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The Role of CRF and CRF-related Peptides in the Dark Side of Addiction

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Abstract

Drug addiction is a chronically relapsing disorder characterized by a compulsion to seek and take drugs, the development of dependence, and the manifestation of a negative emotional state when the drug is removed. Activation of brain stress systems is hypothesized to be a key element of the negative emotional state produced by dependence that drives drug-seeking through negative reinforcement mechanisms, defined as the "dark side" of addiction. The focus of the present review is on the role of corticotropin-releasing factor (CRF) and CRF-related peptides in the dark side of addiction. CRF is a key mediator of the hormonal, autonomic, and behavior responses to stressors. Emphasis is placed on the role of CRF in extrahypothalamic systems in the extended amygdala, including the central nucleus of the amygdala, bed nucleus of the stria terminalis, and a transition area in the shell of the nucleus accumbens, in the dark side of addiction. The urocortin/CRF_2 systems have been less explored, but results suggest their role in the neuroadaptation associated with chronic drug use, sometimes in opposition to the effects produced by the CRF_1 receptor. Compelling evidence argues that the CRF stress system, including its activation of the hypothalamic-pituitary-adrenal axis, plays a key role in engaging the transition to dependence and maintaining dependence once it is initiated. Understanding the role of the CRF systems in addiction not only provides insight into the neurobiology of the dark side of addiction, but also provides novel targets for identifying vulnerability to addiction and the treatment of addiction.

Conceptual Framework: Addiction, Stress, and the Dark Side

Drug addiction is a chronically relapsing disorder characterized by (*i*) compulsion to seek and take the drug, (*ii*) loss of control in limiting intake, and (*iii*) emergence of a negative emotional state (e.g., dysphoria, anxiety, irritability) reflecting a motivational withdrawal syndrome when access to the drug is prevented (defined here as dependence) (Koob and Le Moal, 1997Koob and Le Moal, 2008). Addiction has been conceptualized as an evolving disorder that comprises three stages—*preoccupation/anticipation*, *binge/intoxication*, and *withdrawal/negative affect*—in which impulsivity often dominates at the early stages and compulsivity dominates at terminal stages. As an individual moves from impulsivity to compulsivity, a shift occurs from positive reinforcement driving the motivated behavior to negative reinforcement driving the motivated behavior (Koob, 2004). Negative reinforcement can be defined as the process by which removal of an aversive stimulus (e.g., negative emotional state of drug withdrawal) increases the probability of a response (e.g., dependence-induced drug intake). These three

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stages are conceptualized as interacting with each other, becoming more intense, and ultimately leading to the pathological state known as addiction (Koob and Le Moal, 1997).

The present review focuses on the role of corticotropin-releasing factor (CRF) in what has been described as the "dark side" of the addiction cycle (i.e., the *withdrawal/negative affect* stage of the addiction cycle and the elements of the *preoccupation/anticipation stage*). Different drugs produce different patterns of addiction with emphasis on different components of the addiction cycle, but all addictive drugs show some common elements relevant to the dark side of addiction. The common elements include profound malaise, dysphoria, and anxiety during withdrawal, a protracted abstinence syndrome characterized by a low-level anxiety/dysphoric state and a high vulnerability to relapse when subjected to an acute stressor. CRF is hypothesized to play a key role in the anxiety/stress-like effects of acute withdrawal, anxiety/stress-like effects of protracted abstinence, and relapse to drug taking during protracted abstinence induced by stressors.

The role of CRF in the dark side component of the addiction cycle is predicated on opponent process theory, which been expanded into the domains of the neurobiology of drug addiction from a neurocircuitry perspective. An allostatic model of the brain motivational systems has been proposed to explain the persistent changes in motivation that are associated with dependence in addiction (Koob and Le Moal 2001Koob and Le Moal 2008). In this formulation, addiction is conceptualized as a cycle of increasing dysregulation of brain reward/anti-reward mechanisms that results in a negative emotional state contributing to the compulsive use of drugs. Counteradaptive processes that are part of the normal homeostatic limitation of reward function fail to return within the normal homeostatic range. These counteradaptive processes are hypothesized to be mediated by two mechanisms: within-system neuroadaptations (changes in reward pathways) and between-system neuroadaptations (the recruitment of the brain stress systems) (Koob and Bloom, 1988; Koob and Le Moal, 1997, 2008). The recruitment of the brain stress systems, of which CRF is perhaps the prominent component, provides one key part of the negative reinforcement processes that drive the compulsivity of addiction (Koob, 2008).

Corticotropin-Releasing Factor

CRF is a 41-amino acid polypeptide that has a major role in coordinating the stress response of the body by mediating hormonal, autonomic, and behavioral responses to stressors. CRF (also termed corticotropin-releasing hormone, although the International Union of Pharmacology designation is CRF) was identified by classic techniques of peptide sequencing (Vale et al., 1981). Subsequently, genes encoding three paralogs of CRF—urocortins 1, 2, and 3 (Ucn 1, Ucn 2, Ucn 3), with Ucn 2 and Ucn3 also referred to as stresscopin-related peptide and stresscopin, respectively—were identified by modern molecular biological approaches. CRF agonists can be found in fish (urotensin), frogs (sauvagine), and mammals (urocortin). Urocortin was named for its sequence similarity to carp urotensin I (63%, "uro") and mammalian CRF (45%, "cort"). Two G-protein-coupled receptors (CRF_1, CRF_2) that the CRF/Ucn peptides bind and activate with varying affinities were similarly identified (Bale and Vale, 2004; Fekete and Zorrilla, 2007). Pharmacological and transgenic studies show that brain and pituitary CRF_1 receptors mediate many of the functional stress-like effects of the CRF system (Heinrichs and Koob, 2004). Previous reviews by ourselves and others have surveyed the biology of CRF systems (Bale and Vale, 2004; Heinrichs and Koob, 2004).

