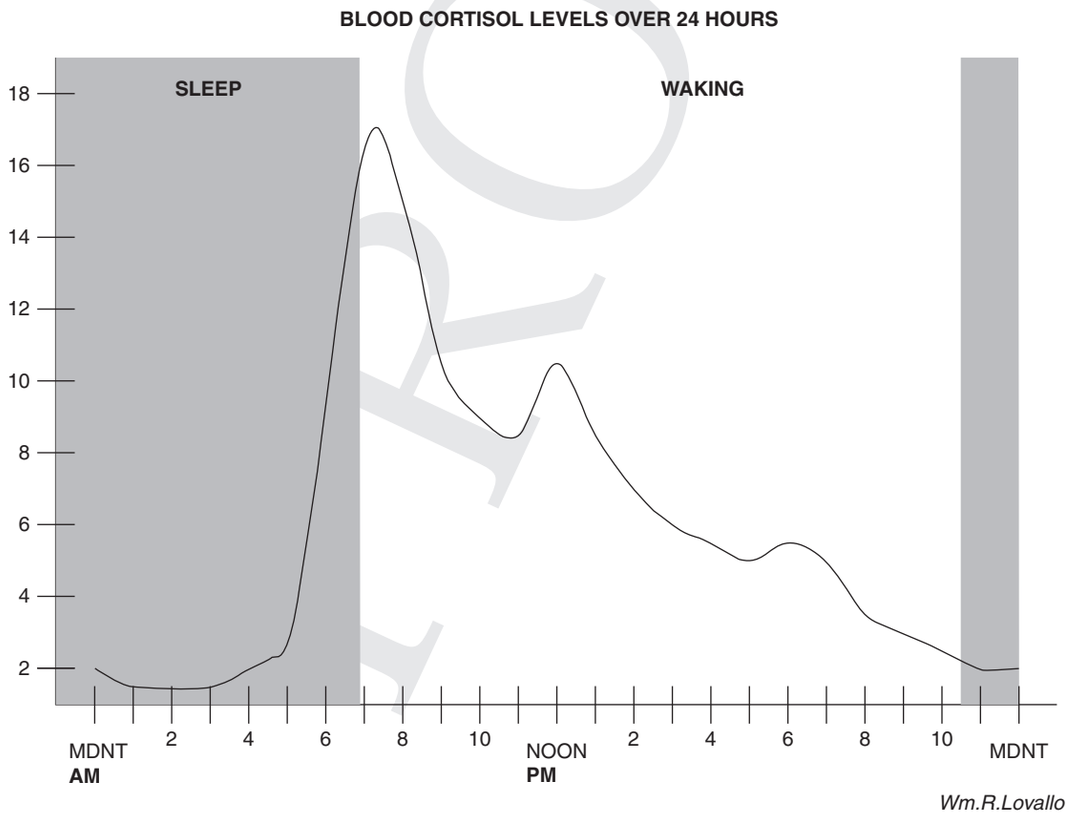

Figure 21.1 Cortisol's diurnal cycle. The daily cycle of cortisol release in normal sleepers is characterized by: (a) a nadir in secretion falling between 11:00 p.m. and 5:00 a.m., (b) a rapid rise prior to awakening with a post-awakening peak between 7:00 and 7:30 a.m., (c) a gradual decline across the waking hours, and (d) minor rises following the midday and evening meals.

Table 21.1 Effects of glucocorticoid dysregulation

Function	Deficiency	Excess
Global		
Mortality	+	+
Stress tolerance	–	+
Energy balance		
Appetite	–	+
Blood glucose	–	+
Weight	–	+
Autonomic regulation		
αand β receptor synthesis	–	0
Temperature regulation	–	0
Sodium/water balance	–	0
Blood volume	–	0
Blood pressure	–	+
Pressure regulation	–	0
Cardiac function	–	0
Vascular permeability	+	0
Immune and blood		
clotting	–	+
Red cell count	–	0
White cell count	+	–
Immune function	–	–
Thymus	+	–
Lymph nodes	+	–
Autoimmunity	+	–
Behavioral and CNS		
Exertion	–	0
Locomotion	–	0
EEG	0	–
Sensory threshold	–	+
Learning	–	–
Memory	–	–
Mood swings	0	+
Euphoria	0	+
Depression	0	+
Anger	0	+

Note: Symptoms of deficiency and excess of cortisol are taken from clinical observations of severe glucocorticoid dysregulation in patients with Addison's disease and Cushing's syndrome or who used high levels of steroid medications. These effects may differ from physiological responses to diurnal variations in cortisol or acute stress responses. Taken from Cake & Litwack, 1975.

responses during acute stress episodes and across the normal diurnal cycle (Joels, Sarabdjitsingh, & Karst, 2012; Lovallo et al., 2010b).

CORT binds to the MR with 10 times the affinity that it has for the GR. According to de Kloet and colleagues (Reul & de Kloet, 1985), the high affinity of CORT for the MR suggests that CORT–MR actions regulate normal metabolic processes across the day (Reul & de Kloet, 1986). In contrast, CORT–GR actions occur preferentially at the high levels of CORT seen during periods of stress. CORT's regulatory role accordingly counteracts cellular activation during a given stress episode, and it programs the hippocampus, amygdala, and prefrontal cortex to respond differently when faced with future psychological and behavioral challenges (de Kloet, Joels, & Holsboer, 2005a; de Kloet, Sibug, Helmerhorst, & Schmidt, 2005b).

Due to CORT's pervasive actions on gene expression, an excess or deficiency has severe consequences, as shown in Table 21.1 (Cake & Litwack, 1975). Too little CORT, as in Addison's disease, or an excess, as in Cushing's syndrome, have broad physiological consequences, including disturbances of cognition and emotion regulation (Starkman, Giordani, Gebarski, & Schteingart, 2003; Starkman, Schteingart, & Schork, 1981). A key physiological role for CORT is postulated to derive from its morning peak. Buijs and Goncharuk have proposed that the morning CORT spike coordinates gene expression across peripheral tissues and entrains their activity to the CNS in synchrony with the sleep–wake cycle (Buijs, van Eden, Goncharuk, & Kalsbeek, 2003). By extension, a flattening of the CORT diurnal cycle, particularly the loss of a sharp morning peak, is thought to diminish the precision of this entrainment, with a consequent loss of coordinated tissue function and a negative impact on health.

Feedforward and feedback control of CORT secretion. CORT secretion is regulated by the hypothalamic–pituitary–adrenocortical axis (HPA) as shown in Plate 33. The paraventricular nucleus (PVN) of the hypothalamus contains neurosecretory cells that synthesize corticotropin releasing factor (CRF) and arginine vasopressin (AVP). These neurosecretory cells send axons to the median eminence of the hypothalamus where their specialized terminals secrete CRF and AVP into the portal circulation of the pituitary stalk, which then carries CRF and AVP to the anterior pituitary gland (Vale, Spiess, Rivier, & Rivier, 1981). Here CRF regulates the cleavage of the precursor protein, proopiomelanocortin, into the endogenous opioid, beta-endorphin, and the pituitary hormone, adrenocorticotropin (ACTH) (Guilleman et al., 1977). In turn, ACTH is released into the systemic circulation whereby it travels to the cortex of the adrenal gland to increase the rate of CORT production and its release into the systemic circulation.

CORT release is restrained by exerting negative feedback at the pituitary and hypothalamus, as emphasized in Figure 21.2 (Jacobson & Sapolsky, 1991; Kovacs, Szabo, Sarnyai, & Telegdy, 1987). Circulating CORT reaches the pituitary, where it slows the rate of POMC synthesis, and also the hypothalamus, where it inhibits CRF production; both effects leading to less ACTH secretion by the pituitary and lower CORT release into the systemic circulation. CORT release varies across the day (Figure 21.1) in part because of changes in these feedback relationships. In the morning hours, the adrenal cortex is more sensitive to ACTH stimulation, and the pituitary is less sensitive to negative feedback, contributing to the morning surge in CORT secretion.

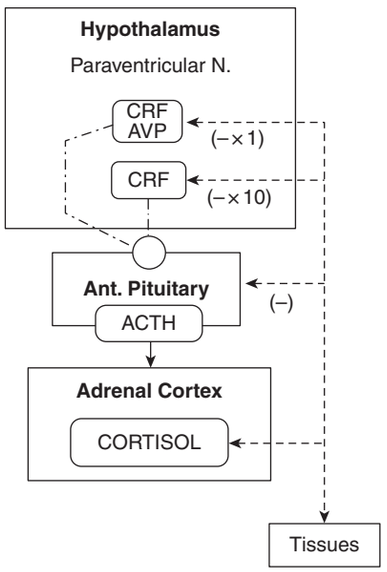

Figure 21.2 The HPA and negative feedback loops. The release of CORT into the systemic circulation results in actions on peripheral tissues and feedback to the pituitary and paraventricular nucleus of the hypothalamus. Here it exerts a greater negative feedback effect on CRF-only cells (–×10) and a much smaller effect on CRF/AVP cells (–×1).

Secretion during stress. The nature of CORT's release and feedback also changes when the system must switch from normal homeostasis to a stress mode of action, as proposed by Munck, Guyre, and Holbrook (1984). At normal times, the PVN uses CRF as the primary peptide hormone to stimulate the pituitary. During periods of stress, the PVN releases arginine vasopressin (AVP) along with CRF, and this combination stimulates about three times more ACTH release by the pituitary, resulting in very high release of CORT into circulation from the adrenal gland. The higher level of CORT secretion during stress is paralleled by diminished negative feedback sensitivity because CRF-AVP neurons respond less to feedback than CRF-only fibers. This shift from a normal homeostatic mode of regulation to a stress level of activity is useful for meeting short-term emergencies, but frequent or prolonged use of the emergency mechanism may have a cost in the form of negative health outcomes.

Interactions with forebrain structures. This classic model of stress activation and negative feedback regulation has been amplified by the finding that the HPA receives inputs from higher brain centers, in particular the hippocampus, amygdala, and medial prefrontal cortex (Swanson, Sawchenko, Rivier, & Vale, 1983). These forebrain regions are in turn regulated by CORT feedback. Initial studies showed that corticosteroid receptors were found in the hippocampus (McEwen et al., 1968) and that hippocampal activity varied with corticosteroid secretion across the diurnal cycle (Pfaff, Silva, & Weiss, 1971). The hippocampus was later shown to exert a tonic inhibitory action on the CRF neurons of the PVN (Joels, 2001), and destruction of the hippocampus in rats or monkeys caused prolonged CORT elevations (Joels, 2001; Sapolsky, Zola-Morgan, & Squire, 1991). Humans with medial temporal lobe damage involving the hippocampus show CORT abnormalities, including an absence of the morning CORT rise (Buchanan, Kern, Allen, Tranel, & Kirschbaum, 2004) and a loss of CORT response to psychosocial stress (Buchanan, Tranel, & Kirschbaum, 2009). Cortisol interactions with the hippocampus play an important role in memory formation and consolidation (Buchanan & Lovallo, 2001; Henckens et al., 2012) and retrieval (de Quervain & McGaugh, 2014). The amygdala is also affected by exogenous CORT, with acute administration causing diminished activation (Henckens, van Wingen, Joels, & Fernandez, 2010; Lovallo et al., 2010b) and long-term exposure leading to increases in amygdaloid reactivity (Shepard, Barron, & Myers, 2000).

The two-way communication between CORT and forebrain structures constitutes a form of of behavioral regulation over stress hormone release, with significant implications for understanding stress mechanisms and their impact on long-term behavior and health.

