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Review

A Role for Brain Stress Systems in Addiction

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Drug addiction is a chronically relapsing disorder characterized by compulsion to seek and take drugs and has been linked to dysregulation of brain regions that mediate reward and stress. Activation of brain stress systems is hypothesized to be key to the negative emotional state produced by dependence that drives drug seeking through negative reinforcement mechanisms. This review explores the role of brain stress systems (corticotropin-releasing factor, norepinephrine, orexin [hypocretin], vasopressin, dynorphin) and brain antistress systems (neuropeptide Y, nociceptin [orphanin FQ]) in drug dependence, with emphasis on the neuropharmacological function of extrahypothalamic systems in the extended amygdala. The brain stress and antistress systems may play a key role in the transition to and maintenance of drug dependence once initiated. Understanding the role of brain stress and antistress systems in addiction provides novel targets for treatment and prevention of addiction and insights into the organization and function of basic brain emotional circuitry.

1. Drugs, Addiction, and Stress: Introduction and Definitions

1.2. Dynamics of Addiction

Drug addiction is a chronically relapsing disorder characterized by compulsive drug use and loss of control over drug intake. Addiction 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). These three stages are conceptualized as feeding into one other, becoming more intense, and ultimately leading to the pathological state known as addiction (Koob and Le Moal, 1997). The *preoccupation/anticipation* (craving) stage of the addiction cycle has long been hypothesized to be a key element of relapse in humans and defines addiction as a chronic relapsing disorder (Tables 1 and 2).

Different drugs produce different patterns of addiction that engage different components of the addiction cycle, depending on dose, length of use, and even cultural factors. With opioids, the classic drugs of addiction, a pattern of compulsive intravenous or smoked drug taking evolves that includes intense intoxication, the development of tolerance, escalation in intake, and profound dysphoria, physical discomfort, and somatic and emotional withdrawal signs during abstinence. A pattern develops in which the drug must be obtained to avoid the severe dysphoria and discomfort experienced during abstinence. Alcohol addiction or alcoholism can follow a similar trajectory, but the pattern of oral drug taking often is characterized by binges of alcohol intake that can be daily episodes or prolonged days of heavy drinking and is characterized by a severe somatic and emotional withdrawal syndrome. Nicotine addiction contrasts with the above patterns, with little obvious signs of the *binge/intoxication* stage, and has a pattern of intake characterized by highly titrated intake of the drug except during periods of sleep and negative emotional states during abstinence, including dysphoria, irritability, and intense craving. Marijuana addiction follows a pattern similar to opioids and tobacco, with a significant intoxication stage, but as chronic use continues, subjects begin to show a pattern of use characterized by chronic intoxication during waking hours followed by a withdrawal that includes dysphoria, irritability, and sleep disturbances. Psychostimulant addiction (cocaine and amphetamines) shows a pattern with a salient *binge/intoxication* stage. Such binges can be hours or days in duration and often are followed by a withdrawal (“crash”) characterized by extreme dysphoria and inactivity. Intense craving for all drugs can anticipate withdrawal (i.e., with opioids, alcohol, nicotine) or often occurs after acute withdrawal when craving is driven by both environmental cues signifying the availability of the drug and internal states linked to negative emotional states and stress.

Animal models of the symptoms of addiction on specific drugs such as stimulants, opioids, alcohol, nicotine, and Δ^9-tetrahydrocannabinol can be defined by models relevant to different stages of the addiction cycle (Shippenberg and Koob, 2002) (Table 2). Animal models for the *binge/intoxication* stage of the addiction cycle can be conceptualized as measuring acute drug reward, in which reward can be defined as a positive reinforcer with some additional emotional value, such as pleasure (Table 1). Animal models of reward and reinforcement are extensive and well validated and include intravenous drug self-administration, conditioned place preference, and decreased brain reward thresholds. Animal models of the *withdrawal/negative affect* stage include conditioned place aversion (rather than preference) to precipitated withdrawal or spontaneous withdrawal from chronic administration of a drug, increases in brain reward thresholds, and dependence-induced increases in drug seeking (Table 2). Rodents will increase intravenous or oral self-administration of drugs with extended access to the drugs and during withdrawal from the dependent state, measured both by increased drug administration and increased work to obtain the

Table 1. Definitions

Term	Definition
Addiction	Also known as *substance dependence*, defined as a chronically relapsing disorder characterized by (1) compulsion to seek and take the drug and (2) loss of control in limiting intake. A third key element included by some, and particularly relevant to the present review, is the emergence of a negative emotional state (e.g., dysphoria, anxiety, irritability) when access to the drug is prevented (defined here as dependence) (Koob and Le Moal, 1997, 2008). *Addiction* throughout this article will be used interchangeably with *substance dependence* (as currently defined by the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition; American Psychiatric Association, 1994) and "dependence" with a lower-case "d" will be used to define the manifestation of a withdrawal syndrome when chronic drug administration is stopped (Koob and Le Moal, 2006).
Impulsivity	Defined behaviorally as a tendency toward rapid, unintended reactions to internal and external stimuli without regard for the negative consequences of these reactions.
Compulsivity	Defined as elements of behavior that result in perseveration in responding in the face of adverse consequences or perseveration in the face of incorrect responses in choice situations.
Positive reinforcer	Defined as any event that increases the probability of a response.
Negative reinforcer	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).
Opponent process	Hedonic, positive affective, or negative affective emotional states, once initiated, were hypothesized to be modulated automatically by the central nervous system with mechanisms that reduce the intensity of the emotional state. For addiction, the *a-process* consists of positive hedonic responses and occurs shortly after presentation of the drug and correlates closely with the stimulus intensity and quality and duration of the reinforcer and shows tolerance. In contrast, the *b-process* reflects a negative emotional state (dysphoria) that appears after the *a-process* has terminated. The *b-process* is sluggish in onset, slow to build up to an asymptote, slow to decay, and shows sensitization (i.e., becomes larger with repeated exposure).
Stress	Defined as responses to demands (usually noxious) upon the body (Selye, 1936) that historically have been defined by various physiological changes that include activation of the hypothalamic-pituitary-adrenal (HPA) axis. This activation is characterized by the release of adrenal steroids triggered by the release of adrenocorticotropic hormone (ACTH) from the pituitary. ACTH release, in turn, is controlled by the liberation of hypothalamic corticotropin-releasing factor (CRF) into the pituitary portal system of the median eminence. Another widely adopted definition of stress is any alteration in psychological homeostatic processes (Burchfield, 1979). The construct of stress subsequently has been linked to the construct of arousal and as such may represent the extreme pathological continuum of overactivation of the body's normal activational or emotional systems (Hennessy and Levine, 1979; Pfaff, 2006).
Within-system neuroadaptation	Repeated drug administration elicits an opposing reaction within the same system in which the drug elicits its primary reinforcing actions. For example, if the synaptic availability of the neurotransmitter dopamine is responsible for the acute reinforcing actions of cocaine, then the within-system opponent process neuroadaptation would be a decrease in synaptic availability of dopamine.
Between-system neuroadaptation	Repeated drug administration recruits a different neurochemical system, one not involved in the acute reinforcing effects of the drug but that when activated or engaged acts in opposition to the primary reinforcing effects of the drug. For example, chronic cocaine may activate the neuropeptide dynorphin, and dynorphin produces dysphoric-like effects that would be opposite to those of dopamine.

drug. Such increased self-administration in dependent animals has been observed with cocaine, methamphetamine, nicotine, heroin, and alcohol (Ahmed et al., 2000; Ahmed and Koob, 1998; Kitamura et al., 2006; O'Dell and Koob, 2007; Roberts et al., 2000). This model will be a key element for the evaluation of the role of brain stress systems in addiction outlined below.

Animal models of craving (*preoccupation/anticipation* stage) involve reinstatement of drug seeking following extinction from the drugs themselves, by cues linked to the drug, and from exposure to stressors (Shaham et al., 2003) (Table 1). Drug-induced reinstatement first involves extinction and then a priming injection of the drug. Latency to reinitiate responding or the amount of responding on the previously extinguished lever are hypothesized to reflect the motivation for drug-seeking behavior. Similarly, drug-paired or drug-associated stimuli can reinitiate drug-seeking behavior (cue-induced reinstatement). Stress-induced reinstatement involves the application of acute stressors that reinitiate drug-seeking behavior in animals that have been extinguished from the drug. These stressors can include physical stressors such as footshock, psychological stressors such as restraint, or pharmacological stressors such as yohimbine (Shaham et al., 2003). In rats with a history of dependence, protracted abstinence can be defined as a period after acute physical withdrawal has disappeared in which elevations in ethanol intake over baseline and increased stress responsivity persist (e.g., 2–8 weeks postwithdrawal from chronic ethanol). Protracted abstinence has been linked to increased

Table 2. Stages of the Addiction Cycle

Stage	Source of Reinforcement	Animal Models
Binge/intoxication	positive reinforcement	conditioned place preference, drug self-administration, decreased reward thresholds
Withdrawal/negative affect	negative reinforcement	increased anxiety-like responses, increased reward thresholds, conditioned place aversion, increased self-administration in dependence
Preoccupation/anticipation	conditioned positive reinforcement	drug-induced reinstatement, cue-induced, reinstatement, stress-induced reinstatement
	conditioned negative reinforcement	protracted abstinence

brain reward thresholds and increases in sensitivity to anxiety-like behavior that have been shown to persist after acute withdrawal in animals with a history of dependence. Stress-induced reinstatement of drug seeking and stress-induced reinstatement of anxiety-like states during protracted abstinence will be used in the present review to explore the role of the brain stress systems in the *preoccupation-anticipation* (craving) stage of the addiction cycle (Table 2).

The thesis of this review is that a key element of the addiction process involves a profound interaction with brain stress systems and dysregulation of brain antistress systems to produce the negative emotional state that becomes the powerful motivation for drug seeking associated with compulsive use in the *withdrawal/negative affect* and *preoccupation/anticipation* (craving) stages of the addiction cycle. Chronic use of drugs of abuse has long been associated with exaggerated responses to stressors, and these exaggerated responses contribute to addiction (Himmelsbach, 1941). Delineation of key elements of not only hormonal but also brain stress neurocircuits have laid the foundation for new insights into the pathophysiology of addiction.