CRF has a wide distribution throughout the brain but particularly high concentrations of cell bodies in the paraventricular nucleus of the hypothalamus, the basal forebrain (notably the extended amygdala), and the brainstem (Swanson et al., 1983). Central administration of CRF mimics the behavioral response to activation and stress in rodents, and administration of

competitive CRF receptor antagonists generally has anti-stress effects (Heinrichs et al., 1994; Menzaghi et al., 1994; Spina et al., 2000; for reviews, see Dunn and Berridge, 1990; Koob et al., 1994, 2001; Sarnyai et al., 2001) (Table 1). Of the two major CRF receptors that have been identified, CRF_1 receptor activation is associated with increased stress responsiveness (Koob and Heinrichs, 1999), and CRF_2 receptor activation is associated with decreases in feeding and decreased stress responsiveness (Spina et al., 1996; Pelleymounter et al., 2000; but see Ho et al., 2001; Takahashi et al., 2001; Fekete and Zorrilla, 2007). Numerous blood-brain barrier-penetrating, selective CRF_1 receptor antagonists have been developed, but no small-molecule brain-penetrating CRF_2 antagonists have been developed (Zorrilla and Koob, 2007). As a result, an extensive amount of work has been done to elucidate the role of CRF_1 receptors in addiction with limited work on the CRF_2 receptor (see below).

Hormonal Stress Systems: Hypothalamic-Pituitary-Adrenal Axis

A key element of the body's response to stress relevant to addiction is the hypothalamic-pituitary adrenal (HPA) axis, a system largely controlled by CRF in the paraventricular nucleus of the hypothalamus (Figure 1). The HPA axis is composed of three major structures: the paraventricular nucleus of the hypothalamus, the anterior lobe of the pituitary gland, and the adrenal gland (for review, see Smith and Vale, 2006). Neurosecretory neurons in the medial parvocellular subdivision of the paraventricular nucleus synthesize and release CRF into the portal blood vessels which enter the anterior pituitary gland. Binding of CRF to the CRF_1 receptor on pituitary corticotropes induces the release of adrenocorticotropic hormone (ACTH) into the systemic circulation. ACTH in turn stimulates glucocorticoid synthesis and secretion from the adrenal cortex. The HPA axis is finely tuned via negative feedback from circulating glucocorticoids that act on glucocorticoid receptors in two main brain areas: the paraventricular nucleus of the hypothalamus and the hippocampus. The hypophysiotropic neurons of the paraventricular nucleus of the hypothalamus are innervated by numerous afferent projections, including from the brainstem, other hypothalamic nuclei, and forebrain limbic structures.

Extrahypothalamic CRF Systems

CRF is also located outside of the HPA axis to control autonomic and behavioral responses to stressors. Substantial CRF-like immunoreactivity is present in the neocortex, extended amygdala, medial septum, hypothalamus, thalamus, cerebellum, and autonomic midbrain and hindbrain nuclei, including the ventral tegmental area (Charlton et al., 1987; Swanson et al., 1983). The distribution of Ucn 1 projections overlaps with CRF but also has a different distribution, including visual, somatosensory, auditory, vestibular, motor, tegmental, parabrachial, pontine, median raphe, and cerebellar nuclei (Zorrilla and Koob, 2005). The CRF_1 receptor has abundant, widespread expression in the brain that overlaps significantly with the distribution of CRF and Ucn 1.

The endogenous selective CRF_2 agonists—the type 2 urocortins Ucn 2 (Reyes et al., 2001) and Ucn 3 (Lewis et al., 2001)—differ from Ucn 1 and CRF in their neuropharmacological profiles. Ucn 2 and Ucn 3 show high functional selectivity for the CRF_2 receptor and have neuroanatomical distributions that are distinct from those of CRF and Ucn 1 (Figure 2). Ucn 2 and Ucn 3 are notably salient in hypothalamic nuclei that express the CRF_2 receptor, including the supraoptic nucleus, magnocellular neurons of the paraventricular nucleus, and forebrain, including the ventromedial hypothalamus, lateral septum, bed nucleus of the stria terminalis, and medial and cortical amygdala (Li et al., 2002). The $CRF_{2(a)}$ receptor isoform is localized neuronally in brain areas distinct from those of the CRF/Ucn 1/CRF_1 receptor system, such as the ventromedial hypothalamic nucleus, paraventricular nucleus of the hypothalamus, supraoptic nucleus, nucleus tractus solitarius, area postrema, lateral septum, and bed nucleus of the stria terminalis.

Construct of the Extended Amygdala: Interface of CRF and the Dark Side of Addiction

Recent neuroanatomical data and new functional observations have provided support for the hypothesis that the neuroanatomical substrates for many of the motivational effects associated with the dark side of addiction may involve a common neural circuitry that forms a separate entity within the basal forebrain, termed the “extended amygdala” (Alheid and Heimer, 1988). The extended amygdala represents a macrostructure composed of several basal forebrain structures: the bed nucleus of the stria terminalis, central medial amygdala, and a transition zone in the posterior part of the medial nucleus accumbens (i.e., posterior shell) (Johnston, 1923; Heimer and Alheid, 1991). These structures have similarities in morphology, immunohistochemistry, and connectivity (Alheid and Heimer, 1988), and they receive afferent connections from limbic cortices, the hippocampus, basolateral amygdala, midbrain, and lateral hypothalamus. The efferent connections from this complex include the posterior medial (sublenticular) ventral pallidum, ventral tegmental area, various brainstem projections, and perhaps most intriguing from a functional point of view, a considerable projection to the lateral hypothalamus (Heimer et al., 1991). Key elements of the extended amygdala include not only neurotransmitters associated with the positive reinforcing effects of drugs of abuse, such as dopamine and opioid peptides, but also major components of the extrahypothalamic CRF systems associated with negative reinforcement mechanisms (Koob and Le Moal, 2005; see below).