Stress Physiology and CORT Secretion

The HPA responds to both physiological and psychological stressors. Physiological stressors activate the HPA through reflex, or bottom-up, signals from the body such as cold, pain, or threats to homeostasis. Psychological challenges activate the HPA through top-down signals originating from forebrain structures, including the amygdala and prefrontal cortex (Brady, Porter, Conrad, & Mason, 1958; Davis, 2000; Lundberg & Frankenhaeuser, 1980; Mason, 1968; Rolls, 2015). This process is summarized in Figure 21.3. During periods of psychological stress, signals from the prefrontal cortex and limbic system act on the HPA and the SNS in parallel, releasing CORT and EPI into circulation (Figure 21.4). As a result, both arms of the stress endocrine system act in concert and reinforce one another.

Hormone secretion during psychological stress results from a complex of CNS events including: (a) interactions between the prefrontal cortex and the amygdala during the appraisal process, (b) the resulting experience of emotion and initiation of behavioral coping, and (c) autonomic and endocrine outputs from the hypothalamus, along with (d) descending signals to the brainstem and spinal cord. In considering the initiation of psychological stress and its effects on the stress hormones, we have noted that Lazarus and Folkman (Folkman, 1984; Lazarus & Folkman, 1984) postulate primary and secondary appraisals of an event's threat value and available coping resources. It is clear that these appraisals must involve interactions between the prefrontal cortex and the amygdala. The amygdala has extensive inputs from association areas of the cerebral cortex, and these convey sensory

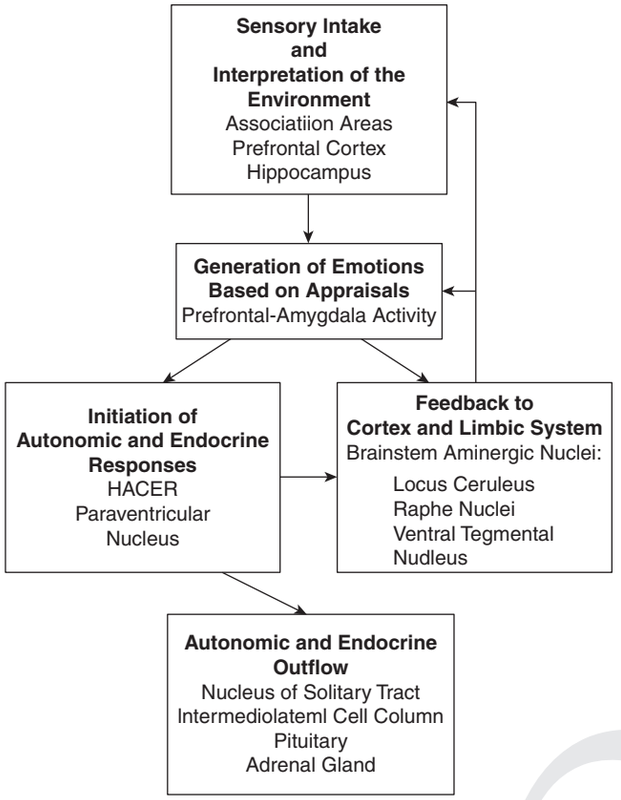

Figure 21.3 The generation of stress responses. The flowchart emphasizes major steps in generating HPA responses and sympathetic nervous system responses to environmental inputs. HACER (hypothalamic area controlling emotional responses) refers to lateral hypothalamic areas that receive inputs from the prefrontal cortex in the generation of cardiovascular responses during states of fight-of-flight (Smith, DeVito, & Astley, 1982).

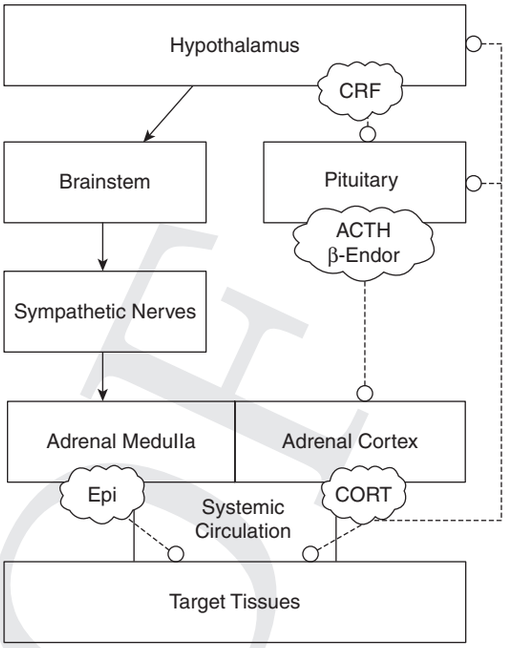

Figure 21.4 Parallel hypothalamic outputs during stress. HPA outputs leading to CORT responses during stress are parallel to outputs via the SNS causing epinephrine (EPI) release into the circulation.

information that has been enhanced by long-term memories (Amaral, Price, Pitkanen, & Carmichael, 1992; Halgren, 1992; Rolls, 2015; Swanson & Petrovich, 1998). Amygdala responses to these inputs may be innately programmed, such as young primates' innate fear of snakes, a response that is abolished by bilateral lesions of the amygdala, or they are acquired through Pavlovian conditioning, a learning process that requires an intact amygdala (Amaral, 2002; Davis, 2000). In turn, the amygdala and hippocampus interact extensively with the medial prefrontal cortex and anterior cingulate gyrus. These prefrontal–amygdala interactions ultimately serve in evaluation of threats and in shaping descending outputs to the HPA and brainstem in the course of stress endocrine secretion.

Figure 21.5 shows an expanded model of stress hormone regulation that emphasizes the role of higher inputs to the HPA and brainstem that depend on prefrontal–amygdaloid processing of sensory inputs. In the left of the diagram, the central nucleus of the amygdala sends CRF neurons to the lateral hypothalamus, the PVN, and the hippocampus. As shown in the lower left, CRF neurons from the PVN extend to the brainstem to act on: (a) the nucleus paragigantocellularis that then sends inputs to the locus coeruleus (LC), (b) the nucleus of the solitary tract (NTS), a center for autonomic integration, and (c) the intermediolateral cell column which sends SNS outputs to the body. This system of HPA and brainstem outputs is responsible for coordinated fight-or-flight responses.

The Central CRF System, Stress Hormones, and Behavior

Although the amygdala is essential for initiating psychological stress responses, the fight-or-flight state depends on an integrated systems response, and the central CRF system serves this purpose. As noted, CRF acts as a peptide hormone at the pituitary to stimulate ACTH production, but it is also a CNS neurotransmitter in a widely dispersed network of CRF-synthesizing neurons. This CRF neuronal network serves to tie stress mechanisms into a larger pattern of approach and avoidance motivation underlying adaptive behavior. The CRF fiber system stimulates activity in the prefrontal cortex, limbic system, and brainstem to organize CORT output during stress with autonomic outputs and behavior. This network also responds to CORT feedback at the PVN, limbic system, and prefrontal cortex (Swanson et al., 1983). The central CRF system has been described as "a widespread and complex system that appears to be dedicated, perhaps uniquely among chemically defined neurons, to a single task, the regulation and coordination of the body's endocrine, autonomic, metabolic, behavioral, and emotional responses to stressful stimuli" (Petrusz & Merchenthaler, 1992, p. 169).

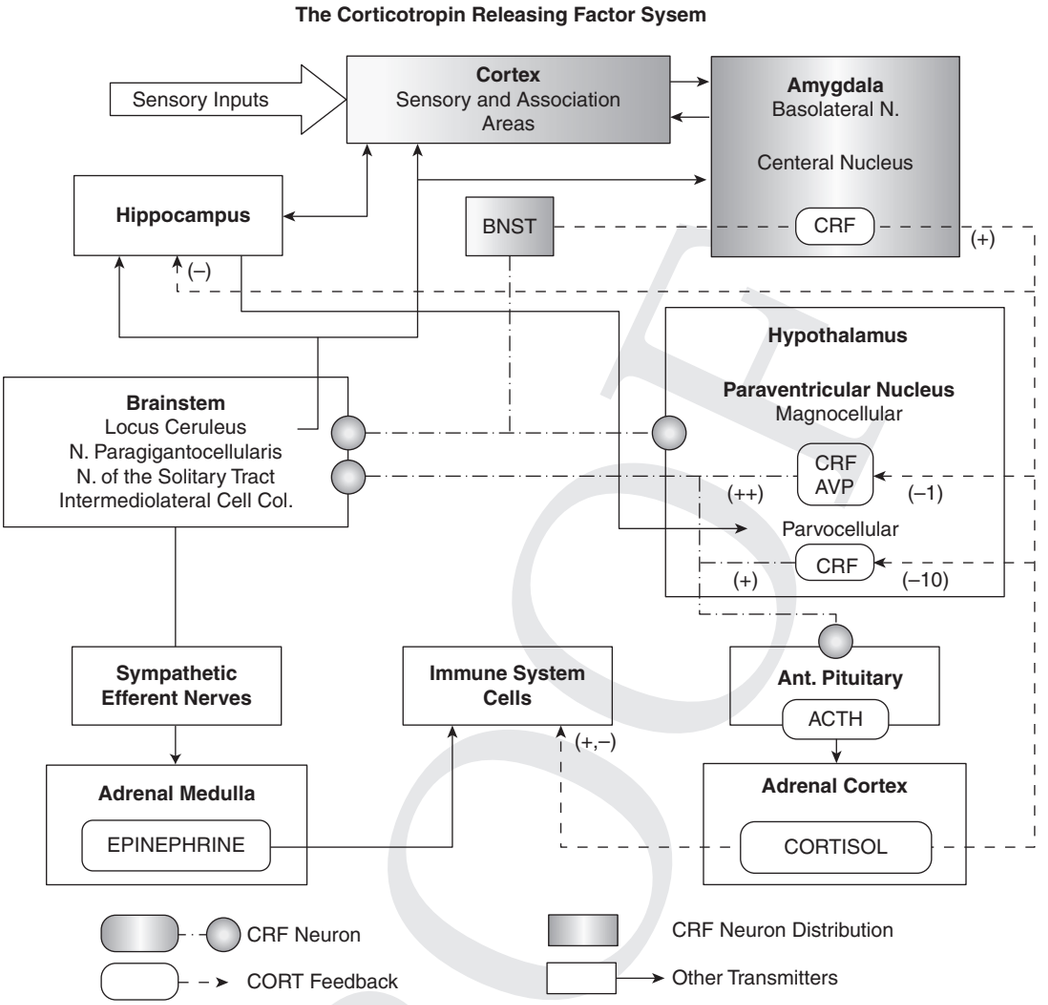

Figure 21.5 The corticotropin releasing factor system. The CRF neuronal system is heavily represented in the cortex, amygdala, and bed nucleus of the stria terminalis (BNST). CRF neurons from the central nucleus of the amygdala act on the PVN of the hypothalamus and on brainstem centers responsible for SNS outflow and on brainstem locus ceruleus that projects back to the cortex, hippocampus, and amygdala during states of stress, thus setting up a coordinated activational state of the CNS during states of fight-or-flight.