1.3. Motivation, Opponent Process, and Stress

Motivation is a state that guides behavior in relationship to changes in the environment (Hebb, 1949) and shares key common characteristics with our concepts of arousal (Pfaff, 2006). Motivational states gain energy both from the external milieu (incentives) or internal milieu (central motive states or drives). As such, motivation or motivational states are not constant and vary over time but have long been hypothesized to have homeostatic constraints. In the context of temporal dynamics, Solomon and Corbit inextricably linked the concept of motivation with hedonic, affective, or emotional states in addiction by the opponent process theory of motivation (Solomon and Corbit, 1974) (Table 1).

More recently, opponent process theory has been expanded into the domains of the neurocircuitry and neurobiology of drug addiction from a physiological perspective (Koob and Le Moal, 2008). Counteradaptive processes such as opponent process that are part of the normal homeostatic limitation of reward function are hypothesized to fail to return to the normal homeostatic range and thus produce the reward deficits that are prominent in addiction. These counteradaptive processes were hypothesized to be mediated by two processes: within-system neuroadaptations and between-system neuroadaptations (Koob and Bloom, 1988) (Table 1).

For the present review, the systems activated as between-system neuroadaptations are hypothesized to involve the brain stress systems and the brain antistress systems. These circuits also can be conceptualized as an antireward homeostatic mechanism (Koob and Le Moal, 2008). In this framework, addiction is conceptualized as a cycle of spiraling dysregulation of brain reward/antireward mechanisms that progressively increases, resulting in the compulsive use of the drug. The purpose of this review is to explore the neuroadaptational changes that occur in the brain stress and antistress systems to account for the negative emotional state that provides motivation for the compulsivity of addiction.

1.4 Hypothalamic-Pituitary-Adrenal Axis

The hypothalamic-pituitary-adrenal (HPA) axis is defined by three major structures: the paraventricular nucleus of the hypothalamus, the anterior lobe of the pituitary gland, and the adrenal gland (for review, see Turnbull and Rivier, 1997). Neurosecretory neurons in the medial parvocellular subdivision of the paraventricular nucleus synthesize and release CRF into the portal blood vessels that 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. Vasopressin released from parvocellular neurons of the paraventricular nucleus produces synergistic effects on ACTH release that are mediated by vasopressin V_{1b} receptors. The HPA axis is finely tuned via negative feedback from circulating glucorticoids that act on the glucocorticoid receptor, a cytosolic protein that acts via the nucleus and transcriptional mechanisms, in two main brain areas: the paraventricular nucleus and the hippocampus. The hypophysiotropic neurons of the paraventricular nucleus of the hypothalamus are innervated by numerous afferent projections, including from brainstem, other hypothalamic nuclei, and forebrain limbic structures.

1.5. Extended Amygdala: Interface of Stress and Addiction

New functional observations have provided support for the hypothesis that the neuroanatomical substrates for many of the motivational effects of opponent processes associated with drug dependence may involve a common neural circuitry that forms a separate entity within the basal forebrain, termed the "extended amygdala" (Koob and Le Moal, 2001). The extended amygdala represents a macrostructure that is composed of several basal forebrain structures: the bed nucleus of the stria

terminalis, the central medial amygdala, and a transition zone in the posterior part of the medial nucleus accumbens (i.e., posterior shell) (Heimer and Alheid, 1991). These structures have similarities in morphology, immunohistochemistry, and connectivity, and they receive afferent connections from limbic cortices, 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 and Alheid, 1991). Key elements of the extended amygdala include not only neurotransmitters associated with the positive reinforcing effects of drugs of abuse but also major components of the brain stress systems associated with the negative reinforcement of dependence (Koob and Le Moal, 2005). The role of specific neuropharmacological mechanisms associated with the brain stress systems and the extended amygdala will be explored in the sections below.

2. Brain Stress Systems and Addiction: Corticotropin-Releasing Factor, Norepinephrine, Orexin, Vasopressin, Dynorphin

2.1. Corticotropin-Releasing Factor

Corticotropin-releasing factor is a 41 amino acid polypeptide that controls hormonal, sympathetic, 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 (Swanson et al., 1983) (Figure 1). The CRF_1 receptor has abundant, widespread expression in the brain that overlaps significantly with the distribution of CRF and urocortin 1. The discovery of other peptides with structural homology, notably the urocortin family (urocortins 1, -2, and -3), has suggested broad neurotransmitter roles for the CRF systems in behavioral and autonomic responses to stress (Bale and Vale, 2004) (see Supplemental Data available online). Urocortin 1 binds both to CRF_1 and CRF_2 receptors and has a different neuroanatomical distribution than CRF. The type 2 urocortins, urocortin 2 (Reyes et al., 2001) and urocortin 3 (Lewis et al., 2001), differ from urocortin 1 and CRF in their neuroanatomical, neuropharmacological, and distribution profiles and are endogenous selective CRF_2 agonists.

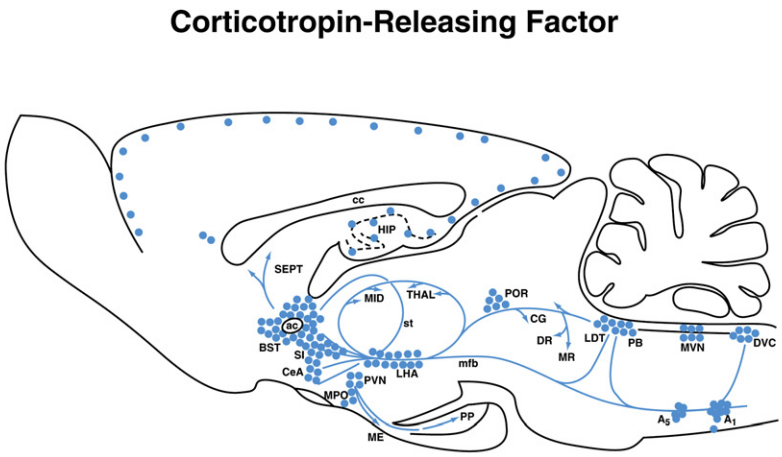
CRF in the paraventricular nucleus of the hypothalamus controls the pituitary adrenal response to stress (Turnbull and Rivier, 1997). Progressive changes in the HPA axis are observed during the transition from acute administration to chronic administration of drugs of abuse. Acute administration of most drugs of abuse in animals activates the HPA axis and may first facilitate activity in the brain motivational circuits, facilitate 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). With repeated administration of cocaine, opiates, nicotine, and alcohol, these acute changes are blunted or dysregulated (Kreek and Koob, 1998; Rasmussen et al., 2000; Goeders, 2002; Koob and Kreek, 2007; Sharp and Matta, 1993; Semba et al., 2004). An early hypothesis was that atypical responsivity to stressors contributes 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 current thesis, high circulating levels of glucocorticoids can feed back to shut off the HPA axis but can "sensitize" CRF systems in the central nucleus of the amygdala and norepinephrine systems in the basolateral amygdala that are 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, while activation of the HPA axis may characterize initial drug use and the *binge/intoxication* stage of addiction, the HPA activation also can lead to subsequent activation of extrahypothalamic brain stress systems that characterize the *withdrawal/negative affect* stage of addiction (Kreek and Koob, 1998; Koob and Le Moal, 2005; Koob and Kreek, 2007) (Figure 2).

Substantial evidence now suggests that brain extrahypothalamic CRF systems are activated during the development of dependence on alcohol, and this activation has motivational significance. During ethanol withdrawal, CRF release increases within the central nucleus of the amygdala and bed nucleus of the stria terminalis of dependent rats (Funk et al., 2006; Merlo-Pich et al., 1995; Olive et al., 2002) (Figures 1B and 2), and this 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, systemic CRF_1 antagonists (Overstreet et al., 2004) or the subtype nonselective CRF receptor antagonists α-helical $CRF_{9\text{-}41}$ and D-Phe $CRF_{12\text{-}41}$ when injected intracerebroventricularly (Baldwin et al., 1991) or directly into the central nucleus of the amygdala (Rassnick et al., 1993) reduced ethanol withdrawal-induced anxiety-like behavior.

Exposure to repeated cycles of chronic ethanol vapor to induce dependence substantially increased ethanol intake in rats, both during acute withdrawal and during protracted abstinence (2 weeks postacute withdrawal) (O'Dell et al., 2004; Rimondini et al., 2002). Intracerebroventricular administration and direct intracerebral administration into the central nucleus of the amygdala of a CRF_1/CRF_2 peptide antagonist selectively blocked the dependence-induced increase in ethanol self-administration during acute withdrawal (Valdez et al., 2004). Systemic injections of small-molecule CRF_1 antagonists also blocked the increased ethanol intake associated with acute ethanol withdrawal (Knapp et al., 2004; Funk et al., 2007; Richardson et al., 2008) (Figure 3). A CRF_2 agonist injected into the central nucleus of the amygdala had a similar effect in reducing the increase in ethanol self-administration associated with acute withdrawal, suggesting a role for CRF_2 receptors opposite to that of CRF_1 receptors in modulating ethanol intake in dependent animals (Funk and Koob, 2007). 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 (Valdez et al., 2004; Funk et al., 2006). None of the CRF antagonists had any effects on ethanol self-administration in nondependent

A



B

Drug	Withdrawal-induced changes in extracellular CRF in CeA	CRF Antagonist Effects: Withdrawal-induced anxiety-like or aversive responses	CRF Antagonist Effects: Baseline self-administration or place preference	CRF Antagonist Effects: Dependence-induced increases in self-administration	CRF Antagonist Effects: Stress-induced reinstatement
Cocaine	↑	↓	—	↓	↓
Opioids	↑	↓	—	↓	↓
Ethanol	↑	↓	—	↓	↓
Nicotine	↑	↓	—	↓	↓
Δ^9-THC	↑	↓			

—, no effect; blank entries indicate not tested. CeA, central nucleus of the amygdala.