CRF, the HPA Axis, and Addiction

From the perspective of addiction, progressive changes in the HPA axis are observed during the transition from acute administration to chronic administration of drugs of abuse. Acute administration of most drugs of abuse in animals activates the HPA axis and may first facilitate activity in the brain motivational circuits and drug reward and as a result facilitate acquisition of drug-seeking behavior (Piazza et al., 1993; Goeders, 1997; Piazza and Le Moal, 1997; Fahlke et al., 1996). Relevant for the role of CRF in the dark side of addiction, these acute changes are blunted or dysregulated with repeated administration of cocaine, opioids, nicotine, and alcohol (Kreek and Koob, 1998; Rasmussen et al., 2000; Goeders, 2002; Koob and Kreek, 2007; Sharp and Matta, 1993; Semba et al., 2004). An atypical responsivity to stressors has been hypothesized to contribute to the persistence and relapse to cycles of opioid dependence, and subsequently this hypothesis was extended to other drugs of abuse (Kreek and Koob, 1998).

Importantly for the role of CRF in the dark side of the addiction process, high circulating levels of glucocorticoids can feedback to shut off the HPA axis but can “sensitize” CRF systems in the central nucleus of the amygdala and basolateral amygdala known to be involved in behavioral responses to stressors (Imaki et al., 1991; Makino et al., 1994; Swanson and Simmons, 1989; Schulkin et al., 1994; Shepard et al., 2000). Thus, although activation of the HPA axis may characterize initial drug use and the *binge/intoxication* stage of addiction, such activation also can lead to subsequent activation of extrahypothalamic brain stress systems that characterize the *withdrawal/negative affect* stage (Kreek and Koob, 1998; Koob and Le Moal, 2005; Koob and Kreek, 2007).

Role of CRF in Animal Models of Addiction

Chronic administration of drugs with dependence potential dysregulates the stress responses mediated by CRF, including not only the HPA axis, but also the brain extrahypothalamic stress system. Responses common to all drugs of abuse and alcohol include, during acute withdrawal, an activated HPA stress response reflected in elevated ACTH and corticosteroids and an

activated brain stress response with increased amygdala CRF release. However, with repeated cycles of addiction, a blunted HPA response occurs but with a sensitized extrahypothalamic CRF stress system response (Koob and Kreek, 2007; Koob, 2008).

In vivo microdialysis during acute withdrawal following chronic administration or self-administration of drugs of abuse produces increases in extracellular CRF in the extended amygdala, a stress-like response (Merlo-Pich et al., 1995; Richter et al., 2000). During alcohol withdrawal, extrahypothalamic CRF systems become hyperactive, with an increase in extracellular CRF within the central nucleus of the amygdala and bed nucleus of the stria terminalis of dependent rats (Merlo-Pich et al., 1995; Olive et al., 2002). Extracellular CRF also increased in the central amygdala during precipitated withdrawal from chronic nicotine (George et al., 2007), withdrawal from binge cocaine self-administration (Richter and Weiss, 1999), and precipitated withdrawal from opioids (Weiss et al., 2001) and cannabinoids (Rodriguez de Fonseca et al., 1997). Amygdala CRF tissue content was reduced during acute withdrawal from ethanol exposure and from binge cocaine self-administration (Zorrilla et al., 2001; Funk et al., 2006; Koob, 2009).

Another common response to acute withdrawal and protracted abstinence from all major drugs of abuse is the manifestation of a negative emotional state, including anxiety-like responses. Animal models in which the dependent variable is often a passive response to a novel and/or aversive stimulus, such as the open field, elevated plus maze, defensive withdrawal test, or social interaction test, or an active response to an aversive stimulus, such as defensive burying of an electrified metal probe, have shown anxiety-like responses to acute withdrawal from all major drugs of abuse. Withdrawal from repeated administration of cocaine, alcohol, nicotine, cannabinoids, and benzodiazepines produces an anxiogenic-like response in the elevated plus maze, defensive withdrawal, or defensive burying test, and these effects are reversed by administration of CRF antagonists (Sarnyai et al., 1995; Basso et al., 1999; Knapp et al., 2004; Overstreet et al., 2004; Tucci et al., 2003; George et al., 2007; Rodriguez de Fonseca et al., 1997; Skelton et al., 2007).

Moreover, the decreased brain reward function associated with drug withdrawal is CRF_1 receptor-dependent. Elevation of reward thresholds during nicotine withdrawal is blocked by CRF_1 antagonists (Bruijnzeel et al., 2007, 2009). Using the place aversion model, a CRF_1 antagonist also blocked the development of conditioned place aversion induced by precipitated opioid withdrawal in opioid-dependent rats (Stinus et al., 2005). Studies with microinjections of noradrenergic and CRF antagonists have provided evidence for a role of the bed nucleus of the stria terminalis (Delfs et al., 2000) and central nucleus of the amygdala (Heinrichs et al., 1995), respectively, in the place aversions produced by precipitated opioid withdrawal.

Significant evidence from our laboratory and those of others have demonstrated a key role for CRF in the motivational effects of ethanol in dependence. During ethanol withdrawal, extrahypothalamic CRF systems become hyperactive, with an increase in extracellular CRF within the central nucleus of the amygdala and bed nucleus of the stria terminalis in dependent rats (Funk et al., 2006; Merlo-Pich et al., 1995; Olive et al., 2002). The dysregulation of brain CRF systems is hypothesized to underlie both the enhanced anxiety-like behaviors and enhanced ethanol self-administration associated with ethanol withdrawal. Supporting this hypothesis, subtype-nonselective CRF receptor antagonists, such as α-helical CRF_{9-41} and D-Phe CRF_{12-41} (intracerebroventricularly injected) reduce ethanol withdrawal-induced anxiety-like behavior (Baldwin et al., 1991; see above).