The largest aggregation of CRF fibers is in the PVN. Other major accumulations are in the prefrontal, cingulate, and insular cortices (De Souza et al., 1985) along with the central nucleus of the amygdala, lateral amygdala, septum, and hippocampus. As shown in Figure 21.5, CRF fibers travel from the PVN to the brainstem. One set of these fibers travels to the nucleus paragigantocellularis, the nucleus of the solitary tract, and the intermediolateral cell column of the spinal cord, which together integrate autonomic outflow on the basis of ascending visceral signals and descending outputs from prefrontal–limbic processes (Ennis & Aston-Jones, 1988). CRF fibers to the nucleus paragigantocellularis also activate the LC (Reyes, Bangasser, Valentino, & Van Bockstaele, 2014), which in turn alters the activational state of the entire CNS (Agnati, Bjelke, & Fuxe, 1992).

Injection of physiological levels of CRF into the cerebral ventricles results in integrated physiological responses including: increased ACTH release by the anterior pituitary (Rivier & Vale, 1985), increased firing of the LC (Valentino, Foote, & Aston-Jones, 1983), increased SNS nerve firing, increased EPI in circulation (Brown et al., 1982), a fight-or-flight cardiovascular pattern including increased blood pressure and cardiac output (Davis, 2000), and suppression of the parasympathetic nervous system (PNS). Activation of the central CRF system also evokes stress-related behaviors (Berridge & Dunn, 1989; Hilton, 1982; Sherman & Kalin, 1988; Takahashi, Kalin, Vanden Burgt, & Sherman, 1989) including decreased food intake and sexual activity (Petrusz & Merchenthaler, 1992). The central CRF system interacts with mechanisms of reward and dysphoria because of its

actions on dopaminergic neurons projecting to the striatum. This is thought to be a means by which stress mechanisms influence drug addiction and relapse (Boyson et al., 2014; Vranjkovic, Gasser, Gerndt, Baker, & Mantsch, 2014; Zorrilla, Logrip, & Koob, 2014).

Summary. CORT secretion is regulated during a normal day by the activity of the hypothalamus, the pituitary, and the adrenal cortex with additional participation of forebrain regions such as the PFC, amygdala, and hippocampus. This diurnal pattern of secretion is essential for normal metabolic functions in cells of the body, and its disruption has negative consequences for health. During exposure to physiological or behavioral stressors, the hypothalamus and amygdala are activated and utilize CRF neurons to stimulate the locus ceruleus in the brainstem, which in turn sets off a cascade of activity in noradrenergic neurons and other CRF neurons throughout the CNS. Noradrenergic and CRF activation of the prefrontal cortex, anterior cingulate gyrus, and reward centers, along with glucocorticoid feedback to these same areas, implies a deep involvement of the stress axis in long-term behavioral regulation, with implications for health.

The Catecholamines

The catecholamines, EPI and NE, are released in response to activity of the SNS during normal homeostatic function and during states of stress. NE is released by sympathetic nerve endings at the *sympathetic neuroeffector junction* to act on specific target tissues. EPI is released from the medulla of the adrenal gland to circulate widely during states of stress. The peripheral actions of the catecholamines are shown in Table 21.2. The actions of EPI shown at the top of the table indicate that it increases cardiac output and circulation of the blood to exercising muscles, along with improved airflow to the lungs and increased liberation of energy stores from fat and the liver. The actions of NE, shown at the bottom of the table, indicate that its primary actions are support of cardiorespiratory function and reduced blood flow to nonexercising muscle and visceral organs, along with increased sweating to improve cooling. Resting levels of NE have the following primary sources: lungs (30%), kidneys (25%), skeletal muscle (22%), liver (6%), skin (5%), heart (3%), and adrenal medulla (2%) (Esler, Hasking, Willett, Leonard, & Jennings, 1985).

Stress-Related Regulation of the Catecholamines

NE's responsiveness to many stressors, including exercise, cognitive effort, negative emotions, and immune system activation make its measurement a useful indicator of global SNS outflow. Accordingly, NE measured in circulation reflects its collective rate of release from all sympathetic nerve endings in the preceding minutes, minus its rate of removal (Blombery & Heinzow, 1983). Factors that increase NE entry to the circulation may simultaneously diminish its removal, and the influence of decreased clearance may not be trivial. During the heads-up tilt challenge, a potent SNS stimulus similar to standing from a supine position, increased circulating NE was 40 percent due to increased SNS nerve firing and 60 percent due to reduced renal clearance (Esler et al., 1988).

Tissue actions and distinction between EPI and NE and their hormonal vs. autonomic roles. The catecholamines act on target tissues via alpha- and beta-adrenoreceptors. EPI is primarily effective at beta-receptors and NE acts preferentially at alpha-receptors. In addition, alpha-adrenoreceptors are mainly innervated by the SNS and are found within tissues in smooth muscle and secretory cells. In contrast, beta-adrenoreceptors are generally noninnervated and are therefore located where they are accessible to the blood supply and circulating EPI. This receptor specificity and anatomical arrangement has implications for considering the relative status of NE and EPI as true hormones.

Although blood levels of NE are elevated during stress, this circulating pool is not acting on target tissues; the alpha-adrenoreceptors innervated by the SNS are not readily accessible to NE in the bloodstream (Esler et al., 1984; Hoyle, 1992). Most NE released by sympathetic nerves is taken back in by the SNS terminals, some of it is destroyed in the immediate environment, and the remaining 10–20 percent escapes into the systemic circulation (Esler et al., 1985). This picture is confused by the fact that intravenous injection of high doses of NE (4× to 5× normal physiological concentrations) will cause blood pressure elevations because such high concentrations can diffuse sufficiently from the bloodstream to reach innervated alpha receptors embedded in the blood vessel walls (Mazzeo, Rajkumar, Jennings, & Esler, 1997). Blood pressure responses to NE injections often lead to the misstatement that NE seen in circulation under physiological conditions is "acting" to maintain normal blood pressure or to elevate it during stress. From a practical perspective, the primary value of measuring NE in circulation is that it represents the collective integrated activity of the SNS nerves in the minutes before sampling.

Psychological causes of catecholamine secretion. Sympathetic output is sensitive to purely psychological causes. The two main determinants of SNS outflow that originates in higher brain centers are cognitive effort and emotional arousal. The influence of cognitive effort has been well demonstrated in studies of the effect of so-called "central commands" arising during exercise preparation, in which the pattern of peripheral cardiovascular adjustments mimics that seen during actual exercise (Hobbs, 1982; McArdle, 1967; Smith, Guyton, Manning, & White, 1976). The central commands for exercise originate in the anterior cingulate gyrus and prefrontal cortex and act on programs in the premotor cortex to alter hypothalamic and brainstem

Table 21.2 Tissue actions of the catecholamines

Tissue	Function	Receptor type
	Epinephrine, hormonal actions	
Heart	+ Contractility	beta-1, non-innervated
	+ Pacemaker frequency	"
	+ Conduction velocity	"
Arteries		
Skeletal muscle	+ Dilation	beta-2, non-innervated
Heart	+ Dilation	"
Veins	+ Constriction	"
Bronchioles	+ Dilation	"
Gut	– Motility	"
	+ Sphincter constriction	"
Spleen	– Contraction	"
Exocrine glands		
Parotid	– Protein secretion	"
Adipose tissue	+ Lipolysis	"
Liver	+ Glycogenolysis	"
	+ Gluconeogenesis	"
	Norepinephrine, autonomic neuroeffector actions	
Heart	+ Contractility	beta-1, innervated
	+ Pacemaker frequency	"
	+ Conduction velocity	"
Bronchioles	+ Dilation	"
Arteries	+ Constriction	alpha-1, innervated
Veins	+ Constriction	"
Gut	– Motility	"
	+ Sphincter constriction	"
Spleen	+ Contraction	"
Iris radial muscle	+ Contraction	"
Exocrine glands		
Sweat	+ Secretion	"

pattern generators that shape SNS outflow to the muscles and viscera (Mulert, Menzinger, Leicht, Pogarell, & Hegerl, 2005). Not surprisingly, emotions that call for escape or avoidance also evoke an exercise-like pattern of SNS cardiovascular adjustments in the absence of actual exercise. In a study of emotional imagery, subjects were asked to imagine a state of fear or a state of physical action with no affective component. Both instructions produced similar exercise-like cardiovascular adjustments (Sinha, Lovallo, & Parsons, 1992) that resembled changes following stimulation of the hypothalamic "defense center" in cats (Hilton, 1982). SNS outflow from the brainstem is therefore altered by non-stress-related cognitive intentions to exert effort or by negative emotions calling on fight-or-flight like mechanisms.

HPA and SNS Interactions

Actions of the catecholamines and the HPA complement each other during basal states and during periods of stress. CORT increases gluconeogenesis (the production of glucose from non-carbohydrate substrates) and glycogenolysis (liberation of glucose from glycogen stores) in the liver and muscle, thus complementing these actions by EPI (see

Table 21.2). The importance of CORT to support the exercise component of the fight-or-flight response was demonstrated in measurements in marathoners, who showed saliva CORT values that were elevated 5.6 times relative to a rest day (Harris, Cook, Walker, Read, & Riad-Fahmy, 1989). Other points of interaction are: (a) EPI enhances the HPA responses to stress, as illustrated by impaired stress tolerance after removal of the adrenal medulla (Selye, 1936); (b) administration of CRF by way of the cerebral ventricles increases both HPA and SNS activation (Irwin, Hauger, Brown, & Britton, 1988); (c) entry to the pituitary circulation by systemic EPI increases HPA activation (Proulx, Giguere, Lefevre, & Labrie, 1984); (d) beta-adrenergic neurotransmitter activity in the brainstem increases PVN secretion of CRF (Richardson Morton et al., 1990); (e) CORT supports catecholamine synthesis, and it maintains conformation of beta adrenoreceptors (both of which are permissive actions), improving their response to EPI (Davies & Lefkowitz, 1984); (f) CORT enhances the release and actions of catecholamines (Szabo, Hedler, Schurr, & Starke, 1988); and (g) CORT secretion and SNS outputs are both regulated in common at the level of the hypothalamic PVN.

SOCIAL AND PSYCHOLOGICAL CONTEXT: PSYCHOPHYSIOLOGICAL INTERACTIONS WITH STRESS HORMONES

Major Drivers of Psychological Stress Responses and the Stress Hormones: Threat, Controllability, and Effort vs. Distress

The two major themes in the study of psychological stress are: (a) the threat value of an event in relation to (b) the resources available to cope with the challenge (Folkman, 1984; Lazarus & Folkman, 1984). In a similar formulation, Seligman, Maier, and Solomon (1971) and Averill (1973) recognized that a lack of perceived control over a threatening challenge was a primary determinant of stress responses. In the context of the stress hormones, Ulf Lundberg and Marianne Frankenhaeuser at the Karolinska Institute in Stockholm formulated a theory of stress hormone secretion that incorporated the amount of distress (negative affect) the person experienced along with the amount of coping (behavioral) effort put forth in the situation (Lundberg & Frankenhaeuser, 1980). In their work, persons reporting effort expenditure (reports of effort, concentration, stimulation, and a lack of tiredness and boredom) alone had elevated catecholamine output, while persons reporting effort plus distress (distress, irritation, tiredness, and boredom) were likely to have elevated catecholamines plus CORT. Others in this same tradition distinguished a defensive, fear-driven "CORT factor" and a success-like "catecholamine factor" based on self-reports of men undergoing stressful military training (Ellertsen, Johnsen, & Ursin, 1977). Such evidence in human and animal studies from the field and laboratory suggests that successful cognitive and behavioral coping efforts, in the absence of fear or other negative emotions, will elevate NE preferentially to CORT and that coping efforts associated with negative affect will increase CORT along with catecholamines. These findings fit with a meta-analysis of laboratory stress studies demonstrating that the combination of uncontrollability, motivated performance, and social evaluation were conditions most effective in eliciting a CORT response (Dickerson & Kemeny, 2004).