Figure 1. Localizations and Projections of Brain Stress Systems—Corticotropin-Releasing Factor

(A) The major CRF-stained cell groups (dots) and fiber systems in the rat brain. Most of the immunoreactive cells and fibers appear to be associated with systems that regulate the output of the pituitary and the autonomic nervous system and with cortical interneurons. Most of the longer central fibers course either ventrally through the medial forebrain bundle and its caudal extension in the reticular formation, or dorsally through a periventricular system in the thalamus and brainstem central gray. The direction of fibers in these systems is unclear because they appear to interconnect regions that contain CRF-stained cell bodies. Three adjacent CRF-stained cell groups—laterodorsal tegmental nucleus, locus coeruleus, parabrachial nucleus—lie in the dorsal pons. Uncertain is which of these cell groups contributes to each of the pathways shown, and which of them receives inputs from the same pathways. Modified with permission from Swanson et al. (1983). ac, anterior commissure; BST, bed nucleus of the stria terminalis; cc, corpus callosum; CeA, central nucleus of the amygdala; CG, central gray; DR, dorsal raphe; DVC, dorsal vagal complex; HIP, hippocampus; LDT, laterodorsal tegmental nucleus; LHA, lateral hypothalamic area; ME, median eminence; mfb, medial forebrain bundle; MID THAL, midline thalamic nuclei; MPO, medial preoptic area; MR, median raphe; MVN, medial vestibular nucleus; PB, parabrachial nucleus; POR, perioculomotor nucleus; PP, peripeduncular nucleus; PVN, paraventricular nucleus; SEPT, septal region; SI, substantia innominata; st, stria terminalis.

(B) Role of corticotropin-releasing factor in dependence.

rats (Valdez et al., 2004). 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.

Increased expression of CRF_1 receptors is associated with stress-induced ethanol intake in Marchigian Sardinian (msP) alcohol-preferring rats (Hansson et al., 2006) as well as in nongenetically selected animals in a postdependent state (Sommer et al., 2008). In the genetically selected msP rat line, high ethanol preference was 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 nongenetically 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). Adolescents homozygous for the C allele of R1876831 located on an intron that could potentially influence transcription of the CRF_1 receptor gene 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). 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 and, perhaps more exciting, may predict responsiveness to the use of CRF receptor antagonists in the treatment of alcoholism.

Similar interactions with CRF have been observed with the dependence associated with cocaine, heroin, and nicotine. Chronic administration of cocaine produces an anxiety-like response that is blocked by intracerebroventricular administration of a CRF_1/CRF_2 antagonist (Sarnyai et al., 1995; Basso et al., 1999). A CRF_1/CRF_2 peptide antagonist injected into the central nucleus of the amygdala and systemic administration of CRF_1 antagonists blocked conditioned place aversion associated with precipitated opiate withdrawal (Heinrichs et al., 1995; Stinus et al., 2005). Opioid withdrawal also increased CRF release in the amygdala, measured by in vivo microdialysis (Weiss et al., 2001). CRF_1 knockout mice failed to show conditioned place aversion to opioid withdrawal and failed to show an opioid-induced increase in dynorphin mRNA in the nucleus accumbens (Contarino and Papaleo, 2005). A CRF antagonist injected intracerebroventricularly blocked the anxiogenic-like effects of withdrawal from bolus injections of nicotine (Tucci et al., 2003). The anxiogenic-like effects of precipitated withdrawal from chronic nicotine also were blocked by a CRF_1 receptor antagonist (George et al., 2007) (Figure 2). A CRF_1/CRF_2 peptide antagonist also blocked the nicotine withdrawal-induced increase in brain

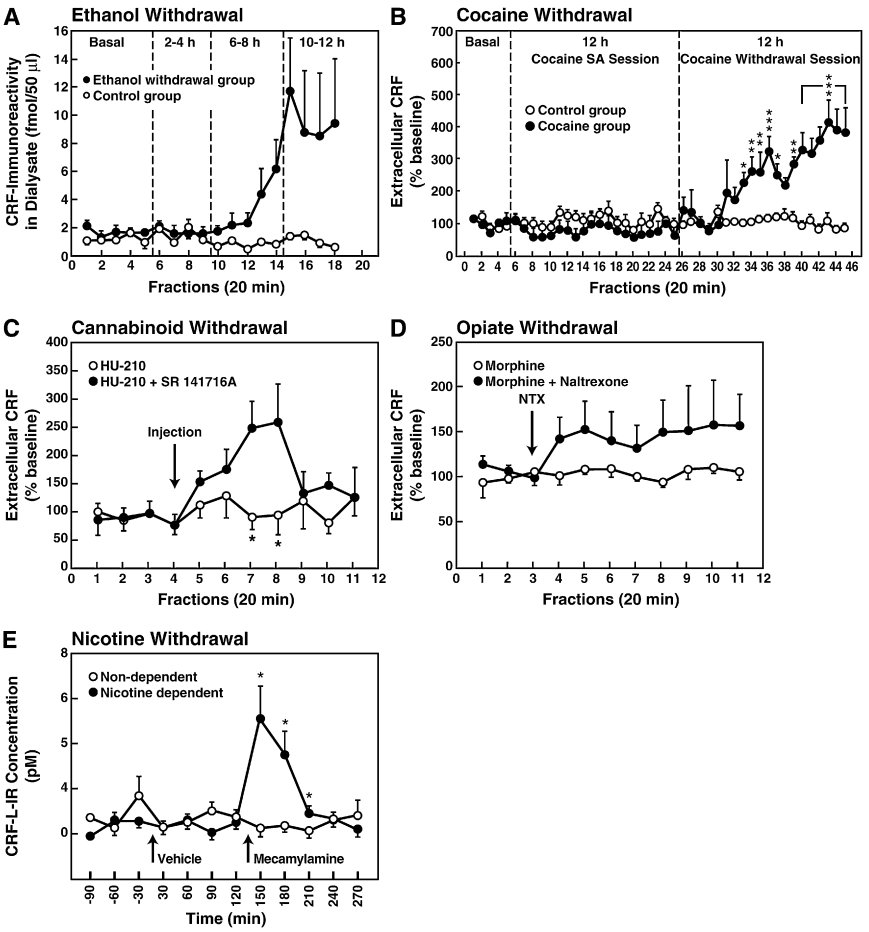


Figure 2. Effects of Drug Withdrawal on CRF Levels in the Amygdala

(A) Effects of ethanol withdrawal on CRF-like immunoreactivity in the rat amygdala determined by microdialysis. Dialysate was collected over four 2 hr periods regularly alternated with nonsampling 2 hr periods. The four sampling periods corresponded to the basal collection (before removal of ethanol), and 2–4 hr, 6–8 hr, and 10–12 hr after withdrawal. Fractions were collected every 20 min. Data are represented as mean ± SEM (n = 5 per group). ANOVA confirmed significant differences between the two groups over time ($p < 0.05$). Taken with permission from Merlo-Pich et al. (1995).

(B) Mean (±SEM) dialysate CRF concentrations collected from the central nucleus of the amygdala of rats during baseline, 12 hr cocaine self-administration, and a subsequent 12 hr withdrawal period (Cocaine group, n = 5). CRF levels in rats with the same history of cocaine self-administration training and drug exposure but not given access to cocaine on the test day (Control group, n = 6). Data are expressed as percentages of basal CRF concentrations. Dialysates were collected over 2 hr periods alternating with 1 hr nonsampling periods shown by the timeline at the top. During cocaine self-administration, dialysate CRF concentrations in the cocaine group were decreased by about 25% compared with control animals. In contrast, termination of access to cocaine resulted in a significant increase in CRF release, which began ~5 hr post-cocaine and reached about 400% of presession baseline levels at the end of the withdrawal session. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Simple effects after overall mixed-factorial analysis of variance. Taken with permission from Richter and Weiss (1999).

(C) Effects of cannabinoid CB_1 antagonist SR 141716A (3 mg/kg) on CRF release from the central nucleus of the amygdala in rats pretreated for 14 days with cannabinoid CB_1 agonist HU-210 (100 mg/kg). Cannabinoid withdrawal induced by SR 141716A was associated with increased CRF release (*$p < 0.005$, n = 5–8). Vehicle injections did not alter CRF release (n = 5–7). Data were standardized by transforming dialysate CRF concentrations into percentages of baseline values based on averages of the first four fractions. Data are shown as mean ± SEM. Taken with permission from Rodriguez de Fonseca et al. (1997).

(D) Effects of morphine withdrawal on CRF release in the central nucleus of the amygdala. Withdrawal was precipitated by administration of naltrexone (0.1 mg/kg) in rats prepared with chronic morphine pellet implants. Data are shown as mean ± SEM. Taken with permission from Weiss et al. (2001).

(E) Effect of mecamylamine (1.5 mg/kg, i.p.) precipitated nicotine withdrawal on CRF release in the central nucleus of the amygdala measured by in vivo microdialysis in chronic nicotine pump-treated (nicotine-dependent, n = 7) and chronic saline pump-treated (nondependent, n = 6) rats. *$p < 0.05$ compared with nondependent. Data are shown as mean ± SEM. Taken with permission from George et al. (2007).

reward thresholds (Bruijnzeel et al., 2007). Continuous access to intravenous self-administration of cocaine for 12 hr, precipitated opioid withdrawal, and precipitated nicotine withdrawal increased CRF release in the amygdala during the withdrawal, measured by in vivo microdialysis (Richter and Weiss, 1999; Weiss et al., 2001; George et al., 2007) (Figure 2). Systemic administration of CRF_1 antagonists reversed the increased self-administration of cocaine, heroin, and nicotine associated with extended access (Specio et al., 2008; George et al., 2007; T.N. Greenwell, C.K. Funk, P. Cottone, H.N. Richardson, S.A. Chen, K. Rice, M.J. Lee, E.P. Zorrilla, and G.F.K., unpublished data).