Exposure to repeated cycles of chronic ethanol vapor produces substantial increases in ethanol intake in rats, both during acute withdrawal and protracted abstinence (2 weeks or more post-acute withdrawal) (O'Dell et al., 2004; Rimondini et al., 2002). Intracerebroventricular

administration of a CRF_1/CRF_2 antagonist blocked the dependence-induced increase in ethanol self-administration during acute withdrawal (Valdez et al., 2004). CRF antagonists had no effect on ethanol self-administration in nondependent animals (Valdez et al., 2004). When administered directly into the central nucleus of the amygdala, CRF antagonists also attenuated anxiety-like behavior produced by ethanol withdrawal (Rassnick et al., 1993) and ethanol self-administration in dependent rats (Funk et al., 2006, 2007). Again, no effect of the CRF antagonists were observed on ethanol self-administration in nondependent animals. CRF_1 small-molecule antagonists selectively reduced the increased self-administration of drugs associated with extended access to intravenous self-administration of cocaine (Specio et al., 2008), nicotine (George et al., 2007), and heroin (Greenwell et al., 2009). These data suggest an important role for CRF, primarily within the central nucleus of the amygdala, in mediating the increased self-administration associated with dependence.

CRF antagonists injected intracerebroventricularly or systemically also blocked the potentiated anxiety-like responses to stressors observed during protracted abstinence (Breese et al., 2005; Valdez et al., 2003) and the increased ethanol self-administration associated with protracted abstinence (Sabino et al, 2006; Funk et al., 2007; Richardson et al., 2008; Chu et al., 2007; Gilpin et al., 2008; Sommer et al., 2008; Gehlert et al., 2007). These results suggest that a residual dysregulation of CRF systems continues into the protracted abstinence associated with the *preoccupation/anticipation* stage. Supporting this hypothesis, both ethanol- and cocaine-withdrawn animals showed reduced CRF-like immunoreactivity in the amygdala followed by a progressive increase culminating in elevated levels 6 weeks post-withdrawal (Zorrilla et al., 2001).

Thus, the brain CRF system has an important role in mediating the shift from positive to negative reinforcement associated with the development of motivational aspects of dependence reflected in increased drug intake with extended access (see Koob and Le Moal, 2008, for further elaboration of this conceptual framework). Data from microdialysis, anxiety-like responses, place conditioning (conditioned place aversion), and extended access to intravenous drug self-administration have converged to provide a neuropharmacological framework for the present hypothesis.

Urocortin and Addiction

A limited number of studies have explored the role of urocortin systems independent of CRF receptors in addiction. A number of studies suggest that urocortin systems may play a role in ethanol self-administration (Ryabinin and Weitemier, 2006). Mouse and rat strains that drink ethanol excessively have higher amounts of urocortin-expressing cells in the Edinger-Westphal nucleus compared with strains that do not drink excessively (Bachtell et al., 2002, 2003; Turek et al., 2005). High alcohol intake induced activity in urocortin cells in the Edinger-Westphal nucleus (Ryabinin et al., 2003). Ucn 1 microinjection into the projection area of the urocortin cells in the Edinger-Westphal nucleus attenuated the increase in limited-access drinking in mice (Ryabinin et al., 2008). Lipopolysaccharide stress also increased the activity of urocortin cells in the Edinger-Westphal nucleus (Kozicz, 2003). Subsequent studies have shown that other stressors and acute administration of psychostimulants activate urocortin cells in the Edinger-Westphal nucleus region, now termed the pIIIu, suggesting that multiple drugs of abuse and stressors can activate the Ucn 1 system in this region (Spangler et al., 2009). However, the locomotor sensitization observed in mice with repeated administration of ethanol was blocked by CRF_1 antagonists and not in Ucn 1 or CRF_2 knockout mice (Pastor et al., 2008). CRF_2 knockout mice also failed to show decreases in ethanol consumption in both 24 h two-bottle choice and limited-access paradigms (Sharpe et al., 2005). Altogether, these results suggest that the Ucn 1 system deriving from the pIIIu in the region of the Edinger-Westphal

nucleus is activated by excessive ethanol consumption. However, its action may be mediated more by CRF_1 receptors than CRF_2 receptors.

In the domain of increased ethanol intake associated with ethanol dependence, a highly selective CRF_2 agonist, Ucn 3, when injected intracerebroventricularly or directly into the central nucleus of the amygdala, had an effect similar to a CRF_1 antagonist in reducing the increase in ethanol self-administration associated with acute withdrawal in dependent rats. However, no effect was observed in nondependent rats (Valdez et al., 2004; Funk and Koob, 2007). Ucn 3 also selectively attenuated the increase in ethanol intake observed in C57BL/6J mice during limited access to ethanol (Sharpe and Phillips, 2009). These results suggest that the Ucn 3 system may block excessive drinking under a number of conditions and suggest a role for CRF_2 receptors that is opposite to the role of CRF and Ucn 1 via CRF_1 receptors in modulating ethanol intake in dependent animals.

Withdrawal-induced enhanced long-term potentiation in hippocampal slices associated with chronic high-dose cocaine exposure was blocked by both CRF_1 and CRF_2 receptor antagonists (Guan et al., 2009). In brain slice recordings from the lateral septum following acute withdrawal from chronic cocaine, a shift in CRF_2 receptor activity from inhibition to facilitation was observed (Liu et al., 2005). Spontaneous somatic withdrawal from chronic opioid administration was blocked in CRF_2 knockout mice (Papaleo et al., 2008), whereas the motivational effects of opioid withdrawal, measured by conditioned place aversion, were blocked in CRF_1 knockout mice (Contarino and Papaleo, 2005). Altogether, these results suggest that during withdrawal from drugs of abuse, the CRF_2 system may become engaged in the neuroplasticity that conveys somatic withdrawal, motivational withdrawal, and aspects of changes in learning. The specific sites that are involved (e.g., septum, amygdala, hippocampus), however, remain to be determined.