Activation vs. distress in stress hormone release. In our research, we have observed the joint and independent impact of coping effort and the experience of distress on NE and CORT secretion. Male volunteers worked on two versions of a reaction time task in counterbalanced order during a single test session. In an explicitly distressing version invoking effort plus distress, slow responses were followed by annoying noise bursts or by occasional harmless electric shocks to the shin (N = 4). In the contrasting task that produced activation without distress, the subjects were asked to respond rapidly to the same number of unpredictable signals but were rewarded with $0.50 bonuses for rapid responses (Lovallo, Pincomb, Brackett, & Wilson, 1990; Lovallo et al., 1985). Self-reports to both tasks were high on Lundberg's (Lundberg & Frankenhaeuser, 1980) "effort" factor, while the aversive task alone was high on the "distress" factor. Both versions of the task produced high NE output, but only the aversive task resulted in a significant CORT response. A similar convergence of aversive experience and elevated CORT was seen using public speaking and mental arithmetic as stressors (al'Absi et al., 1997). We concluded that NE reflected primarily the cognitive effort to support motor preparedness, attention to the respond cue, and rapid responses. CORT, however, appeared to respond more specifically to the negative affect associated with threat of aversive stimulation.

In an explicit test, we measured CORT output while manipulating the aversiveness of the situation across three counterbalanced days in the same subjects using: (a) public speaking contrasted with (b) a humorous and heartwarming video relative to (c) a neutral rest day (Buchanan, al'Absi, & Lovallo, 1999). As expected, CORT was elevated on the speech day and, somewhat surprisingly, was lower than baseline on the positive video day (Plate 34). Self-reports of positive affect using the Positive Affectivity, Negative Affectivity Schedule (PANAS) (Watson, Clark, & Tellegen, 1988) were elevated on both speech and video days but did not predict CORT responses. In contrast, PANAS reports of negative affect were elevated on the speech day and diminished on the positive video day relative to the rest day (Plate 34, inset). These results support the idea that CORT is responsive to fluctuations in negative affect and not to states of activation per se.

Metabolic vs. Psychological Factors as Drivers of Stress Hormone Responses: Physical and Psychological Sources

The foregoing discussion indicates that CORT and the catecholamines respond differently depending on the subject's state of activation, coping resources, and the degree of distress induced by the situation. These differences are understandable if we take account of the respective metabolic demands associated with fight-or-flight responses undertaken for survival vs. the invocation of fight-or-flight like mechanisms in laboratory studies, where survival is not at stake. Fight-or-flight states use the SNS and circulating EPI to immediately increase cardiac output in order to maximize blood flow to exercising muscles, while minimizing blood flow to all other organs. Secondarily, CORT will rise when fuel stores in the exercising muscle are depleted and additional glucose must be liberated to augment these diminishing supplies. For example, we have observed during 15 minutes of increasingly intense exercise that catecholamines are elevated with no change in CORT (Sung, Lovallo, Pincomb, & Wilson, 1990), because this brief exercise duration does not require replenishing fuel stores in exercising muscle. The reason for this dissociation between NE and CORT seems to be that SNS adjustments are necessary under conditions requiring motor activity whether the motive is positive or negative. CORT appears to be differentially responsive to negative emotion states, and will appear during purely behavioral efforts only when metabolic demands call for increased glucose availability (Lovallo, 2016). It is noteworthy that some evidence suggests that EPI may be moderately more responsive than NE to states of distress (Frankenhaeuser & Rissler, 1970), although evidence for this difference is slight, possibly due to the difficulties of EPI sample handling and measurement.

The potential impact of psychological processes on CORT and NE release is illustrated particularly well by current laboratory research using stressors such as mental arithmetic, frustrating cognitive tasks, or public speaking simulations (Kirschbaum, Pirke, & Hellhammer, 1993). In such cases, the subjects are working on purely psychological challenges with minimal physical demands. Nonetheless, these challenges are distressing; they may involve social evaluation by the experimenter or an unpleasant degree of intense cognitive effort (al'Absi et al., 1997). These experimental conditions therefore result in: (a) a diminished level of control on the part of the subject, (b) a degree of uncertainty, a condition we associate with threat, (c) social evaluation, which we social creatures find potentially embarrassing, and (d) negative affect as a result of the first three conditions. The conditions of threat resulting from loss of control, social evaluation, and uncertainty result in elevated CORT and catecholamine secretion, along with cardiovascular adjustments, mimicking a genuine state of fight-or-flight (al'Absi et al., 1997; Kirschbaum et al., 1993; McCann et al., 1993; Sgoutas-Emch et al., 1994; Williams, 1982). Control studies that approximate the cognitive and motor demands of psychologically threatening tasks, but lack their uncontrollability, distress, and social evaluations, elicit no changes in CORT, but they may lead to increased SNS activity in the absence of HPA responses (Het, Rohleder, Schoofs, Kirschbaum, & Wolf, 2009; Wiemers, Schoofs, & Wolf, 2013). Studies outside the lab reinforce these findings. CORT varies along with acute fluctuations in negative affect in the ambulatory setting (Smyth et al., 1998), and it increases in response to daily stressors (Brantley, Dietz, McKnight, Jones, & Tulley, 1988), anticipation of a painful tooth extraction (Goldstein et al., 1982), or impending medical school exams (Francis, 1979; Malarkey, Pearl, Demers, Kiecolt-Glaser, & Glaser, 1995; Sausen, Lovallo, Pincomb, & Wilson, 1992). Chronic stressors may also affect CORT secretion; women having recently experienced a separation from a spouse or significant other had persistent evening CORT elevations during normal workdays (Powell et al., 2002). These examples call attention to the powerful influence of mental events on the physiology of the body. In considering the prefrontal–limbic processes that generate an HPA response to stress, it is worth recalling that these areas are also key targets of glucocorticoid feedback above the hypothalamus (Figures 21.3 and 21.4), and that these same areas are modifiable by experience. The following discussion focuses primarily on these themes.

Individual Differences in Stress Hormone Secretion: Selected Themes

One of the most active areas of psychophysiological research on stress hormones concerns the all-important topic of individual variation in stress reactivity. The forgoing material addressed general issues concerning stress hormone regulation and activation. However, endocrine responses to stress are not consistent across persons. Most theories of stress and its impact on health presume that individual differences in stress hormone response are important because large responses (a) may be damaging to the system or because they (b) indicate subclinical systemic dysfunction underlying increased risk. We have previously outlined a model of the potential sources of individual differences in stress reactivity, with an emphasis on health outcomes (Lovallo & Gerin, 2003). This model specified that persons may have larger or smaller responses to stress because of differences in: (a) psychological reactions to a situation, (b) characteristic degrees of activation at the level of the hypothalamus or brainstem, or (c) how the peripheral tissues respond to a given level of output from the endocrine and autonomic nervous systems.

Exaggerated and Diminished Stress Reactivity

Most research on stress reactivity and health outcomes assumes that large stress responses predict poor health outcomes (Treiber et al., 2003), and indeed there is evidence that this is true, particularly in the area of cardiovascular

disease (Everson, Kaplan, Goldberg, & Salonen, 1996; Hines, 1937; Menkes et al., 1989). This view assumes that stress exerts a form of wear and tear on the system and therefore that small reactions are preferable to large ones (Charvat, Dell, & Folkow, 1964). We recently expressed an alternative view, arguing that stress reactivity ought to be considered on a normal distribution, with a central mean, a normal deviation about that mean, and extreme values representing 95 percent confidence intervals at the high and low ends (Carroll, Lovallo, & Phillips, 2009; Lovallo, 2011). Considered in this way, stress reactions that fall within the mid-range of the distribution are "normative" by definition. Reactions at the high and low ends are, by definition, not normative and become candidates for predicting adverse health outcomes.

This line of thought stemmed in part from consistent findings that patients in treatment for alcoholism had diminished CORT responses to a range of stressors (Bernardy, King, Parsons, & Lovallo, 1996; Errico, Parsons, King, & Lovallo, 1993; Lovallo, Dickensheets, Myers, Thomas, & Nixon, 2000). Further investigation showed that healthy young adults with a family history of alcoholism also had reduced CORT responses to mental stress, particularly if they also had disinhibitory and antisocial tendencies (Sorocco, Lovallo, Vincent, & Collins, 2006). Other work has connected low levels of CORT reactivity to psychiatric manifestations: (a) Persons with posttraumatic stress disorder or victims of violence may have diminished diurnal CORT variation or reduced stress reactivity (Basu, Levendosky, & Lonstein, 2013; Bremner et al., 2003; Bublitz & Stroud, 2013; Kim et al., 2015), although not all studies are in agreement (Inslicht et al., 2006; Laudenslager et al., 2009). (b) Similarly, adult victims of bullying in the workplace display diminished diurnal CORT patterning (Hansen et al., 2006). (c) Flattened diurnal cycles also may be predictive of all-cause mortality (Kumari, Shipley, Stafford, & Kivimaki, 2011). Other work finds diminished CORT reactivity in relation to elevated pro-inflammatory cytokines, suggesting a connection with inflammation-related disorders (Kunz-Ebrecht, Mohamed-Ali, Feldman, Kirschbaum, & Steptoe, 2003). Women with irritable bowel syndrome may also have diminished CORT responses to mental stress (Suarez-Hitz et al., 2012), also suggesting an inflammatory or autoimmune connection.

This initial research on low levels of stress hormone reactivity provides some support for the idea that diminished as well as exaggerated stress reactivity may index altered systems regulation that predicts poor health outcomes, although high vs. low reactivity may predict different categories of disorders (Carroll, Phillips, & Lovallo, 2011; Fries, Hesse, Hellhammer, & Hellhammer, 2005).

Early Life Adversity: Altered Stress Hormone Reactivity and Behavior

The impact of early adverse experience is gaining attention in stress research. In our search for predictors of high vs. low CORT reactivity, we made an empirical search through a large database of CORT responses to combined public speaking and mental arithmetic stressors similar to the Trier Social Stress Test (Kirschbaum et al., 1993). The strongest predictor of HPA response in healthy young adults was the person's self-report of early adverse experience (Lovallo, Farag, Sorocco, Cohoon, & Vincent, 2012a). Persons reporting the most adversity had the smallest CORT and heart rate responses relative to a resting control day, implying a dual impact of adversity on HPA and autonomic outputs. Early adversity also predicted diminished CORT responses in studies by Linda Carpenter and others (Carpenter et al., 2007; Carpenter, Shattuck, Tyrka, Geracioti, & Price, 2011; Voellmin et al., 2015) and also altered diurnal patterns (Gonzalez, Jenkins, Steiner, & Fleming, 2009; Ranjit, Young, & Kaplan, 2005; Sjogren, Leanderson, & Kristenson, 2006). A systematic review found that early adversity may be associated with both enhanced and diminished reactivity (Hunter, Minnis, & Wilson, 2011). Future investigations attempting to establish the direction of this relationship will need to address problems of small sample sizes, differing subject populations, and varying definitions of adversity as key variables.