The role of CRF in stress-induced reinstatement of drug seeking follows a pattern of results similar to its role 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) (Figure 1B). Mixed CRF_1/CRF_2 antagonists injected intracerebroventricularly and/or CRF_1 small-molecule antagonists blocked stress-induced reinstatement of cocaine, opiate, alcohol, and nicotine intake (Erb et al., 1998; Lu et al., 2001; Shaham et al., 1997, 1998; Shalev et al., 2006; Le et al., 2000; Liu and Weiss, 2002; Gehlert et al., 2007; Hansson et al., 2006; Zislis 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, and ventral tegmental area, but not the amygdala or nucleus accumbens (Le et al., 2002; Erb et al., 2001; Erb and Stewart, 1999; Wang et al., 2006, 2007), suggesting that different sites, such as the bed nucleus of the stria terminalis, median raphe, and ventral tegmental area, may be important for stress-induced relapse, in contrast to the role of CRF in dependence-induced drug self-administration that has been localized to the central nucleus of the amygdala (Funk et al., 2006).

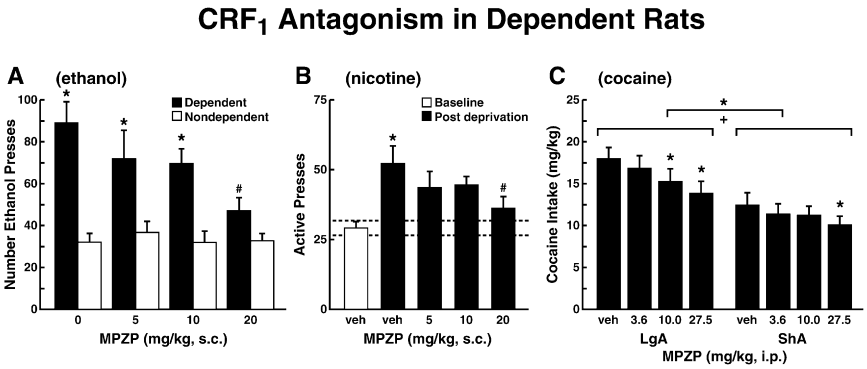


Figure 3. Effect of CRF_1 Receptor Antagonist on Alcohol and Nicotine Self-Administration in Dependent Rats

(A) The effect of small-molecule CRF_1 receptor antagonist MPZP on operant self-administration of alcohol (g/kg) in dependent and nondependent rats. Testing was conducted when dependent animals were in acute withdrawal (6–8 hr after removal from vapors). Dependent animals self-administered significantly more alcohol than nondependent animals. MPZP significantly reduced alcohol self-administration only in dependent animals. MPZP had no effect on alcohol self-administration in nondependent animals. *$p < 0.05$ compared with nondependent controls. #$p < 0.05$ compared with vehicle (0 mg/kg MPZP). Data are shown as mean ± SEM (n = 8 per vapor treatment group). Taken with permission from Richardson et al. (2008).

(B) The effect of small-molecule CRF_1 receptor antagonist MPZP on nicotine self-administration during the active period in rats given extended access to nicotine (*$p < 0.05$ versus baseline, #$p < 0.05$ versus post abstinence vehicle treatment, n = 8). Data are shown as mean ± SEM. Taken with permission from George et al. (2007).

(C) The effect of small-molecule CRF_1 receptor antagonist MPZP on cocaine intake in short-access (ShA) and long-access (LgA) rats. MPZP dose-dependently reduced cocaine intake, achieving a maximal reduction of ~20%, with a greater effect in LgA compared to ShA rats. A main effect for Access (*$p < 0.05$), a main effect for MPZP dose ($p < 0.001$), and a significant access × MPZP dose interaction (+$p < 0.05$) were observed. Data are expressed as mean (+SEM) cocaine intake (mg/kg). Taken with permission from Specio et al. (2008).

In summary, the extrahypothalamic CRF systems play a role in mediating the anxiety-like effects of acute withdrawal, the increase in drug-taking associated with dependence, and stress-induced reinstatement for all major drugs of abuse, including psychostimulants, opioids, ethanol, nicotine, and (with limited studies) cannabinoids. Many of these effects have been localized to the extended amygdala, and acute withdrawal from all major drugs of abuse increased CRF release in the central nucleus of the amygdala, measured by in vivo microdialysis (Figures 1B and 2). This pattern of results suggests a major role for CRF in mediating the negative emotional states that have motivational significance in maintaining the dependent state (Koob and Le Moal, 2005; Bruijnzeel and Gold, 2005).

2.2. Norepinephrine

Norepinephrine is a well established neurotransmitter in the central nervous system with widespread distribution throughout the brain (Figure 4) and has hypothesized functions in arousal, attention, stress, anxiety, and affective disorders (see Supplemental Data). Cell bodies for the brain norepinephrine systems originate in the dorsal pons and brainstem. The locus coeruleus in the dorsal pons is the source of the dorsal noradrenergic pathway to the cortices and hippocampus, and the brainstem projections converge in the ventral noradrenergic bundle to innervate the basal forebrain and hypothalamus.

Norepinephrine binds to three distinct families of receptors—α_1, α_2, and β-adrenergic—each with three receptor subtypes (Rohrer and Kobilka, 1998). The α_1 receptor family comprises α_{1a}, α_{1b}, and α_{1d}. Each subtype activates phospholipase C and α_2 and are coupled to the inositol phosphate second messenger system via the G protein G_q. A centrally active α_1 receptor antagonist used in drug dependence research is prazosin. The α_2 family comprises α_{2a}, α_{2b}, and α_{2c}. Each subtype inhibits adenylate cyclase via coupling to the inhibitory G protein G_i. Two α_2 drugs commonly used in drug-dependence research are the α_2 agonist clonidine and the α_2 antagonist yohimbine. Because the α_2 receptor is hypothesized to be presynaptic, these drugs inhibit and facilitate noradrenergic function, respectively. The β-adrenergic receptor family comprises β_1, β_2, and β_3. Each subtype activates adenylate cyclase via coupling to the G protein G_s. Few β-adrenergic drugs have been explored in drug-dependence research, with the exception of the β-adrenergic antagonist propranolol, presumably because of poor brain bioavailability.

Precipitated morphine withdrawal increases norepinephrine release in the central nucleus of the amygdala and bed nucleus of the stria terminalis (Watanabe et al., 2003; Fuentealba et al., 2000). The noradrenergic α_2 agonist clonidine, a functional norepinephrine antagonist with presynaptic actions, blocked the suppression in responding for food during opioid withdrawal, a measure of the motivational component of opioid withdrawal (Sparber and Meyer, 1978) and the aversive stimulus effects (conditioned place aversions) of opioid withdrawal (Schulteis et al., 1998). Increased anxiety-like behavior was observed during cocaine and morphine withdrawal in rats and was blocked by the β-adrenergic antagonists propranolol and atenolol (Harris and Aston-Jones, 1993; Gold et al., 1980). Similar effects were observed with direct injections of a β-adrenergic antagonist directly into the central nucleus of the amygdala (Rudoy and van Bockstaele, 2007). Norepinephrine functional antagonists (β_1 antagonist and α_2 agonist) injected into the lateral bed nucleus of the stria terminalis blocked precipitated opiate withdrawal-induced place aversions (Delfs et al., 2000), and β-adrenergic antagonists produced similar effects when injected into the central nucleus of the amygdala (Watanabe et al., 2003). Studies that further localized the effects of norepinephrine in driving opioid withdrawal showed that ventral noradrenergic bundle lesions attenuated opioid withdrawal (Delfs et al., 2000), but virtually complete lesions of the dorsal noradrenergic bundle from the locus coeruleus with the neurotoxin 6-hydroxydopamine failed to block the place aversion produced by opioid withdrawal-induced place aversion (Caille et al., 1999). Consistent with the studies of the aversive effects of opioid withdrawal, the α_1

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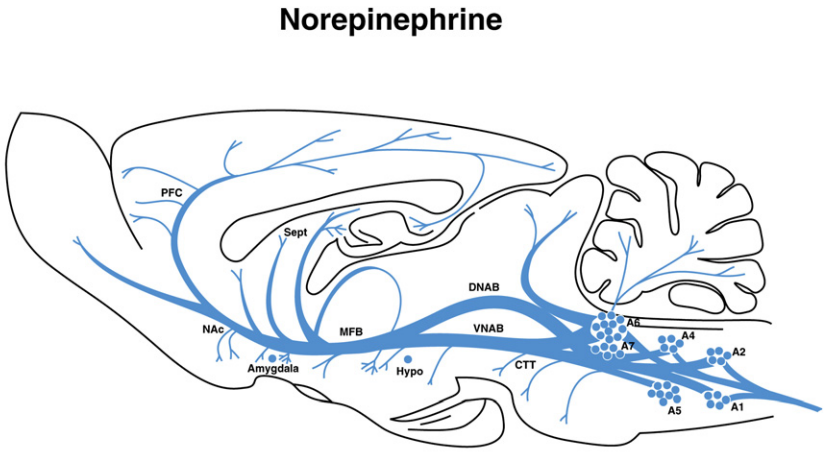

B

Drug	Withdrawal-induced changes in extracellular norepinephrine in CeA	Noradrenergic Antagonist Effects: Withdrawal-induced anxiety-like or aversive responses	Baseline self-administration or place preference	Dependence-induced increases in self-administration	Stress-induced reinstatement
Cocaine		↓		↓	↓
Opioids	↑	↓		↓	↓
Ethanol		↓	↓	↓	↓
Nicotine					↓

—, no effect; blank entries indicate not tested. CeA, central nucleus of the amygdala.

Figure 4. Localizations and Projections of Brain Stress Systems—Norepinephrine

(A) Origin and distribution of central noradrenergic pathways in the rat brain. Note noradrenergic cell groups A1–A7, including the locus coeruleus (A6). Modified with permission from Robbins and Everitt (1995). PFC, prefrontal cortex; Sept, septum; NAc, nucleus accumbens; MFB, medial forebrain bundle; Hypo, hypothalamus; DNAB, dorsal noradrenergic ascending bundle; VNAB, ventral noradrenergic ascending bundle; CTT, central tegmental tract.