Stress-Induced Reinstatement

A state of stress and stressor exposure have long been associated with relapse and vulnerability to relapse (Koob and Kreek, 2007; Marlatt and Gordon, 1980). In human alcoholics, numerous symptoms that can be characterized by negative emotional states such as dysphoria, malaise, irritability, and anxiety, persist long after acute physical withdrawal from alcohol (Alling et al., 1982). These symptoms, post acute withdrawal, often precede relapse (Hershon, 1977; Annis et al., 1998). Negative emotion, including elements of anger, frustration, sadness, anxiety, and guilt, is a key factor in relapse (Zywiak et al., 1996) and was the leading precipitant of relapse in a large-scale replication of Marlatt's taxonomy (Lowman et al., 1996). Negative affect, stress, or withdrawal-related distress also increases drug craving (Childress et al., 1994; Cooney et al., 1997; Sinha et al., 2000).

The role of CRF in stress-induced reinstatement of drug-seeking follows a pattern of results somewhat parallel to the role of CRF in the anxiety-like effects of acute withdrawal and dependence-induced increases in drug intake (for reviews, see Shaham et al., 2003; Lu et al., 2003). Mixed CRF_1/CRF_2 antagonists injected intracerebroventricularly and/or CRF_1 small-molecule antagonists blocked stress-induced reinstatement of cocaine, opioid, alcohol, and nicotine seeking behavior (see Liu and Weiss, 2002; Shaham et al., 2003; Lu et al., 2003; Le et al. 2000; Shaham et al. 1998; Gehlert et al., 2007; Bruijnzeel et al., 2009; Marinelli et al., 2007). These effects have been replicated with intracerebral injections of a mixed CRF_1/CRF_2 antagonist or small-molecule CRF_1 antagonist into the bed nucleus of the stria terminalis, median raphe nucleus, and ventral tegmental area, but not the amygdala or nucleus accumbens (see Shaham et al., 2003; Lu et al., 2003), suggesting that different sites, such as the bed nucleus of the stria terminalis, median raphe nucleus, and ventral tegmental area, may be important for stress-induced relapse, in contrast to the role of CRF in dependence-induced increases in drug

self-administration which to date has been localized primarily to the central nucleus of the amygdala (Funk et al., 2006). Notice that stress-induced reinstatement occurs independent of stress-induced activation of the HPA axis (Erb et al., 1998; Le et al., 2000; Shaham et al., 1997).

For example, CRF systems have also been identified in the ventral tegmental area, and footshock stress can release CRF into the ventral tegmental area and has a role in stress-induced reinstatement (Wang et al., 2005). Footshock-induced reinstatement of cocaine-seeking was blocked by administration of a CRF_2 receptor antagonist, and CRF agonists with strong affinity for the CRF binding protein mimicked the effects induced by footshock, suggesting the involvement of both CRF_2 receptors and the CRF binding protein in the ventral tegmental area in stress-induced reinstatement (Wang et al., 2007). These results complement the role of the CRF_1 system in the extended amygdala in stress-induced reinstatement (Shaham et al., 1998). Other brain stress systems implicated in stress-induced reinstatement possibly linked to brain CRF systems include norepinephrine, orexin, vasopressin, and nociceptin (see Shaham et al., 2003; Lu et al., 2003).

Thus, the brain stress systems may impact both the *withdrawal/negative affect* stage and *preoccupation/anticipation* stage of the addiction cycle, albeit by engaging different components of the extended amygdala emotional system (central nucleus of the amygdala *vs.* bed nucleus of the stria terminalis; see above), and the dysregulations that comprise the negative emotional state of drug dependence persist during protracted abstinence to set the tone for vulnerability to "craving" driven by activation of the drug-, cue-, and stress-induced reinstatement neurocircuits.

CRF, the Dark Side, and Addiction: A Conceptual Framework for Linking Stress Systems and Addiction

All drugs of abuse engage the HPA axis during acquisition of drug-taking and again during acute withdrawal from the drug via activation of CRF in the paraventricular nucleus of the hypothalamus. As the cycle of drug taking and withdrawal continues, the HPA axis response becomes blunted, but the repeated exposure of the brain to high levels of glucorticoids can continue to have profound effects on the extrahypothalamic brain stress systems (Figure 3). Strong evidence suggests that glucocorticoids "sensitize" the CRF system in the amygdala (Imaki et al., 1991;Makino et al., 1994;Swanson and Simmons, 1989). Thus, the first component of the contribution of CRF to the dark side is activation of the HPA axis and glucocorticoids, which are linked initially to high responsivity to novelty and facilitation of reward. Subsequently, sensitization of CRF systems in the extended amygdala occurs in which they contribute to a stress component of the shift from homeostasis to pathophysiology associated with drug addiction. This stress component may reflect a component of the opponent process response to excessive activation of reward systems, termed anti-reward (Koob and Le Moal, 2008).

Opponent process, between-system neuroadaptations are hypothesized to involve neurochemical systems other than those involved in the positive rewarding effects of drugs of abuse that are recruited or dysregulated by chronic activation of the reward system (Koob and Bloom, 1988). A between-system neuroadaptation is a circuitry change in which another different circuit (anti-reward circuit) is activated by the reward circuit and has opposing actions, again limiting reward function. Therefore, recruitment of the CRF system during the development of dependence for all drugs of abuse would have key motivational significance. Additional between-system neuroadaptations associated with motivational withdrawal of a between-system opponent process include activation of the dynorphin/κ-opioid system, norepinephrine brain stress system, extrahypothalamic vasopressin system, and possibly the

orexin system. Brain anti-stress systems, such as neuropeptide Y and nociceptin, may also be compromised during the development of dependence, thus removing a mechanism for restoring homeostasis (Koob and Le Moal, 2008). Additionally, activation of the brain stress systems may not only contribute to the negative motivational state associated with acute abstinence, but also may contribute to the malaise, persistent dysphoria, and vulnerability to stressors observed during protracted abstinence in humans. These results suggest that the motivation to continue drug use during dependence not only includes a change in the function of neurotransmitters associated with the acute reinforcing effects of drugs of abuse during the development of dependence, such as dopamine, opioid peptides, serotonin, and γ-aminobutyric acid, but also recruitment of the brain stress systems and/or disruption of the brain anti-stress systems (Koob, 2008; Koob and Le Moal, 2008).