Altered stress reactivity becomes interesting insofar as it might predict changes in general behavioral tendencies, health behaviors, and health outcomes. Following the logic laid out in Figures 21.3 and 21.5, we reasoned that if diminished CORT reactivity to stress reflected altered prefrontal–limbic relationships due to adversity, then adversity should also predict changes in other domains including mood regulation, behavioral impulsivity, and risk for substance abuse, and this proved to be the case (Lovallo et al., 2013; Sorocco, Carnes, Cohoon, Vincent, & Lovallo, 2015). Persons exposed to adversity had greater mood instability, showed more rapid discounting of future rewards, along with impaired cognitive performance, and began drinking at an earlier age and were more likely to smoke and experiment with drugs (Lovallo, 2013). In keeping with this model of the impact of adversity on stress reactivity, others have reported hippocampal and amygdala changes (Hanson et al., 2015) as well as prefrontal cortical functional alterations (Gianaros et al., 2007; Sheridan, Sarsour, Jutte, D'Esposito, & Boyce, 2012) in children exposed to abuse and neglect or having come from low socioeconomic circumstances.

Changes in stress hormone reactivity, regardless of direction, therefore suggest that early adverse experience has a long-term impact on prefrontal–limbic processing of stressful events resulting in changed outputs at the level of the PVN and brainstem. Not surprisingly, theories of gene–environment interactions would imply differential vulnerability to early adversity depending on genotype, as some have reported (Caspi et al., 2002, 2003; Enoch, Steer, Newman, Gibson, & Goldman, 2010; Moffitt, Caspi, & Rutter, 2006). Reviews tend to agree that early adverse experience permanently alters behavioral and emotional

dispositions, with altered stress endocrine reactivity being one manifestation (Ehlert, 2013; Obradovic, Bush, Stamperdahl, Adler, & Boyce, 2010). Miller and Chen have commented that early life adversity leaves a "biological residue" in later life (Miller et al., 2009), with significant consequences for health behaviors and biological risk for disease.

CORT and Catecholamine Feedback to the CNS: Cognitive and Behavioral Effects with Implications for Stress Research

CORT influences on cognition and behavior. Most work on CORT as a stress hormone focuses on its secretion during stressful episodes. The second dimension of CORT's involvement in stress-related processes concerns its feedback to the CNS during and after acute stress episodes (Figure 21.5). Since the hippocampus, amygdala, and prefrontal cortex have CORT receptors, these structures should respond to CORT feedback, with implications for understanding the effects of stress on behavior. Studies of CORT's effects on memory and behavior have used three approaches: (a) examining the after-effects of CORT responses to stress in the lab, (b) testing persons with naturally occurring CORT excess or deficiency, and (c) systematically manipulating CORT effects by administration of synthetic corticosteroids, such as hydrocortisone.

CORT effects on cognition have been tested in humans by observing effects in persons with spontaneously high levels of stress CORT reactivity who are presumed to have greater levels of acute feedback to the brain (Wolf, 2009). Stress levels of corticosteroids resulting in occupancy of low-affinity GR in the hippocampus have three effects relevant to memory function: (a) temporary reduction in long-term potentiation, (b) suppression of normal hippocampal neurogenesis, and (c) potential atrophy of apical dendrites (McEwen, 1997). We measured CORT responses to a mental arithmetic stressor and found that persons with higher levels of CORT output committed more errors on the mental arithmetic task itself, although they had improved auditory comprehension on a subsequent dichotic listening task (al'Absi et al., 1997; al'Absi, Hugdahl, & Lovallo, 2002; al'Absi, Lovallo, McKey, & Pincomb, 1994). Others have shown that acute CORT stress responses result in poorer recall of word lists following a five-minute retention interval (Kirschbaum, Wolf, May, Wippich, & Hellhammer, 1996). Recent studies have emphasized the dynamic relationship between CORT feedback and memory by manipulating the timing of stress exposure in relation to tests of learning and retrieval (Schwabe & Wolf, 2013) in an attempt to separate CORT's rapid, membrane (MR) and longer-term, genomic (MR + GR) effects. A limitation of this approach is that cognitive effects associated with individual differences in spontaneous stress reactivity necessarily confound stress mechanisms with presumed feedback effects.

Human clinical research supports the effects of altered CORT secretion on cognition. Cognitive deficits and loss of hippocampal volume have been observed in naturally occurring states of CORT excess, including Cushing's disease, depression, normal aging with elevated CORT, and Alzheimer's disease (de Leon et al., 1988; Lupien et al., 1994, 1998; Starkman, Gebarski, Berent, & Schteingart, 1992). Elderly volunteers with high levels of CORT that trended upward over several years had impaired spatial and declarative memory associated with their degree of hippocampal atrophy (Lupien et al., 1998). Others have reported cognitive deficits and loss of hippocampal volume in adults with experience of childhood trauma due to physical or sexual abuse and warfare experience with presumed excess CORT secretion under stress (Bremner, 2005; Bremner et al., 1995). Studies of naturally occurring CORT deficiency, as in Addison's disease, have also found deficits on measures of memory and cognitive function (Henry, Thomas, & Ross, 2014; Schultebraucks, Wingenfeld, Heimes, Quinkler, & Otte, 2015). Most such studies are limited by small sample sizes due to the scarcity of suitable patient populations and the inability to systematically manipulate CORT exposure. Nonetheless, they point to the importance of CORT levels for maintaining normal cognitive function.

A third experimental model avoids the limitations of spontaneous alterations in CORT activity by administration of hydrocortisone (synthetic CORT) vs. placebo in healthy volunteers and observing effects on cognition and behavior. In a dramatic illustration of glucocorticoid effects on the brain, young rats exposed to high levels of corticosterone suffered loss of hippocampal neurons that mimicked the losses associated with old age (McEwen & Sapolsky, 1995; Sapolsky, Krey, & McEwen, 1985). Chronic exposure of the rat amygdala to high levels of corticosterone led to elevated gene expression in amygdaloid CRF neurons and long-lasting increases in anxiety behaviors and HPA responses to stress (Shepard, Barron, & Myers, 2003).

In human volunteers, oral administration of hydrocortisone (20 mg), chosen to mimic CORT values seen during stress, caused a non-specific diminution of the startle eye blink reflex, an effect potentially mediated by changes in amygdala inputs to the auditory pathway (Buchanan, Brechtel, Sollers, & Lovallo, 2001). We also found that this same hydrocortisone dose enhanced the long-term recall of emotionally salient material one week after initial exposure (Buchanan & Lovallo, 2001). In exploring the timing and targets of CORT feedback to the CNS, we found that a 10 mg intravenous injection of hydrocortisone began to depress activity in the hippocampus and amygdala 15 minutes post-injection (Lovallo et al., 2010b) suggesting that these may be the sites of CORT's immediate effects during naturally occurring stress. Others have made extensive investigations into the effects of corticosteroids and EPI on memory and cognitive function in animal models (Barsegyan, Mackenzie, Kurose, McGaugh, & Roozendaal, 2010; Introini-Collison & McGaugh, 1986; McIntyre & Roozendaal, 2007;

Zalachoras et al., 2013). This line of work is interesting for its basic neuroscience implications but also because it suggests ways of understanding the real-world effects of stress on the brain and behavior.

Catecholamine influences on cognition and behavior. In addition to glucocorticoid effects on cognition and behavior, EPI acting on peripheral beta-adrenoreceptors is able to influence storage and retrieval of declarative memories (de Quervain & McGaugh, 2014; McGaugh, 1983). Since EPI does not cross the blood–brain barrier, peripheral EPI effects must act via receptors on nerve fibers transmitting information back to the brain, and there is good evidence that beta-receptors on the vagus nerve serve this function. Vagal afferents project to the nucleus of the solitary tract, which extends rostrally to the hypothalamus and sends projections to the PVN and the basolateral amygdala (McGaugh & Roozendaal, 2002). Pharmacologic manipulation of this afferent beta-adrenergic receptor system affects discriminations learned under aversive circumstances (Liang, Juler, & McGaugh, 1986) such that EPI effects are antagonized by beta antagonists.

Since EPI is elevated in the bloodstream specifically during periods of fight-or-flight, and because the afferent vagal fibers in question appear to have primary effects on the PVN and amygdala, it is reasonable to ask if EPI is effective primarily in the storage and retrieval of aversive and traumatic events (Hermans et al., 2014). Emotional arousal alters functional connectivity between the amygdala and hippocampus, suggesting a pathway by which stressful experience may influence encoding and storage of emotional events (Fastenrath et al., 2014). Since hydrocortisone administration also increases memory for both positive and negative emotional material (Buchanan & Lovallo, 2001), it is potentially the case that glucocorticoids and EPI work together in the formation of emotional memories during periods of stress (Atsak et al., 2015).

Sex Differences: Sex Steroids and CORT Secretion

Women may have significant differences from men in CORT secretion during stress, and evidence points to a sex difference in the endogenous opioid system along with variations in sex steroid hormones occurring over the menstrual cycle and across the lifespan. By extension, the effects of these sex differences on mood and cognition have attracted recent attention.

CORT secretion across the day is equivalent for men and women, as shown in studies carrying out repeated sampling from waking to bedtime (Kudielka et al., 2009; Lovallo, Farag, & Vincent, 2010a) (Figure 21.6). This comparability of diurnal secretion implies that fundamental HPA regulation, including adrenal sensitivity and feedback at the pituitary and hypothalamus, is similar in men and women. On the other hand, CORT reactivity to psychological stress consistently shows smaller responses in women compared to men (Kudielka et al., 2009; Lovallo et al., 2010a). There are two major sources for this difference, ovarian hormones and differences in endogenous opioid function. Stress CORT release is higher in postmenopausal women than in premenopausal women (Kudielka et al., 2009; Saab, Matthews, Stoney, & McDonald, 1989), and CORT responses in saliva are smaller in women using hormonal contraceptives or when tested during the follicular phase of the menstrual cycle, when estradiol levels are high and progesterone levels are lower (Kudielka et al., 2009). This blunted response is seen in saliva samples, which contain only CORT, representing about 5 percent of the total, but this blunting is not seen in CORT obtained from blood samples, where approximately 95percent is bound to CORT binding globulin (CBG) (Kumsta, Entringer, Hellhammer, & Wust, 2007). CBG levels vary as a function of the menstrual cycle and with hormonal contraceptive use (Wiegratz et al., 2003), and as such hormonally linked CBG variations may affect estimates of saliva CORT responses.

Hormonal fluctuations may not account entirely for sex differences in CORT stress reactivity. We have compared men and women on their CORT responses to the mu-opioid receptor antagonist, naltrexone. The HPA is under partial control of endogenous opioids that diminish CRF release at the PVN. Blocking this opioid effect unmasks underlying levels of HPA activation (Mendelson, Mello, Cristofaro, Skupny, & Ellingboe, 1986) leading to a CORT response, and larger CORT responses should indicate greater degrees of endogenous opioid activity. Accordingly, we tested CORT responses to oral naltrexone (50 mg) in men and women in a double blind, placebo-controlled, crossover trial with serial saliva samples taken over 180 minutes. Women had CORT responses to naltrexone that were approximately four to five times larger than in men (Lovallo et al., 2012b) in agreement with other studies showing larger responses in women (Klein et al., 2000; Roche, Childs, Epstein, & King, 2010). This suggests that women have a higher level of basal opioid tone, with a greater endogenous mu-opioid restraint on the HPA in comparison to men. Endogenous opioid activity may therefore act in concert with menstrual cycle variation to contribute to sex differences in CORT responsivity (Roche & King, 2015; Roche, King, Cohoon, & Lovallo, 2013). Women also have smaller catecholamine responses to stress than men (Frankenhaeuser et al., 1978; Johansson & Post, 1974), also in keeping with opioid mechanisms.