(B) Role of norepinephrine in dependence.

norepinephrine antagonist prazosin reduced heroin self-administration in dependent rats with extended access (Greenwell et al., 2008). Prazosin also selectively blocked the increased motivation to intravenously self-administer cocaine on a progressive-ratio schedule in rats with extended access to the drug (a procedure that is hypothesized to produce dependence) (Wee et al., 2008). The extended-access rats showed a decreased number of neurons with α_1 adrenergic-like immunoreactivity in the bed nucleus of the stria terminalis, suggesting that the α_1 noradrenergic system in the bed nucleus of the stria terminalis also may be involved in cocaine dependence (Wee et al., 2008).

Substantial evidence also has accumulated suggesting that, in animals and humans, central noradrenergic systems are activated during acute withdrawal from ethanol and may have motivational significance. Alcohol withdrawal in humans is associated with activation of noradrenergic function, and the signs and symptoms of alcohol withdrawal in humans are blocked by postsynaptic β-adrenergic blockade (Romach and Sellers, 1991). Alcohol withdrawal signs also are blocked in animals by administration of α_1 antagonists and β-adrenergic antagonists and selective blockade of norepinephrine synthesis (Trzaskowska and Kostowski, 1983). In dependent rats, the α_1 antagonist prazosin selectively blocked the increased drinking associated with acute withdrawal (Walker et al., 2008). Thus, converging data suggest that disruption of noradrenergic function blocks ethanol reinforcement, that noradrenergic neurotransmission is enhanced during ethanol withdrawal, and that noradrenergic functional antagonists can block aspects of ethanol withdrawal.

Chronic nicotine self-administration (23 hr access) increases norepinephrine release in the paraventricular nucleus of the hypothalamus and the amygdala, measured by in vivo microdialysis (Fu et al., 2001, 2003). However, during the late maintenance phase of 23 hr access to nicotine, norepinephrine release was no longer elevated in the amygdala, suggesting some desensitization/tolerance-like effect (Fu et al., 2003).

The role of norepinephrine in stress-induced reinstatement also follows a pattern of results similar to its role 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). The α_2 adrenergic agonist clonidine decreased stress-induced reinstatement of cocaine, opiate, alcohol, and nicotine seeking (Le et al., 2005; Erb et al., 2000; Shaham et al., 2000; Zislis et al., 2007). The α_2 antagonist yohimbine reinstated drug seeking (Lee et al., 2004). Limited studies with intracerebral injections also have localized the effects of functional blockade of norepinephrine system on stress-induced reinstatement of morphine conditioned place preferences to the bed nucleus of the stria terminalis (Wang et al., 2001). β-adrenergic antagonists administered systemically also blocked stress-induced reinstatement of cocaine seeking (Leri et al., 2002).

2.3. Dynorphin/κ Opioid System

Dynorphins are opioid peptides that derive from the prodynorphin precursor and contain the leucine (leu)-enkephalin sequence at the N-terminal portion of the molecule and are the presumed endogenous ligands for the κ opioid receptor (Chavkin et al., 1982). Dynorphins have widespread distribution in the central nervous system (Watson et al., 1982) (Figure 5) and play a role in a wide variety of physiological systems, including neuroendocrine regulation, pain regulation, motor activity, cardiovascular function, respiration, temperature regulation, feeding behavior, and stress responsivity (Fallon and Leslie, 1986) (see Supplemental Data). Possible products of prodynorphin processing include dynorphin A(1-17), dynorphin A(1-8), and dynorphin B(1-29). Immunocytochemical distribution of dynorphin A and -B shows significant cell bodies and terminals in addiction-relevant brain areas such as the nucleus

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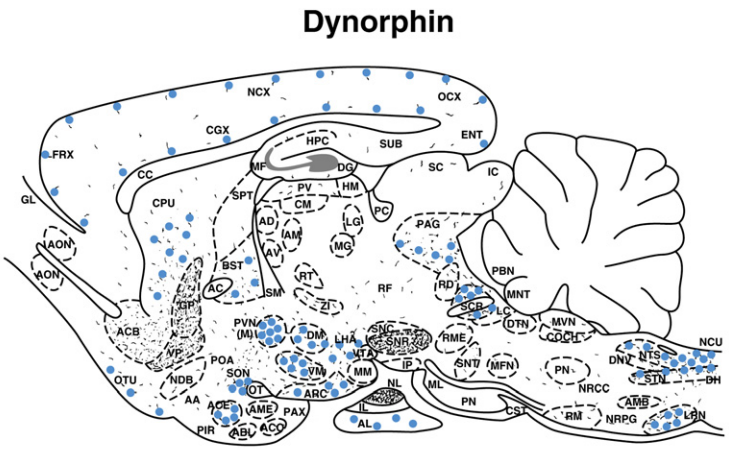

B

Drug	Withdrawal-induced changes in dynorphin peptide and prodynorphin mRNA in NAc	κ-Opioid Antagonist Effects			
		Withdrawal-induced anxiety-like or aversive responses	Baseline self-administration or place preference	Dependence-induced increases in self-administration	Stress-induced reinstatement
Cocaine	↑		—		↓
Opioids	↑		—	↓	
Ethanol	↑		↑	↓	
Δ^9-THC			↓		

—, no effect; blank entries indicate not tested. NAc, nucleus accumbens.

Figure 5. Localizations and Projections of Brain Stress Systems—Dynorphin

(A) Schematic representation of the distribution of prodynorphin-derived peptides in the rat central nervous system determined by immunohistochemistry. Prodynorphin codes for several active opioid peptides containing the sequence of [Leu]enkephalin, including dynorphin A, dynorphin B, and α-neoendorphin. This precursor is distributed in neuronal systems found at all levels of the neuraxis. Like their proenkephalin counterparts, the prodynorphin neurons form both short- and long-tract projections often found in parallel with the proenkephalin systems. Neuronal perikarya are shown as solid circles, and fibers-terminals as short curved lines and dots. Modified with permission from Khachaturian et al. (1985). AA, anterior amygdala; ABL, basolateral nucleus of amygdala; AC, anterior commissure; ACB, nucleus accumbens; ACE, central nucleus of the amygdala; ACO, cortical nucleus of amygdala; AD, anterodorsal nucleus of thalamus; AL, anterior lobe of pituitary; AM, anteromedial nucleus of thalamus; AMB, nucleus ambiguus; AME, medial nucleus of the amygdala; AON, anterior olfactory nucleus; ARC, arcuate nucleus; AV, anteroventral nucleus of thalamus; BST, bed nucleus of the stria terminalis; CC, corpus callosum; CGX, cingulate cortex; CM, central-medial nucleus of thalamus; COCH, cochlear nuclear complex; CPU, caudate-putamen; CST, corticospinal tract; DH, dorsal horn of spinal cord; DG, dentate gyrus; DM, dorsomedial nucleus of hypothalamus; DNV, dorsal motor nucleus of vagus; DTN, dorsal tegmental nucleus; ENT, entorhinal cortex; FN, fastigial nucleus of cerebellum; FRX, frontal cortex; GL, glomerular layer of olfactory bulb; GP, globus pallidus; HM, medial habenular nucleus; HPC, hippocampus; IC, inferior colliculus; IL, intermediate lobe of pituitary; IP, interpeduncular nuclear complex; LC, nucleus locus coeruleus; LG, lateral geniculate nucleus; LHA, lateral hypothalamic area; LRN, lateral reticular nucleus; MF, mossy fibers of hippocampus; MFN, motor facial nucleus; MG, medial geniculate nucleus; ML, medial lemniscus; MM, medial mammillary nucleus; MNT, mesencephalic nucleus of trigeminal; MVN, medial vestibular nucleus; NCU, nucleus cuneatus; NCX, neocortex; NDB, nucleus of diagonal band; NL, neural lobe of pituitary; NRGC, nucleus reticularis gigantocellularis; NRPG, nucleus reticularis paragigantocellularis; NTS, nucleus tractus solitarius; OCX, occipital cortex; OT, optic tract; OTU, olfactory tubercle; PAG, periaqueductal gray; PAX, periamygdaloid cortex; PBN, parabrachial nucleus; PC, posterior commissure; PIR, piriform cortex; PN, pons; POA, preoptic area; PP, perforant path; PV, periventricular nucleus of thalamus; PVN(M), paraventricular nucleus (pars magnocellularis); PVN(P), paraventricular nucleus (pars parvocellularis); RD, nucleus raphe dorsalis; RE, nucleus reuniens of thalamus; RF, reticular formation; RM, nucleus raphe magnus; RME, nucleus raphe medianus; SC, superior colliculus; SCP, superior cerebellar peduncle; SM, stria medullaris thalami; SNC, substantia nigra (pars compacta); SNR, substantia nigra (pars reticulata); SNT, sensory nucleus of trigeminal (main); SON, supraoptic nucleus; SPT, septal nuclei; STN, spinal nucleus of trigeminal; SUB, subiculum; VM, ventromedial nucleus of hypothalamus; VP, ventral pallidum; ZI, zona incerta.

(B) Role of dynorphin in dependence.

accumbens, central nucleus of the amygdala, bed nucleus of the stria terminalis, and hypothalamus (Fallon and Leslie, 1986). Dynorphins bind to all three opioid receptors but show a preference for κ receptors (Chavkin et al., 1982). Activation of the dynorphin/κ receptor system produces actions similar to other opioids but often actions that are opposite to those of μ opioid receptors in the motivational domain, in which dynorphins produce aversive dysphoric-like effects in animals and humans (Shippenberg et al., 2007).

Dynorphin has long been hypothesized to mediate negative emotional states. κ receptor agonists produce place aversions (Shippenberg et al., 2007) and depression and dysphoria in humans (Pfeiffer et al., 1986). The activation of dynorphin systems in the nucleus accumbens has long been associated with activation of the dopamine systems by cocaine and amphetamine. Activation of dopamine D_1 receptors stimulates a cascade of events that ultimately leads to cAMP response-element binding protein (CREB) phosphorylation and subsequent alterations in gene expression, notably the activation of expression of protachykinin and prodynorphin mRNA. The subsequent activation of dynorphin systems could contribute to the dysphoric syndrome associated with cocaine dependence and also feedback to decrease dopamine release (Nestler, 2005). Activation of dynorphin systems also may mediate a dysphoric component of stress (Land et al., 2008; McLaughlin et al., 2003).