Thus, the activity of neural circuits involving CRF normally involved in appropriate responses to acute stressors contributes to the aversive emotional state that drives the negative reinforcement of addiction. The *withdrawal/negative affect* stage defined above consists of key motivational elements, such as chronic irritability, emotional pain, malaise, dysphoria, alexithymia, and loss of motivation for natural rewards, and is characterized in animals by increases in reward thresholds during withdrawal from all major drugs of abuse (Koob, 2008). A key component of the dark side of addiction is the concept of anti-reward (i.e., processes put in place to limit reward) (Koob and Le Moal, 1997Koob and Le Moal, 2005Koob and Le Moal, 2008). As dependence and withdrawal develop, brain anti-reward systems such as CRF are hypothesized to be recruited to produce stress-like, aversive states (Koob and Le Moal, 2001; Nestler, 2005; Aston-Jones et al., 1999).

An overall conceptual framework throughout this review is that engagement of a key brain stress system mediated by CRF represents more than a simple break with homeostasis in the context of addiction, but rather the development of allostasis. Allostasis is defined as “stability through change” and is different from homeostasis because feed-forward, rather than negative feedback, mechanisms are hypothesized to be engaged (Sterling and Eyer, 1988). However, precisely this ability to mobilize resources quickly and to use feed-forward mechanisms leads to an allostatic state if the systems do not have sufficient time to reestablish homeostasis. An *allostatic state* can be defined as a state of chronic deviation of the regulatory system from its normal (homeostatic) operating level.

The brain stress systems respond rapidly to anticipated challenges to homeostasis but are slow to habituate or do not readily shut off once engaged (Koob, 1999). Thus, the very physiological mechanism that allows a rapid and sustained response to environmental challenge becomes the engine of pathology if adequate time or resources are not available to shut off the response. Drug addiction, similar to other chronic physiological disorders such as high blood pressure, worsens over time, is subject to significant environmental influences (e.g., external stressors), and leaves a residual neural trace that allows rapid “re-addiction” even months and years after detoxification and abstinence. These characteristics of drug addiction have led to a reconsideration of drug addiction as more than simply homeostatic dysregulation of emotional function, but rather as an allostatic state with CRF activation as a key contributor. This state of compulsive drug seeking represents a combination of chronic elevation of reward set point fueled by numerous neurobiological changes, including decreased function of reward circuits, loss of prefrontal cortex executive control, facilitation of striatal stimulus-response associations, and recruitment of the CRF brain stress system (Koob and Le Moal, 2008). Finally, it is becoming increasingly clear that genetic vulnerability may also play a role in the dark side axis of compulsivity.

An association was found between haplotype tagging single-nucleotide polymorphisms of the CRF_1 gene with patterns of alcohol consumption in binge drinking in adolescents and alcohol-

dependent adults (Treutlein et al., 2006). In a subsequent study, adolescents homozygous for the C allele of the R1876831 single-nucleotide polymorphism drank more alcohol per occasion and had higher lifetime rates of heavy drinking in relation to negative life events than subjects carrying the T-allele (Blomeyer et al., 2008). In a follow-up study, homozygotes of the C allele of rs1876831, as well as carriers of the A allele of rs242938, when exposed to stress, exhibited significantly higher drinking activity than carriers of other alleles (Schmid et al., 2009). In the genetically selected Marchigian-Sardinian preferring rat line, high ethanol preference correlated with a genetic polymorphism of the *crhr1* promoter and an increase in CRF_1 density in the amygdala, as well as increased sensitivity to stress and increased sensitivity to a CRF_1 antagonist (Hansson et al., 2006). In non-genetically selected rats exposed to repeated cycles of ethanol intoxication and dependence, a CRF_1 antagonist blocked the increased ethanol intake associated with protracted abstinence, an effect that coincided with upregulation of the CRF_1 gene and downregulation of the CRF_2 gene in the amygdala (Sommer et al., 2008). Altogether these results suggest the exciting possibility that certain single-nucleotide polymorphisms in the human population may predict vulnerability to certain subtypes of excessive drinking syndromes associated with the dark side.