The differences between men and women in CORT reactivity to stress may also result in differences in CORT feedback. This raises questions for the role of long-term changes in free CORT availability as a function of menarche and menopause (Lupien et al., 2009). This topic has been reviewed extensively (Herrera & Mather, 2015).

INFERENTIAL CONTEXT: ISSUES IN RESEARCH DESIGN AND METHODS

Psychophysiological investigations incorporating stress hormone measurements will most often be concerned

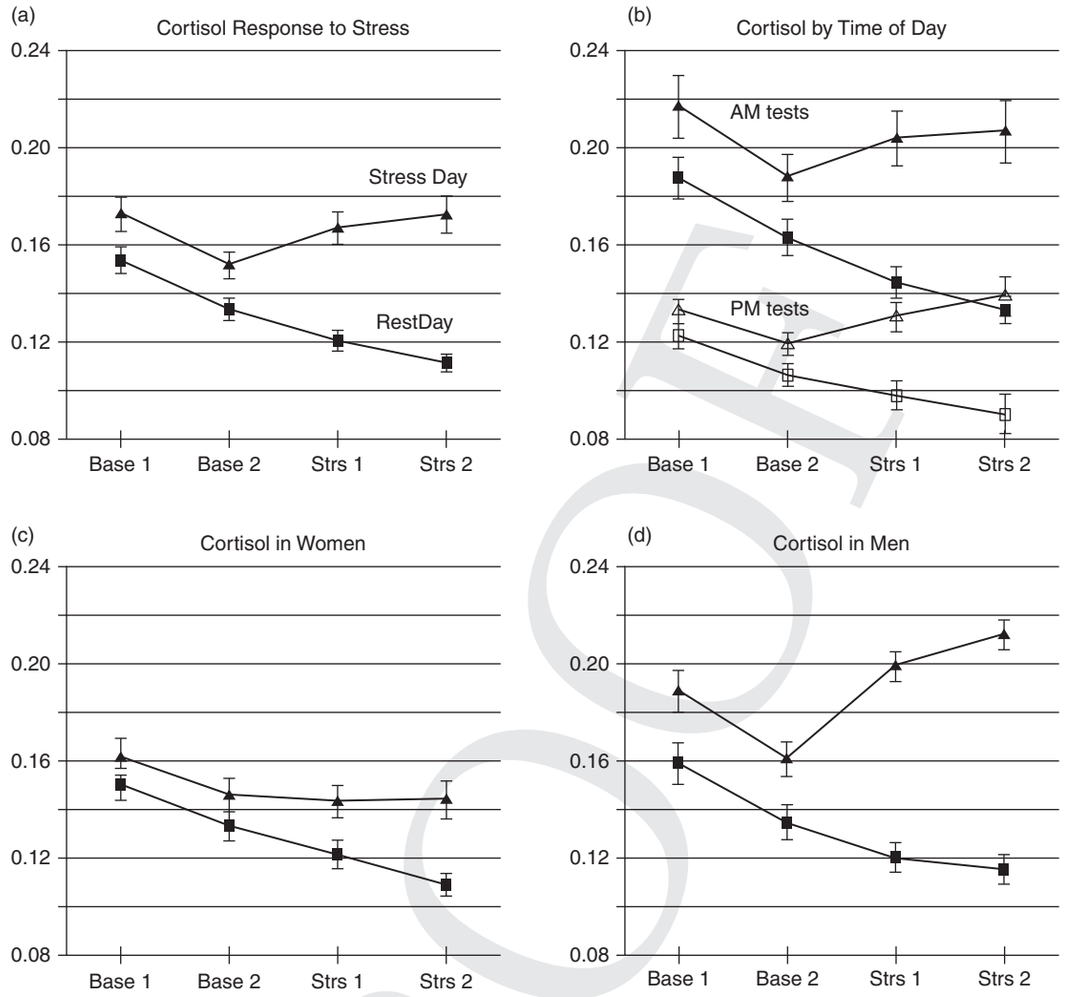

Figure 21.6 CORT responses to stress relative to a resting control day. Panel A: Stress response relative to control measurements in healthy young adults (N = 324). Stress day was the first day in the lab, mimicking a typical stress study in which the stress day is the only time the subject visits the lab. Note the anticipation effect during the Base 1 and Base 2 samples on the stress day, reflecting exposure to a novel environment and anticipation of a stress procedure. Panel B: The effect of time of day of testing on HPA reactivity. Persons tested in the morning between 9:00 a.m. and noon (N = 155) have larger CORT responses than persons tested in the afternoon from 1:00 p.m. to 4:00 p.m. (N = 169). Panels C and D: CORT responses in women (N = 187) and men (N = 137) (Lovallo et al., 2010a).

with measuring hormone changes from a defined baseline in response to a behavioral, emotional, or cognitive manipulation. In many instances, the goal will be to compare stress responses from one group to another in the interests of answering a question about individual differences. As the previous discussion suggests, the choice of a stressor that manipulates varying levels of effort, controllability, and distress might determine whether the subjects produce high levels of catecholamine output, cortisol output, or both. Stressors are not all created equal, and the choice of a stressor challenge to be used in the lab should be based on how well the stressor fits with the anticipated endocrine response.

What follows is information on methods in stress hormone research based on published work and our own labs' experiences in such investigations. This sort of methodological discussion is always a blending of art and science, and our recommendations should be considered in light of the reader's own experience and the unique circumstances prevailing in a given research project. Finally, since psychophysiologists will usually find themselves working with an endocrinologist or clinical chemist in carrying out assay work, information in this section is intended to be an aid to communication with such collaborators. As a general practice, in using blood or any other specimen source, the investigator is advised to establish *in advance* a collaboration with a laboratory that will carry out the assays in order to follow that lab's protocol for sample collection, preparation, storage, and shipping. These procedures *must be adhered to* in order to properly match the

resulting sample to the specific requirements of the assay in question and the practices of the lab carrying out the assay.

CORT

CORT is the hormone of choice for studying psychological stress responses and individual differences in stress reactivity. CORT in circulation represents the fraction bound to CBG and albumen (90–95 percent) (Perogamvros, Ray, & Trainer, 2012) and the unbound 5–10 percent that is free to act on target cells (Faix, 2013). In contrast, CORT in saliva and urine represents only the biologically available, unbound fraction. Sample sources and assay methods to be used will depend on the experimental question and how it is best addressed in relation to CORT's secretion, clearance, and diurnal phase.

Collection and Measurement in Blood, Saliva, Hair, and Urine

Blood. Blood is a useful specimen source in studies where the goal is to measure total CORT content, both bound and unbound, or to note changes in total content due to acute stress. It is also possible to measure free CORT in blood using assays that are capable of separating and measuring bound from unbound fractions (Faix, 2013). An additional consideration is that men and women differ in the proportions of bound and unbound fractions in blood, a factor that will need to be taken into account when comparing sex groups. Estrogen variation across the menstrual cycle is a source of varying CBG binding and cycle phase will affect measurements of unbound CORT in women (Perogamvros, Aarons, Miller, Trainer, & Ray, 2011). Blood sampling has several drawbacks in behavioral studies of stress. A blood draw will require venipuncture, for a single sample, or intravenous catheterization for repeated sampling, as called for in most stress protocols. Venipuncture itself is stressful, and is therefore an experimental confound (Weckesser et al., 2014), and it requires specific training, limiting its use in labs lacking the necessary personnel. The use of sharps in blood sampling is a safety hazard. CORT can also be assayed from small quantities of blood obtained by a finger stick (Fryer et al., 2014). Handling and processing any quantity of blood requires attention to universal safety precautions (see www.OSHA.gov).

Saliva. Saliva currently is the sample source of choice for CORT assessment in behavioral studies (Kirschbaum & Hellhammer, 1989). Sample collection is non-invasive and it can be precisely timed and therefore tied in to laboratory protocols or related to the occurrence of discrete events outside the lab. In addition to its well-documented use in adults, saliva can be used successfully to measure CORT values in infants (Francis et al., 1987; Read & Riad-Fahmy, 1992), and sampling is well accepted by older children (Bauer et al., 2011). Extensive validation work was done to establish that saliva contains the unbound, bioavailable, fraction of circulating CORT, since this fraction readily enters the saliva through the salivary glands (Riad-Fahmy, Read, & Walker, 1983). Saliva values correspond well with circulating CORT (Harris, Read, Walker, & Riad-Fahmy, 1988; Harris et al., 1990). A frequent question concerns whether CORT values are affected by saliva flow rates. Extensive testing over a wide range of stimulated and unstimulated saliva flow rates showed that the relationship between saliva and blood values was unchanged (Riad-Fahmy, Read, Walker, & Griffiths, 1982). Tests show that CORT entering the bloodstream arrives in the saliva with a five-minute time lag, indicating a good temporal relationship between these sample sources. Saliva collection is not stressful for the subject. Samples are readily collected by passive drool into a plain collection tube, or by using a commercial collection device, such as the Salivette (www.sarstedt.com) (Walker, Robinson, Roberts, Ford, & Riad-Fahmy, 1990). The Salivette is a plastic storage tube containing an internal plastic carrier holding a cellulose pledget. The pledget is held in the mouth until saturated with saliva and then placed in the carrier-storage tube until processing. The entire device is designed for low-temperature storage and for placement in a centrifuge during sample extraction from the pledget. Salivettes can readily be carried by the subject for collection of samples outside the laboratory (Kudielka, Gierens, Hellhammer, Wust, & Schlotz, 2012). Although cotton pledgets do not affect cortisol determinations, they interfere with analysis of other substances, including alpha amylase, gonadal hormones, and immune markers (Shirtcliff, Granger, Schwartz, & Curran, 2001). Some writers have cautioned against collecting saliva samples immediately after brushing the teeth out of concern for entry of blood cells into the saliva. However, tests of saliva containing blood cells due to tooth brushing have shown that cortisol values are not affected (Kivlighan et al., 2004). Kudielka and colleagues provide a highly informative source of information on saliva collection, storage, and measurement techniques (Kudielka et al., 2012).

Hair. The use of hair as a specimen source for CORT is a recent addition to the stress research toolkit. Hair has been used in forensic analyses of toxic and illegal substances for some time (Baumgartner, Jones, Baumgartner, & Black, 1979) since hair shafts will incorporate numerous substances, including drugs of abuse (Cone, 1996) and steroid hormones (Kintz, Cirimele, Jeanneau, & Ludes, 1999). The artificial corticosteroid prednisone was first analyzed in hair shafts in 1999, suggesting the feasibility of hair CORT analysis (Bevalot, Gaillard, Lhermitte, & Pepin, 2000; Cirimele, Tracqui, Kintz, & Ludes, 1999), as first reported by Davenport in rhesus monkeys (Davenport, Tiefenbacher, Lutz, Novak, & Meyer, 2006) and by Kalra and Kirschbaum in humans (Kalra, Einarson, Karaskov, Van Uum, & Koren, 2007; Kirschbaum, Tietze, Skoluda, & Dettenborn, 2009). Hair CORT has been shown to reflect long-term stress and the

impact of social circumstance (Dettenborn, Tietze, Bruckner, & Kirschbaum, 2010; Dettenborn, Tietze, Kirschbaum, & Stalder, 2012; Staufenbiel, Penninx, Spijker, Elzinga, & van Rossum, 2013). Human hair grows at an average rate of 1.3 ± 0.2 (SD) cm/month (Saitoh, Uzuka, & Sakamoto, 1967), and incorporation of CORT into the hair shaft accordingly creates a record of stress exposure going back weeks to months prior to hair sampling (Gow, Thomson, Rieder, Van Uum, & Koren, 2010; Russell et al., 2015). As a result, clipping hair at the top of the scalp provides a reasonably well time-resolved record of average CORT levels prevailing over the time the shaft grew from the follicle (Gow et al., 2010). Dividing the hair sample into appropriate lengths and analyzing the segments in serial order can therefore provide information about CORT fluctuations over given units of time (Stalder et al., 2012). One consideration concerns the fact that cosmetic hair treatments leach out CORT relative to untreated hair (Jurado, Kintz, Menendez, & Repetto, 1997) and also that normal hair washing will diminish CORT content starting about 3–4 cm from the scalp, thus limiting the maximum useful length of hair shaft for CORT measurements (Dettenborn et al., 2012). Hair appears to be a particularly useful source of CORT sampling when longer-term accumulations are desired in studies of chronic stress (Wosu et al., 2015; Wosu, Valdimarsdottir, Shields, Williams, & Williams, 2013). Suggested assay methods are published elsewhere (Russell et al., 2015).