The evidence for a role of the dynorphin/κ opioid system in the neuroadaptive actions of other drugs of abuse is based both on biochemical and antagonist studies. Substantial evidence suggests that dynorphin peptide and gene expression are activated in the striatum, ventral striatum, and amygdala during acute and chronic administration of cocaine and alcohol (Spangler et al., 1993; Daunais et al., 1993; Lindholm et al., 2000). Chronic binge patterns of cocaine administration increase μ and κ opioid receptor density in the nucleus accumbens, cingulate cortex, and basolateral amygdala (Unterwald et al., 1994).

A highly selective κ agonist, when administered chronically via minipump, potentiated the alcohol deprivation effect in rats with long-term ethanol experience, but acute injection of a κ

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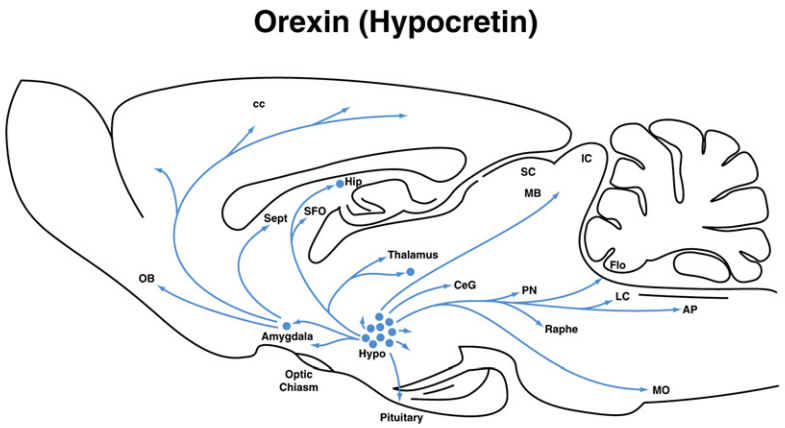

B

Drug	Withdrawal-induced changes in orexin in CeA	Orexin Antagonist Effects: Withdrawal-induced anxiety-like or aversive responses	Baseline self-administration or place preference	Dependence-induced increases in self-administration	Stress-induced reinstatement
Cocaine			—		↓
Opioids			↓		
Ethanol			↓	↓	

—, no effect; blank entries indicate not tested. CeA, central nucleus of the amygdala.

Figure 6. Localizations and Projections of Brain Stress Systems—Orexin (Hypocretin)
(A) Dots indicate the relative location of orexin-immunoreactive neurons, with arrows pointing toward some of the more prominent terminal fields. Modified with permission from Nambu et al. (1999). AP, area postrema; cc, cerebral cortex; CeG, central gray; Flo, flocculus; Hip, hippocampus; Hypo, hypothalamus; IC, inferior colliculus; LC; locus coeruleus; MB, midbrain; MO, medulla oblongata; OB, olfactory bulb; PN, parabrachial nucleus; SC, superior colliculus; Sept, septum; SFO, subfornical organ.
(B) Role of orexin in dependence.

antagonist had no effect, suggesting the possibility that ethanol drinking may be an attempt to overcome the aversive effects of κ agonists (Holter et al., 2000). Direct support for the hypothesis that dynorphin is part of the negative emotional systems recruited in dependence is the observation that nor-binaltorphimine, when injected intracerebroventricularly or systemically, blocked ethanol self-administration in dependent but not in nondependent animals (Walker and Koob, 2008; B.M. Walker and G.F.K., unpublished data). κ knockout mice also drank less ethanol in a two-bottle choice test using escalating doses of ethanol (Kovacs et al., 2005).

Opiate withdrawal has been shown to increase dynorphin levels in the amygdala (Rattan et al., 1992) and nucleus accumbens (Turchan et al., 1997). Animals with a history of heroin self-administration showed increased levels of dynorphin A and -B in the striatum at a time point just before the next scheduled self-administration session (Cappendijk et al., 1999). Intracerebroventricular dynorphin A treatment decreased heroin-stimulated dopamine release and significantly increased heroin self-administration in daily 5 hr sessions, whereas a κ antagonist had the opposite effects (Xi et al., 1998).

Stress increases dynorphin activity, suggesting a potential interaction with CRF systems. Blockade of dynorphin activity, either via κ receptor antagonism or prodynorphin gene disruption, blocked stress-induced reinstatement of cocaine-induced place preference in mice (McLaughlin et al., 2003) and blocked stress-induced reinstatement of cocaine-seeking behavior (Beardsley et al., 2005). Forced swim stress and inescapable footshock produced place aversions in mice that were blocked by a κ antagonist and dynorphin knockout, and here, CRF was hypothesized to produce its aversive effect via a CRF_2 receptor-dynorphin interaction (Land et al., 2008). Evidence also exists showing that reinstatement of drug-seeking behavior via activation of κ opioid receptors is mediated by CRF, and κ agonist-induced reinstatement of cocaine seeking was blocked by a CRF_1 antagonist (Valdez et al., 2007). Thus, the dynorphin/κ system mimics stressor administration in animals in producing aversive effects and inducing drug-seeking behavior, and this aversive response may involve reciprocal interactions with nucleus accumbens dopamine and the brain extrahypothalamic CRF system.

2.4. Orexin

Orexin (also known as hypocretin)-containing neurons derive exclusively from the lateral hypothalamus and project widely throughout the brain (Peyron et al., 1998), with a dense innervation of anatomical sites involved in regulating arousal, motivation, and stress states (Baldo et al., 2003) (Figure 6) (see Supplemental Data). Orexin A and orexin B have actions that are mediated by two G protein-coupled receptors, OX_1 and OX_2 (also referred to as hypocretin 1 and -2, respectively, but orexin A, orexin B, OX_1, and OX_2 are the accepted International Union of Pharmacology nomenclature). OX_1 has higher affinity for orexin A, and OX_2 has equal affinity for both orexin A and -B (Sakurai et al., 1998). The orexin neuropeptides orexin A and orexin B interact with noradrenergic, cholinergic, serotonergic, histaminergic, and dopaminergic systems, in addition to the HPA axis, to mediate sleep-wake regulation, energy homeostasis, and motivational, neuroendocrine, and cardiovascular functions (Sutcliffe and de Lecea, 2002).

A role for the orexin systems in the neuroadaptive processes linked to dependence have been hypothesized based on a brain arousal-stress function. Orexin neurons have been implicated in drug seeking. Orexin neurons in the lateral hypothalamus are activated by cues associated with rewards, such as food or drugs, and exogenous stimulation of lateral hypothalamic orexin neurons reinstates extinguished drug-seeking behavior in rodents (Harris et al., 2005). Injection of an OX_1 antagonist decreased the place preference produced by morphine (Narita et al., 2006).

Using an intravenous cocaine self-administration model, administration of orexin A reinstated previously extinguished cocaine-seeking behavior, but rather than potentiating reward,

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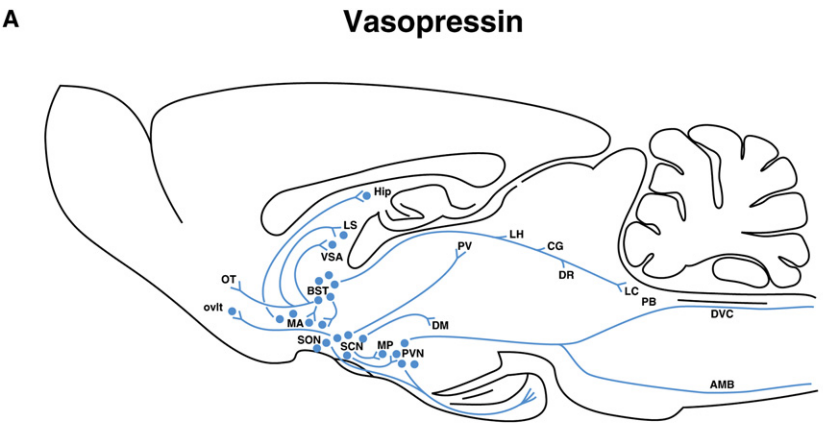

B

Drug	Withdrawal-induced changes in vasopressin immunoreactivity in CeA	Vasopressin Antagonist Effects: Withdrawal-induced anxiety-like or aversive responses	Baseline self-administration or place preference	Dependence-induced increases in self-administration	Stress-induced reinstatement
Cocaine			↑		
Opioids					↓
Ethanol	↓		—		

—, no effect; blank entries indicate not tested. CeA, central nucleus of the amygdala.

Figure 7. Localizations and Projections of Brain Stress Systems—Vasopressin

Schematic of the most prominent vasopressin-immunoreactive projections. Modified with permission from de Vries and Miller (1998). AMB, ambiguus nucleus; BST, bed nucleus of the stria terminalis; CG, midbrain central gray; DM, dorsomedial nucleus of the hypothalamus; DR, dorsal raphe nucleus; DVC, dorsal vagal complex; Hip, ventral hippocampus; LC, locus coeruleus; LH; lateral habenular nucleus; LS; lateral septum; MA, medial nucleus of the amygdala; MP; medial preoptic area; OT, olfactory tubercle; ovlt; organum vasculosum laminae terminalis; PB, parabrachial nucleus; PV, periventricular nucleus of the hypothalamus; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; VSA, ventral septal area.