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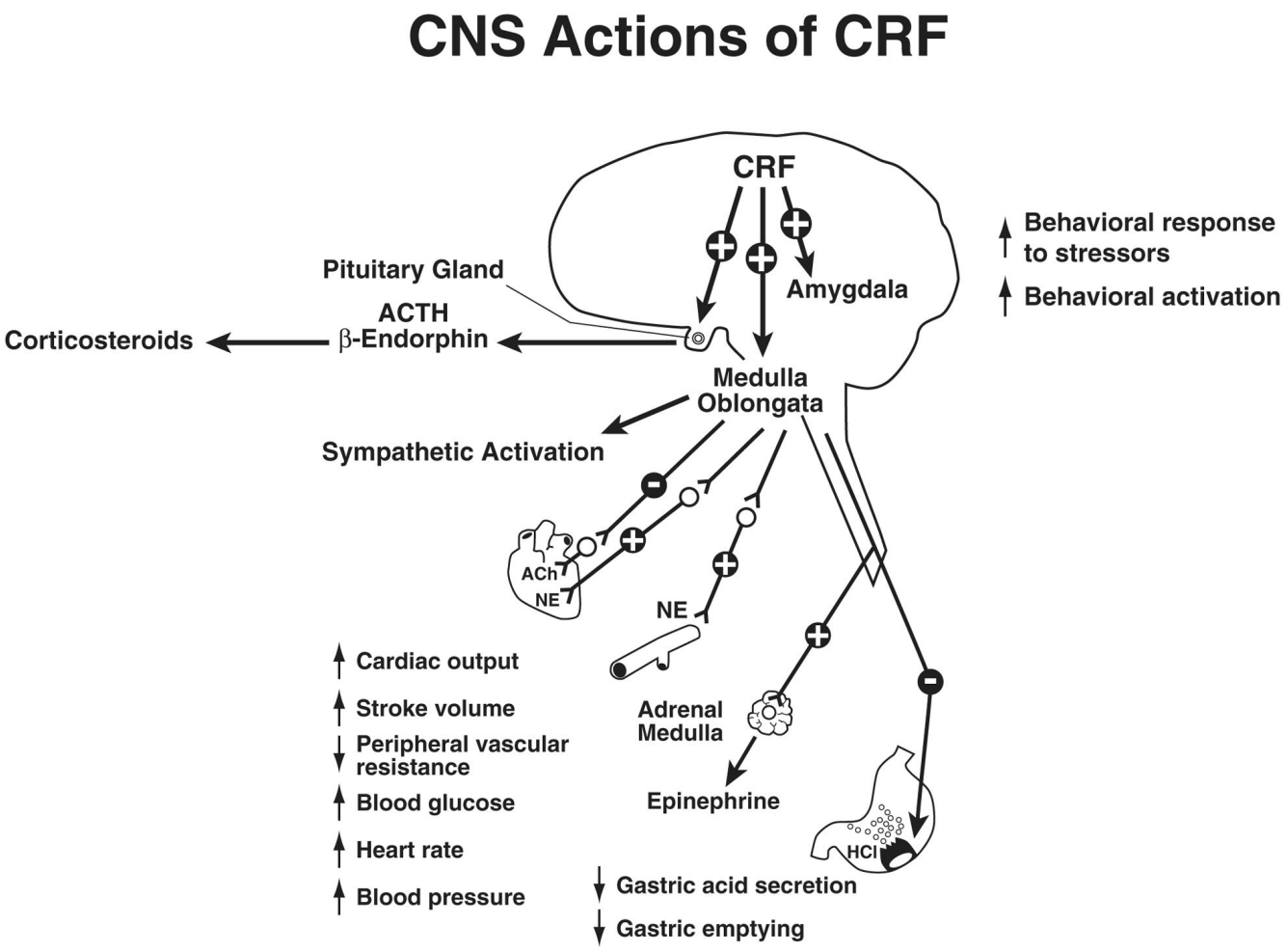

Figure 1.

Diagram illustrating the multiple actions of CRF in mediating stress responses in the body. CRF drives the hypothalamic-pituitary adrenal axis by acting to release adrenocorticotropic hormone (ACTH) in the portal system of the pituitary. CRF activates the sympathetic system by actions in the brainstem and mediates arousal and behavioral responses to stressors by actions in the amygdala, other basal forebrain regions, and ventral midbrain such as the ventral tegmental area. Ach, acetylcholine; NE, norepinephrine.

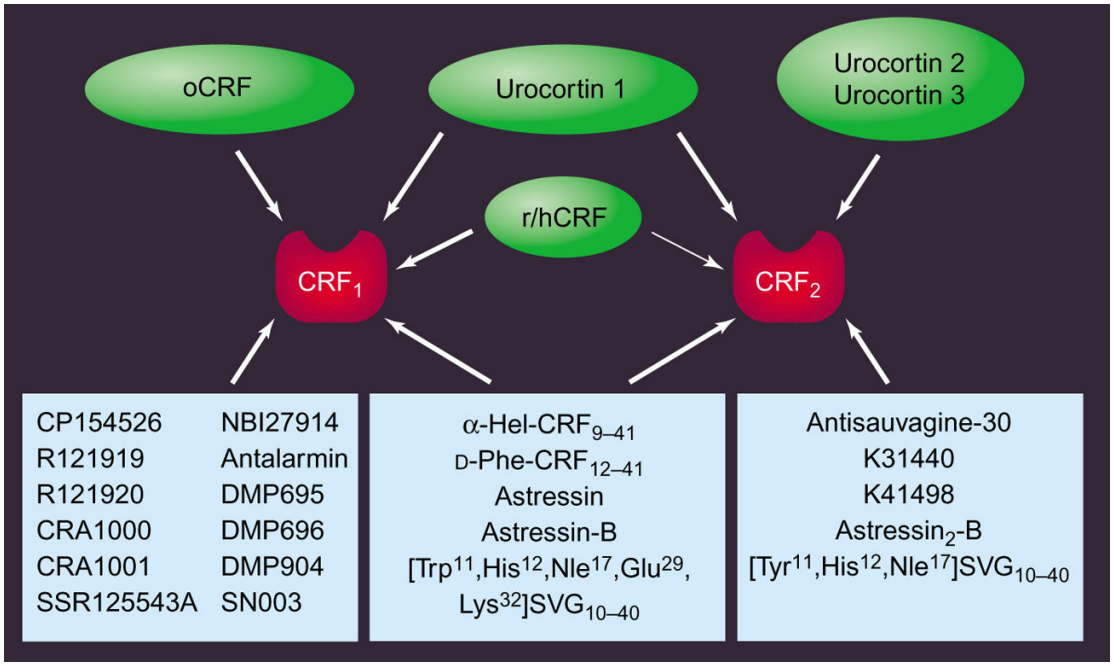

Figure 2.