Urine. Unbound CORT passes through the kidneys to be excreted in urine, and therefore urine can provide a useful specimen source for bioactive CORT in circulation and representing a collection period of minutes to hours (Trainer, McHardy, Harvey, & Reid, 1993). CORT in urine is used less frequently in behavioral research, although this was once a method of choice because of the limited availability of assay methods (Johansson & Frankenhaeuser, 1973). Urine is primarily used in clinical endocrinology to assess 24-hour urinary output for diagnostic purposes or in persons undergoing chronic stress where longer-term assessments are desired. The time course of urine accumulation and the inconvenience of sampling limit its utility in lab studies where precise sample timing is desirable. Sampling logistics and subject compliance similarly limit its use in behavioral studies outside the lab. CORT concentration in urine is affected by the rate of urine flow from the kidney, and samples should be corrected for urine volume per unit of time of collection or else standardized to creatnine clearance, which corrects for urine flow rate.

What to Measure: Acute Change from Baseline, Awakening Response, Diurnal Slope, Total Area under the Curve

Change from baseline. In most laboratory studies the CORT response is measured as a change from a pre-stress baseline to a post-stress value. Although this method is well accepted and will find continued use in stress research, it has the major limitation that the underlying baseline declines across the stress protocol because of the diurnal cycle, affecting interpretation of stress effects and comparison between groups (Figure 21.1). We have found it useful to test each person on two days, a stress day and a resting control day (Lovallo et al., 2010a). As shown in Figure 21.6, the declining baseline captured on the resting control day allows a more accurate measure of the time-locked difference in CORT from the baseline day to the stress day than does a comparison of pre-stress/post-stress change scores on a single day. For example, as shown in Figure 21.6(C), data from women taken from pre- to post-stress would suggest a near lack of CORT response. On the other hand, the women show a robust response when their post-stress value is compared to the same time period on the resting control day. This comparison between rest and stress days is also applicable in ambulatory studies (Pincomb, Lovallo, Passey, Brackett, & Wilson, 1987; Wolfram, Bellingrath, Feuerhahn, & Kudielka, 2013). Testing each person on a second day adds expense and logistical difficulty, but the value of the added precision should be weighed against the costs.

CORT awakening response (CAR). As shown in Figure 21.1, CORT begins rising in the early morning hours and peaks 30–45 minutes after awakening. Given its timing, the CAR appears to be under control of hypothalamic clock genes in the suprachiasmatic nucleus and the PVN. The biological purpose of the CAR is not established (Fries, Dettenborn, & Kirschbaum, 2009), although one theory holds that the morning peak serves as a diurnal signal to entrain gene expression across peripheral tissues (Buijs et al., 2003), and others have assumed that it prepares the person for the physical and cognitive demands of the day to come. For example, the size of the CAR in children is positively associated with afternoon and evening cognitive performance, at home and in the lab (Baumler et al., 2014a, 2014b), and patients with hippocampal amnesia fail to show a CAR (Buchanan et al., 2004; Wolf, Fujiwara, Luwinski, Kirschbaum, & Markowitsch, 2005).

Despite its timing, the CAR is not a simple function of awakening and beginning the day (Federenko et al., 2004). The CAR's connection to sleep is not fully established, with one study showing that it is unaffected by multiple awakenings on three successive nights (Dettenborn, Rosenloecher, & Kirschbaum, 2007), although the CAR was inversely related to measured sleep quality in children (Lemola et al., 2015). It is nonetheless a major feature of CORT's diurnal cycle, and as such it has been studied in relation to personal and situational factors that may affect the HPA, including psychosocial status, life stress, and health behaviors (Stawski, Cichy, Piazza, & Almeida, 2013). The CAR is diminished in: persons who report chronic feelings of fatigue (Kumari et al., 2009), those who are exposed to chronic stress at school (Duan et al.,

2013), persons brought up in urban areas vs. rural settings (Steinheuser, Ackermann, Schonfeld, & Schwabe, 2014), women reporting greater perceived stress (Sjors, Ljung, & Jonsdottir, 2014), and in boys with conduct disorder (von Polier et al., 2013). The CAR was elevated in medical students on the day of stressful exams (Gonzalez-Cabrera, Fernandez-Prada, Iribar-Ibabe, & Peinado, 2014). A meta-analysis found that the CAR was not related to the magnitude of acute CORT stress reactivity in the lab (Kidd, Carvalho, & Steptoe, 2014), and the association of the CAR to general psychological stress reactivity across individuals is generally unknown (Petrowski, Herold, Joraschky, Wittchen, & Kirschbaum, 2010).

In behavioral studies, the CAR is virtually always measured from saliva samples taken by the subject at home, beginning with a sample taken in bed immediately upon awakening and continuing with two or three additional samples during the first hour afterward (Wust et al., 2000). This method does not capture the CORT rise that begins prior to awakening (Figure 21.1), and as such the exact magnitude and positioning of the CAR in relation to the total diurnal cycle are not determined by this technique. The onset of a CORT rise prior to awakening suggests that subjects awakening at earlier times will show greater CARs than those awakening later since later awakenings occur closer to the peak (Chida & Steptoe, 2009; Federenko et al., 2004; Kidd et al., 2014). The brevity of the CAR peak points to the difficulty of capturing it precisely by using only two or three samples after awakening and of relying on subject compliance with sample collection instructions (Kunz-Ebrecht, Kirschbaum, Marmot, & Steptoe, 2004). Nonetheless, a CAR taken the morning of a non-stress day and the morning prior to an acute stress procedure found that the CAR was significantly higher on the stress day, showing a clear anticipation of the stressor and suggesting its value in assessing background stress effects on the diurnal cycle (Wetherell, Lovell, & Smith, 2015). The CAR will remain a measure of considerable interest to researchers wishing to study the impact of psychosocial variables on diurnal characteristics of the HPA (Chida & Steptoe, 2009; Fries, Dettenborn, & Kirschbaum, 2009). Measurement issues and the uncertain state of knowledge concerning the CAR's precise response to life stress or its relationship to stress reactivity in the lab suggest that it is currently an object of study in itself rather than a tool with known properties for assessing stress effects.

Diurnal curve and diurnal slope. As shown in Figure 21.1, CORT levels decline across the day, from the morning peak to the early morning hours during the sleep period. Diurnal curves provide useful measures of the integrity of HPA regulation. A reasonable estimate of a diurnal curve can be obtained from a saliva sample taken upon awakening, two or three samples taken over the next hour to approximate the daily peak, followed by samples in the late afternoon and immediately before bed. Obtaining a diurnal curve on a non-stress day can be a useful adjunct in interpreting acute stress responses. For example, if two groups have comparable diurnal curves, then it is reasonable to conclude that they have similar levels of intrinsic HPA regulation and feedback sensitivity. In this case, a group difference in stress reactivity is not likely to be due to a difference in intrinsic HPA regulation. Group differences in acute stress reactivity may then be attributable to differences in magnitude of descending inputs to the HPA from frontal-limbic processes (Lovallo et al., 2010a, 2012a).

A number of researchers have used saliva samples to examine the slope of the diurnal CORT curve from the morning peak to bedtime. Following the theory of Buijs (Buijs et al., 2003), that the morning peak may coordinate gene transcription and metabolic function across the organs of the body, the morning peak relative to the nightly nadir is seen to form a signal that is stronger and more precise when the diurnal slope is greatest. By extension, a flattened diurnal curve may indicate a degraded diurnal signal and poorer systems integrity. A number of studies have reported that the slope is flatter in persons with a range of poor health behaviors or life stressors, due to a diminished morning peak or an elevated nighttime value or both. A flatter diurnal slope is associated with poorer cognitive outcomes in older adults, including memory performance and risky decision-making (Evans et al., 2011; Weller et al., 2014). The diurnal slope has been measured either from the first sample upon awakening to the bedtime sample or from the peak of the CAR to bedtime. As in studies of the CAR, issues of subject compliance are significant in obtaining accurate information.

Area under the curve (AUC) and peak response. Endocrine researchers are often concerned with the total level of activity in a system over time. However, total accumulation is rarely measured directly in stress research. Instead discrete samples are usually collected, as in the case of saliva specimens, and the output over a unit of time can be estimated by calculating the AUC using standard formulas (Pruessner et al., 1997). A contrasting measure of stress reactivity is to take the value of the peak response following stressor onset, measured as change from baseline or difference from the same timed sample taken on a non-stress control day (Lovallo et al., 2010a). Note that the peak response and AUC are related, but not identical, measures. Depending on the shape of the output curve over time, identical AUCs can be achieved by a slower but longer response vs. a short response with a high peak. In contrast, the peak response measured in these two cases will yield different values reflecting maximum reactivity. The choice of measure is up to the investigator but care should be taken in interpretation.

Collection, Storage, and Quantification

Collection techniques will naturally vary with the specimen source. CORT values can be affected by some

medications and by low blood sugar levels. Our experience has been that satisfactory results can be obtained in CORT studies by incorporating the following procedures: (a) The time of day of testing should be recorded, or preferably standardized across subjects, and if possible, stress days should be contrasted with separate rest days. (b) Unless sleep habits or shift work are the subjects of study, we test only persons with a normal nighttime sleep schedule to avoid confounds due to variation in sleep habits. (c) We avoid low blood sugar by providing a small, standardized meal to each subject before the test protocol. (d) We obtain a good picture of the diurnal curve by having subjects collect a morning sample upon awakening and another at bedtime. An approximate CAR can then be obtained by taking two or three additional samples in the first hour after arising.

CORT in saliva and urine can be safely held prior to refrigeration for a period of hours to days, although normal refrigeration is preferred. Storage at –20°C is effective for up to six months in polypropylene tubes. Samples may be stored at lower temperatures (–40°C to –85°C) for longer periods using appropriate cryogenic storage tubes. CORT can be assayed from all biological fluids using enzyme-linked immunosorbent assay (ELISA) methods following appropriate sample collection and extraction techniques, as determined by the specimen source. ELISA has largely replaced previous techniques. ELISA kits are available from several well-established commercial laboratories, and information is readily available on the Web using the search terms *CORT* and *ELISA*. Characteristics and recommendations are available regarding different immunoassay techniques for salivary cortisol measurement (Miller, Plessow, Rauh, Groschl, & Kirschbaum, 2013). As noted earlier, collection methods are often determined by the requirements of a specific research project. The details of collection, storage, and assay are best left to collaborative interaction with the lab conducting the assay. In analyzing CORT data, the shape of the data distribution may be a consideration. Due to the presence of a small number of large values, or large change scores in the case of reactivity measurements, CORT data often depart from normality and display a substantial positive skew. Some labs advocate carrying out logarithmic transformations to bring the shape of the distribution closer to normality (Miller & Plessow, 2013).