(B) Role of vasopressin in dependence.

orexin A induced a long-lasting brain reward deficit (Boutrel et al., 2005). The reinstatement of cocaine-seeking behavior by orexin also was blocked by noradrenergic or CRF receptor antagonists. Antagonism of OX_1 receptors prevented footshock-induced reinstatement of cocaine-seeking behavior in rats (Boutrel et al., 2005). Additionally, footshock stress elicited a selective effect on activation of orexin neurons in the perifornical-dorsomedial hypothalamus, leading to the hypothesis that orexin neurons in the lateral hypothalamus mediate reward activation/arousal, whereas orexin neurons in the perifornical-dorsomedial hypothalamus mediate stress activation/arousal/memory (Harris and Aston-Jones, 2006). Orexin A, possibly from the perifornical-dorsomedial hypothalamus, activates CRF-expressing neurons in the paraventricular nucleus of the hypothalamus and the central nucleus of the amygdala (Sakamoto et al., 2004). CRF neurons innervate orexin neurons, possibly from the extended amygdala (Winsky-Sommerer et al., 2004), suggesting a novel reciprocal stress-activation system. Overall, these results suggest a dynamic relationship between orexin and reward/stress pathways in regulating the reinstatement of previously extinguished drug-seeking behaviors. Studies on the role of specific orexin peptide receptors and specific brain sites on the motivational aspects of drug dependence remain to be explored.

2.5. Vasopressin

The neurohypophysial peptide vasopressin has actions in the central nervous system in addition to its classic role as an antidiuretic hormone derived from the posterior pituitary (see Supplemental Data). Vasopressin is widely distributed in the brain outside of the hypothalamus, and the highest vasopressin concentrations are in the suprachiasmatic and supraoptic nuclei, but substantial levels also have been observed in the septum and locus coeruleus (Figure 7). Vasopressin neurons innervating the extended amygdala are hypothesized to derive from cell bodies in the medial bed nucleus of the stria terminalis (de Vries and Miller, 1998). Vasopressin binds to three different G protein-coupled receptor subtypes: V_{1a}, V_{1b}, and V_2. The V_2 receptor is expressed almost exclusively in the kidney, where it mediates the antidiuretic action of vasopressin. The V_{1a} and V_{1b} receptors are localized to the brain, and the distribution of vasopressin receptor binding is prominent in the rat extended amygdala, with high concentrations in the lateral and supracapsular bed nucleus of the stria terminalis, the central nucleus of the amygdala, and the shell of the nucleus accumbens (Veinante and Freund-Mercier, 1997).

Vasopressin mRNA levels were increased selectively in the amygdala during early spontaneous withdrawal from heroin, and a selective V_{1b} receptor antagonist, SSR149415, blocked footshock-induced reinstatement of heroin-seeking behavior, suggesting that vasopressin systems in the amygdala may be a key component of the aversive emotional consequences of opioid withdrawal (Zhou et al., 2008). Prolonged or chronic ethanol exposure decreased vasopressin-like immunoreactivity in the hypothalamus and the bed nucleus of the stria terminalis projection to the lateral septum (Gulya et al., 1991). A selective V_{1b} receptor antagonist dose-dependently blocked the increase in ethanol self-administration during withdrawal in dependent rats but had no effect in nondependent animals (S. Edwards et al., 2008, Soc. Neurosci., abstract). To date, few studies have explored the motivational effects of vasopressin antagonists in animal models of dependence or stress-induced reinstatement with other drugs of abuse. However, the literature suggesting that V_{1b} antagonists have anxiolytic-like profiles (see Supplemental Data) and that vasopressin and its receptors are highly expressed in the extended amygdala lends credence to the hypothesis that vasopressin systems in the extended amygdala may have a role in the increased alcohol intake associated with dependence.

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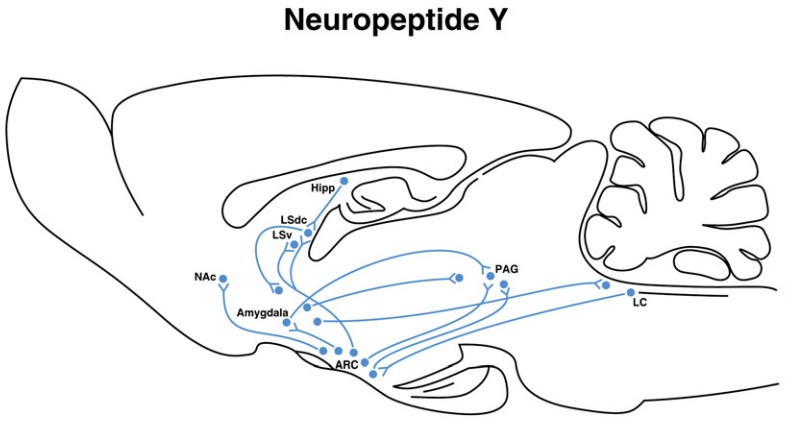


B

Drug	Withdrawal-induced changes in NPY mRNA and protein in CeA	NPY Agonist Effects: Withdrawal-induced anxiety-like or aversive responses	NPY Agonist Effects: Baseline self-administration or place preference	NPY Agonist Effects: Dependence-induced increases in self-administration	NPY Agonist Effects: Stress-induced reinstatement
Cocaine					
Opioids					
Ethanol	↓	↓		↓	

—, no effect; blank entries indicate not tested. CeA, central nucleus of the amygdala.

Figure 8. Localizations and Projections of Brain Antistress Systems—Neuropeptide Y
(A) NPY pathways hypothesized to be involved in NPY effects related to stress and emotionality. Modified with permission from Heilig (2004). ARC, arcuate nucleus; Hipp, hippocampus; LC, locus coeruleus; LSdc, lateral septum-dorsocaudal; LSv, lateral septum-ventral; NAc, nucleus accumbens; PAG, periaqueductal gray matter.
(B) Role of NPY in dependence.

3. Brain Antistress Systems and Addiction: Neuropeptide Y and Nociceptin

3.1. Neuropeptide Y

Neuropeptide Y (NPY) is a 36 amino acid polypeptide with powerful orexigenic and anxiolytic-like actions (see Supplemental Data). NPY is distributed widely throughout the central nervous system but with high concentrations in the extended amygdala (Adrian et al., 1983) (Figure 8). Multiple NPY receptor subtypes have been identified, with the Y_1 and Y_2 subtypes most implicated in stress and drug actions. The Y_1 receptor has a wide distribution throughout the rat brain, where it is most abundantly found in the cortex, olfactory tubercle, hippocampus, hypothalamus, and thalamus (Parker and Herzog, 1999). The distribution of Y_2 receptors is similar to that of Y_1 receptors, although Y_2 receptor expression is less abundant in the cortex and thalamus and more abundant in the hippocampus (Parker and Herzog, 1999). Y_1 receptors are hypothesized to be postsynaptic and Y_2 receptors presynaptic (Heilig and Thorsell, 2002).

NPY administered intracerebroventricularly blocked ethanol withdrawal (Woldbye et al., 2002). Subsequent studies using animal models of dependence-induced drinking in rodents showed that NPY administered intracerebroventricularly reduced limited-access alcohol intake in Wistar rats if they had a history of alcohol dependence produced by chronic intermittent exposure to alcohol vapor (Thorsell et al., 2005). Intracerebroventricularly administered NPY also suppressed alcohol intake in rats selectively bred for high alcohol preference but did not alter alcohol intake in their low alcohol-preferring counterparts (Badia-Elder et al., 2001, 2003). The suppressive effects of intracerebroventricularly administered NPY on ethanol drinking in P rats is enhanced and prolonged following periods of imposed alcohol abstinence (Gilpin et al., 2003). Intracerebroventricular administration of NPY did not affect limited-access nondependent alcohol intake by Wistar rats (Badia-Elder et al., 2001).

Given the evidence that the anti-anxiety-like effects of NPY are mediated by the central or basolateral amygdala complex (Heilig et al., 1994), a logical site for exploring the NPY-induced decrease in excessive ethanol intake is the central nucleus of the amygdala. Ethanol withdrawal decreased NPY protein in the central and medial nuclei of the amygdala (Roy and Pandey, 2002). Infusion of a viral vector encoding prepro-NPY directly into the central nucleus of the amygdala reduced continuous-access alcohol drinking by Long-Evans rats that exhibited anxiety-like behavior in the elevated plus maze (Primeaux et al., 2006). In Wistar rats with a history of dependence and multiple abstinence periods, viral vector-induced amygdala NPY overexpression reduced anxiety-like behavior and produced long-term suppression of alcohol drinking (Thorsell et al., 2007). In P rats with a long history of alcohol consumption, infusions of NPY directly into the central nucleus of the amygdala suppressed alcohol drinking only in P rats that were subjected to periods of imposed alcohol abstinence (Gilpin et al., 2008). P rats have been shown to have lower basal levels of NPY in the central nucleus of the amygdala and correlationally higher anxiety-like behavior compared with alcohol-nonpreferring rats (Suzuki et al., 2004; Pandey et al., 2005). Increases in NPY activity in the central nucleus of the amygdala, produced via alterations in CREB function or direct administration of NPY, decreased ethanol intake and anxiety-like behavior in P rats with a short history of self-administration (Pandey et al. 2005). Exogenous NPY administered into the central nucleus of the amygdala also significantly decreased alcohol drinking by alcohol-dependent rats but not in nondependent controls (Gilpin et al., 2008), confirming the results observed with viral vector-induced induction of NPY activity (Thorsell et al., 2007).

Both Y_1 and Y_2 receptor subtypes are involved in the excessive drinking associated with alcohol dependence. Y_1 receptor knockout mice show increased alcohol consumption (Thiele et al., 2002). In contrast, Y_2 receptor knockout mice drink significantly less alcohol (Thiele et al., 2004). Pharmacological studies have confirmed that blockade of Y_1 receptors increases ethanol intake in C57BL/6 high-drinking mice (Sparta et al., 2004) and blockade of Y_2 receptors decreases ethanol intake in dependent

animals (Rimondini et al., 2005) and in animals responding for ethanol in a sweet solution (Thorsell et al., 2002). Y_1 knockout mice and Y_1 antagonists show an anxiogenic-like profile, and Y_2 knockout mice and Y_2 antagonists show an anxiolytic-like profile, thus providing an important link between the NPY system, anxiety-like responses, and alcohol intake in dependent animals (Valdez and Koob, 2004). Combined with the extensive work in dependent animals, these studies suggest that the NPY system may change its impact on drinking during the transition from nondependent to dependent drinking.