Schematic of mammalian corticotropin-releasing factor (CRF) receptors (red polygons), their putative natural ligands (green ovals), and synthetic receptor antagonists (blue squares). Black arrows indicate receptor affinity. Grouped ligands are broadly ordered from top (earliest) to bottom in chronological order of reported discovery. oCRF, ovine CRF; r/hCRF, rat/human CRF. [Taken with permission from Zorrilla et al., 2003.]

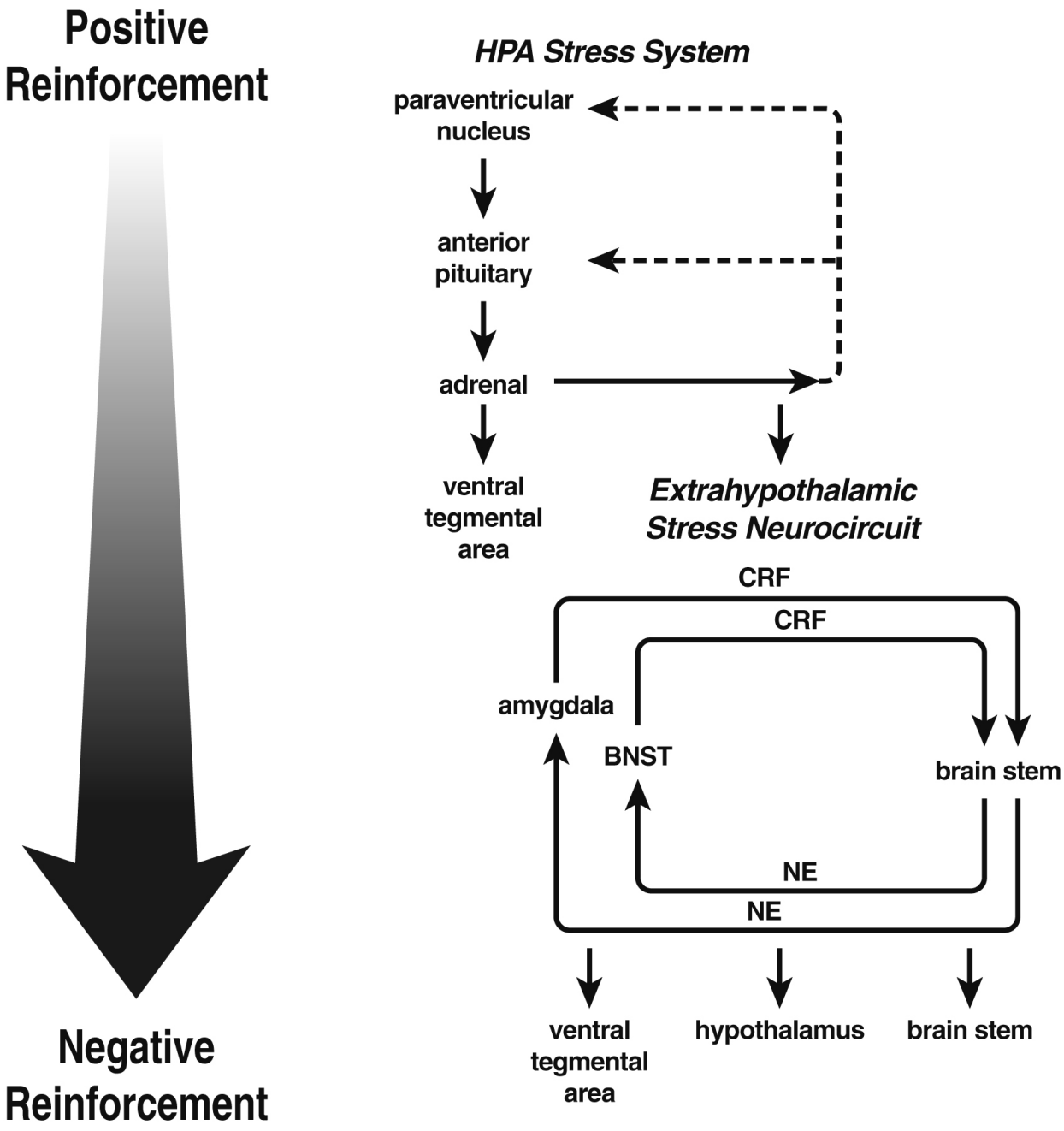

Figure 3.

Brain circuits hypothesized to be recruited at different stages of the addiction cycle as addiction moves from positive reinforcement to negative reinforcement. The top right circuit refers to the hypothalamic-pituitary-adrenal (HPA) axis which (*i*) feeds back to regulate itself, (*ii*) activates the brain reward neurocircuit, and (*iii*) facilitates the extrahypothalamic stress neurocircuit. The bottom right circuit refers to the extrahypothalamic brain stress circuits in feed-forward loops from the brain stem, some of which may be noradrenergic. The extrahypothalamic brain stress system outputs via the hypothalamus, brain stem, and ventral tegmental area to engage stress-like and fear-like behavioral responses. BNST, bed nucleus of the stria terminalis; NE, norepinephrine. [Adapted from Koob and Le Moal, 2004.]

Table 1

Behavioral effects of centrally administered CRF peptides

CRF Agonist	Paradigm	CRF Antagonist
• Suppresses exploration of unfamiliar environment	Elevated plus-maze	• Reverses stress-, drug-, and genotypically induced suppression of exploration
• Facilitates startle	Acoustic startle	• Blocks fear-potentiated startle
• Induces conditioned fear	Conditioned Emotional Response	• Blocks acquisition of conditioned emotional response
• Enhances stress-induced freezing	Cued electric shock	• Attenuates stress-induced freezing
• Decreases food intake	Deprivation-induced eating	• Reverses stress- and drug-induced anorexia
• Produces aversion	Taste/Place conditioning	• Weakens drug-induced place aversion
• Enhances sensitization	Amphetamine stereotypy	• Attenuates stress-induced sensitization
• Enhances defensive burying	Shock-probe	• Reduces defensive burying