The Catecholamines

Catecholamine responses to stress may be measured in urine, blood, or saliva. The value of catecholamine measurement in stress research is that NE output reflects the collective activity of the SNS and, together with EPI responses, forms a key indicator that a state of fight-or-flight has occurred.

Blood is the specimen source of choice for catecholamine measurement. Serial samples can be timed precisely to events in a study protocol. The speed of collection provides an ideal window into the immediate state of the SNS and adrenal output of EPI for each sample. Catecholamines have a relatively short half-life in blood, placing a premium on rapid handling. The time course of catecholamine entry into urine (minutes) and saliva (one hour) limits their use in detecting changes after the onset of acute stressors (Kennedy, Dillon, Mills, & Ziegler, 2001). In addition, the catecholamine values measured in saliva and urine are not perfect measures of the central pool in circulation. NE released from local SNS terminals in the saliva glands is a significant confound when interpreting the source of saliva NE values. Catecholamines in urine contain high levels of EPI (43 percent) produced by the kidney, therefore not representing the adrenal gland (Ziegler, Aung, & Kennedy, 1997). However, urinary secretion of NE is a good source of information about global SNS activity when longer time periods are being sampled, as in studies of chronic stress (Powell et al., 2002; Steptoe, 1987).

Some simple precautions should be taken in measuring catecholamines. Setting up an intravenous line for serial blood sampling causes stress since it calls for a needle stick and placement of a flexible catheter or butterfly needle. Venipuncture is quite stressful for many subjects, often more so than commonly the behavioral stressor under study. Intravenous line placement should be done 30 minutes or more prior to making baseline measurements. As a general rule, subjects in stress studies relying on catecholamine measures should be free of any medications that can influence catecholamine secretion. Since this caveat covers a wide range of medications, we exclude use of all medications in our studies. The increasing use of cannabis will be a factor to deal with in future studies, and use of urine drug screens is likely to become a routine control procedure. Adults commonly consume caffeine, and caffeine will increase catecholamine release, particularly EPI. Caffeine has an average half-life of 3.5 to 4.5 hours in adults. Non-caffeine constituents in coffee affect some assays, such as high performance liquid chromatography. Subjects should be advised to avoid caffeine for at least 4 hours or more prior to arrival at the lab. An alternative is to ask subjects to consume their habitual amount of caffeine on the day of testing and to obtain a report of intake using a caffeine questionnaire. Depending on the assay to be used, subjects may be required to abstain from caffeine starting at dinner the night before testing. Consultation with the lab doing the assays is advised.

Even more than in the case of CORT, the use of catecholamines in behavioral research will necessitate a close collaboration with an experienced clinical lab that has a record of high quality catecholamine measurement suitable for publishable data. Although catecholamines have been studied for some time in human subjects, the process of sample collection, handling, storage, and final quantification, remains highly demanding and specific in its requirements. In general, measuring catecholamines in

blood calls for setting up an intravenous line to allow for serial sampling. EPI has a short half-life in blood and is rapidly metabolized. Accordingly, specimens need to be placed on ice immediately upon being drawn, and preferably collected into chilled tubes. Assays call for measurement in either plasma or serum, and in either case, damage to red cells during sample collection or handling will result in incorrectly high catecholamine values. Plasma and serum measurements both require the samples to be centrifuged and pipetted into storage tubes. Short-term storage (1–3 weeks) may be done at –20°C but longer-term storage needs to be at –40°C to –85°C in cryogenic storage tubes. Accordingly, studies using catecholamines in blood will require access to personnel trained in setting up intravenous lines according to proper safety standards. Staff will need training in universal precautions for blood collection and handling. The lab will need ready access to a centrifuge and low temperature storage approved for biological specimens, and as mentioned, a working relationship with the lab doing the quantification. The above considerations make catecholamine measurements expensive relative to the ease of collection, sample handling, and assay costs for CORT. A useful background reference is found in Ziegler (1989).

Assays of catecholamines have relied on mass spectrometry, radioimmunoassay, and high performance liquid chromatography. Each of these techniques is technically demanding, time consuming, expensive, or has safety considerations if radioactive tracers are used. Laboratories that have a history of catecholamine measurement have largely adopted ELISAs (Fauss et al., 2013). ELISA kits are available from a variety of vendors, and these allow sensitive and specific assays to be done in most clinical laboratories.

Salivary Alpha Amylase

Studies of salivary indices of acute stress responses have made increasing use of salivary alpha amylase (sAA), although it is not a stress hormone. SAA is a digestive enzyme produced by the parotid glands that serves to break down starches into simple sugars, thus aiding in energy intake. The production of fluid and protein that make up saliva is regulated by both the SNS and PNS (Busch, Sterin-Borda, & Borda, 2006; Jensen, Brodin, Berg, & Aars, 1991). Some writers have interpreted sAA production as being a measure of SNS output (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996), in keeping with the fact that saliva protein release is relatively more dependent on the SNS, acting on innervated beta-1 adrenoreceptors, while fluid release is relatively more dependent on the PNS. Following early claims of an SNS connection, subsequent research has called into question the strength of the relationship between SNS activity and sAA production (Bosch, de Geus, Veerman, Hoogstraten, & Nieuw Amerongen, 2003). In keeping with the general principle that fight-or-flight states call for liberation and utilization of energy, it seems likely that saliva sAA output would be more attuned to non-stress states favoring energy intake and storage. In fact, activation of sAA secretion is also increased by PNS activity (Bosch et al., 2003), and the SNS and PNS both seem to contribute to its release (Proctor & Carpenter, 2007). The current view is that sAA may be a generalized index of autonomic activation, with both SNS and PNS contributions, during a range of behavioral challenges, but it is not easily interpreted as an unambiguous index of SNS activity (Bosch, Veerman, de Geus, & Proctor, 2011; Nater & Rohleder, 2009). Because saliva samples are routinely collected for CORT measurement in stress research, use of the same specimens for sAA is highly efficient and allows for simultaneous assessment of HPA and general autonomic activity, although absorbent collection devices need to be avoided. A useful review with considerations for sample collection is available (Rohleder & Nater, 2009).

CONCLUDING COMMENTS

Our understanding of the endocrine component of the stress response has passed through three stages: fixed reflex (Selye), modification with experience (Mason), and cognitive and emotional interactions (McEwen and McGaugh). CORT has pervasive effects on every system in the body, and these effects notably include the CNS, particularly limbic system structures and the prefrontal cortex. As a result, the study of CORT effects is of great interest in psychophysiology. In a similar fashion, EPI is of interest particularly because of its actions on the CNS by way of peripheral beta-adrenoreceptors. As stress hormones, CORT and EPI form a perfect synthesis in psychophysiological investigation; their secretion is altered in relation to affective responses during stress, and their resulting peripheral concentrations are capable of modifying short-term and long-term activity in the CNS. The availability of neuroimaging methods is now allowing increasingly mechanistic studies of glucocorticoid effects on the brain, with reference to behavioral consequences of stress. Similarly, the growing availability of gene arrays for assessing genetic polymorphisms is opening up new possibilities for assessing genetic sources of individual differences in stress hormone response and the effect of stress hormones on the brain.

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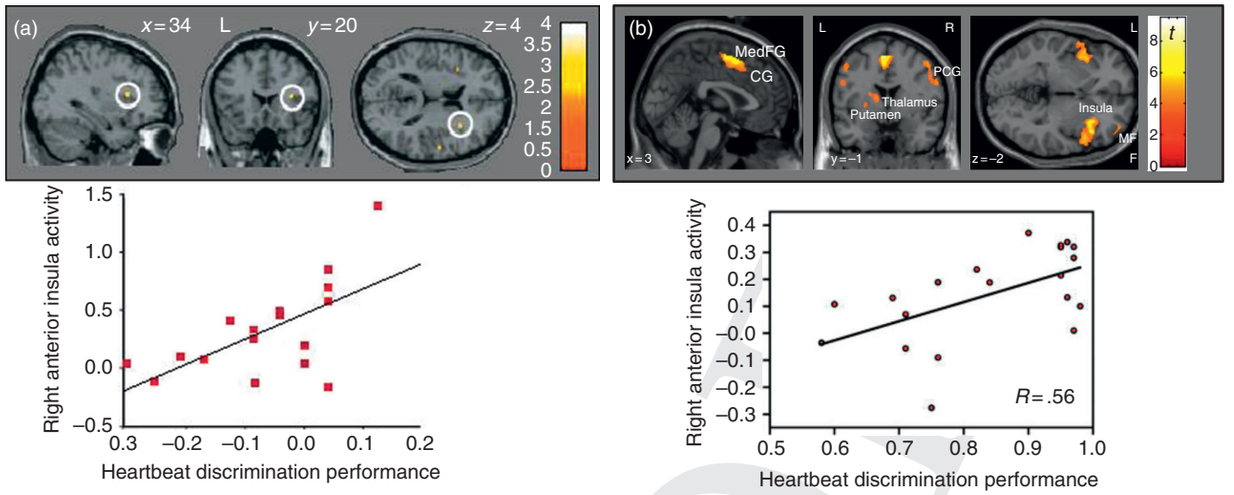

Plate 32 Brain imaging of interoceptive cortex during heartbeat detection. Relative to an exteroceptive control condition, right anterior insula is activated during heartbeat detection conditions (A). Extent of activity in anterior insula predicts heartbeat discrimination performance (A & B). In addition to insula, the interoceptive network also includes anterior cingulate cortex, thalamus, putamen and medial frontal gyrus (B).

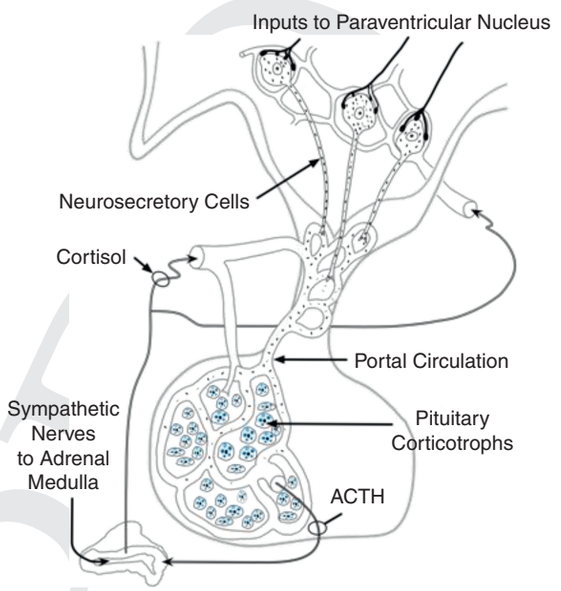

Plate 33 Hypothalamic–pituitary–adrenocortical axis (HPA). Cells of the hypothalamus that synthesize corticotropin releasing factor and arginine vasopressin (CRF, AVP) are shown at the top. These send axon-like processes to specialized neurosecretory terminals that release CRF and AVP into the portal circulation that carries these messengers to the corticotrophs cells of the anterior pituitary. The pituitary releases adrenocorticotropin (ACTH) into the systemic circulation, by which it travels to the adrenal cortex to increase output of CORT. In turn, CORT is carried back to the pituitary and hypothalamus to provide negative feedback regulation of both CRF and ACTH release. Used with permission (Netter, 1953).