These studies suggest that both constitutive and alcohol-induced changes in NPY activity in the amygdala may be involved not only in mediating anxiety-like responses but also in the motivational effects of ethanol dependence. One hypothesis is that decreased activity of NPY, parallel to increased activity of CRF, may provide a motivational basis for increased alcohol self-administration during alcohol withdrawal or protracted abstinence that drives excessive alcohol consumption (Heilig et al., 1994).

NPY has been implicated in dependence on other drugs of abuse, but the extant literature is not as extensive. Chronic heroin treatment increased NPY neuron activity measured by immunohistochemistry in the thalamic paraventricular nucleus and bed nucleus of the stria terminalis (D'Este et al., 2006). NPY administered intracerebroventricularly blocked the somatic signs of withdrawal from morphine precipitated by the opioid antagonist naloxone, and these behavioral changes were accompanied by decreases in c-*fos* expression in the locus coeruleus, lateral septal nucleus, periaqueductal gray, cingulate and frontal cortices, and septohippocampal nucleus (Clausen et al., 2001). NPY and NPY peptide analogs administered intracerebroventricularly decreased naloxone-precipitated withdrawal in rats (Woldbye et al., 1998).

3.2. Nociceptin (Orphanin FQ)

Nociceptin is the endogenous ligand for the nociceptin/orphanin FQ peptide (NOP) receptor (the accepted International Union on Pharmacology nomenclature; the receptor also has been referred to as the orphan opioid receptor or opioid receptor-like-1, or ORL-1 receptor) (Mollereau et al., 1994). Nociceptin is a 17 amino acid polypeptide structurally related to the opioid peptide dynorphin A (Reinscheid et al., 1995; Meunier et al., 1995). Nociceptin does not bind to μ, δ, or κ receptors, and no known opioids bind to the NOP receptor. Brain mapping studies have shown that the neuroanatomical distribution of nociceptin and its receptor are distinct from those of other opioid peptides and probably represent local short projection circuits (Neal et al., 1999) (Figure 9). The highest density of nociceptin and its receptor can be found in the cortex, amygdala, bed nucleus of the stria terminalis, medial prefrontal cortex, ventral tegmental area, lateral hypothalamus, nucleus accumbens, and many brainstem areas, including the locus coeruleus and raphe (Darland et al., 1998; Neal et al., 1999).

NOP receptor agonists, antagonists, and knockouts have numerous functional effects, including blocking stress-induced analgesia, anxiolytic-like effects, and drug reward (see Supplemental Data). Consistent with the role of nociceptin in stress-related responses, the nociceptin system also may modulate dependence via actions on brain emotional systems involved in the brain stress responses. Intracerebroventricular treatment with nociceptin (Ciccocioppo et al., 1999, 2004) or peptidic NOP receptor agonists (Economidou et al., 2006) significantly decreased ethanol consumption in msP rats. These effects were blocked by a nociceptin antagonist (Ciccocioppo et al., 2003). However, NOP knockout mice backcrossed onto a C57BL/6 background also showed decreases in ethanol consumption in a two-bottle choice test (Sakoori and Murphy, 2008), and certain regimens of NOP receptor agonist administration increased ethanol intake (Economidou et al., 2006).

Nociceptin significantly reduced stress-induced reinstatement of ethanol- (but not cocaine-) seeking behavior in Wistar rats (Martin-Fardon et al., 2000) and cue-induced reinstatement in msP rats (Ciccocioppo et al., 2003). In addition, activation of the NOP receptor inhibited drug-induced reinstatement of ethanol- and morphine-induced conditioned place preference in mice (Kuzmin et al., 2003; Shoblock et al., 2005) and prevented relapse-like behavior in the alcohol deprivation model in msP rats (Kuzmin et al., 2007).

Thus, activation of the nociceptin system decreased the acute rewarding effects of drugs of abuse measured by place preference, produced antistress effects, blocked ethanol consumption in a genetically selected line known to be hypersensitive to stressors, and decreased reinstatement of drug-seeking behavior. Investigating the role of nociceptin in dependence-induced drinking and the localization of its site of action for its effects on drinking remains for future work.

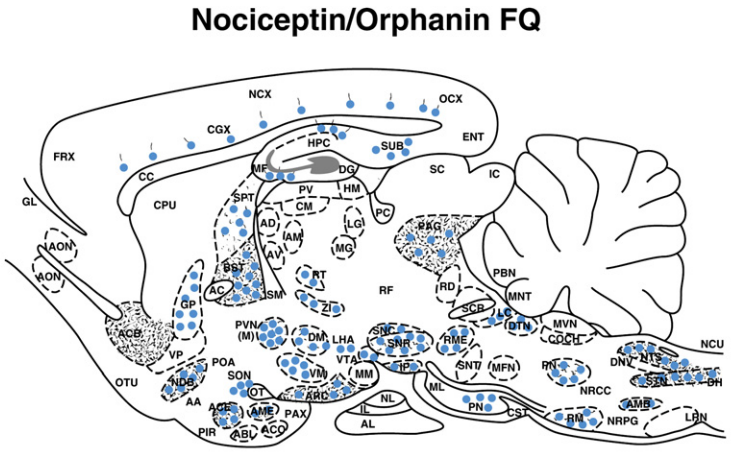
4. Cellular Mechanisms of the Brain Stress Systems in the Extended Amygdala

Elements of the brain stress and antistress systems can be hypothesized to act in series or in parallel on common mechanisms in the extended amygdala to affect emotional states. Cellular studies using electrophysiological techniques have the power to elucidate the common mechanisms. To date, most studies have explored either γ-aminobutyric acid (GABA) or glutamatergic activity within the extended amygdala, and some parallels can be found at the cellular level that appear at the behavioral-neuropharmacological level of analysis.

In the amygdala, CRF is localized within a subpopulation of GABAergic neurons in the bed nucleus of the stria terminalis and central nucleus of the amygdala that are different from those that colocalized with enkephalin (Day et al., 1999). In brain slice preparations, CRF enhanced $GABA_A$ inhibitory postsynaptic potentials (IPSCs) in whole-cell recordings of the central nucleus of the amygdala, and this effect was blocked by CRF_1 antagonists and in CRF_1 knockout mice (Nie et al., 2004). Nociceptin had the opposite effects in the central nucleus of the amygdala—decreasing GABAergic IPSCs (Roberto and Siggins, 2006). Vasopressin also activated cells in the medial part of the central nucleus of the amygdala (Huber et al., 2005). These results show that CRF and vasopressin, which are anxiogenic-like, activate GABAergic interneurons in the central nucleus of the amygdala.

Most neurons in the central nucleus of the amygdala are GABAergic, either inhibitory interneurons with recurrent or feed-forward connections or inhibitory projection neurons to brainstem or downstream regions (e.g., bed nucleus of the stria terminalis). The central nucleus of the amygdala can be identified

A



B

Drug	Withdrawal-induced changes in nociceptin in CeA	Nociceptin Agonist Effects: Withdrawal-induced anxiety-like or aversive responses	Baseline self-administration or place preference	Dependence-induced increases in self-administration	Stress-induced reinstatement
Cocaine			↓		—
Opioids			↓		
Ethanol			↓	↓	↓

—, no effect; blank entries indicate not tested. CeA, central nucleus of the amygdala.

Figure 9. Localizations and Projections of Brain Antistress Systems—Nociceptin/Orphanin FQ

(A) Schematic representation of the distribution of nociceptin peptide in the rat central nervous system determined by immunohistochemistry and in situ hybridization. Neuronal perikarya are shown as solid circles, and fibers-terminals as short curved lines and dots. Data from Neal et al. (1999); very similar results were reported by Anton et al. (1996). AA, anterior amygdala; ABL, basolateral nucleus of amygdala; AC, anterior commissure; ACB, nucleus accumbens; ACE, central nucleus of the amygdala; ACO, cortical nucleus of amygdala; AD, anterodorsal nucleus of thalamus; AL, anterior lobe of pituitary; AM, anteromedial nucleus of thalamus; AMB, nucleus ambiguus; AME, medial nucleus of the amygdala; AON, anterior olfactory nucleus; ARC, arcuate nucleus; AV, anteroventral nucleus of thalamus; BST, bed nucleus of the stria terminalis; CC, corpus callosum; CGX, cingulate cortex; CM, central-medial nucleus of thalamus; COCH, cochlear nuclear complex; CPU, caudate-putamen; CST, corticospinal tract; DH, dorsal horn of spinal cord; DG, dentate gyrus; DM, dorsomedial nucleus of hypothalamus; DNV, dorsal motor nucleus of vagus; DTN, dorsal tegmental nucleus; ENT, entorhinal cortex; FN, fastigial nucleus of cerebellum; FRX, frontal cortex; GL, glomerular layer of olfactory bulb; GP, globus pallidus; HM, medial habenular nucleus; HPC, hippocampus; IC, inferior colliculus; IL, intermediate lobe of pituitary; IP, interpeduncular nuclear complex; LC, nucleus locus coeruleus; LG, lateral geniculate nucleus; LHA, lateral hypothalamic area; LRN, lateral reticular nucleus; MF, mossy fibers of hippocampus; MFN, motor facial nucleus; MG, medial geniculate nucleus; ML, medial lemniscus; MM, medial mammillary nucleus; MNT, mesencephalic nucleus of trigeminal; MVN, medial vestibular nucleus; NCU, nucleus cuneatus; NCX, neocortex; NDB, nucleus of diagonal band; NL, neural lobe of pituitary; NRGC, nucleus reticularis gigantocellularis; NRPG, nucleus reticularis paragigantocellularis; NTS, nucleus tractus solitarius; OCX, occipital cortex; OT, optic tract; OTU, olfactory tubercle; PAG, periaqueductal gray; PAX, periamygdaloid cortex; PBN, parabrachial nucleus; PC, posterior commissure; PIR, piriform cortex; PN, pons; POA, preoptic area; PP, perforant path; PV, periventricular nucleus of thalamus; PVN(M), paraventricular nucleus (pars magnocellularis); PVN(P), paraventricular nucleus (pars parvocellularis); RD, nucleus raphe dorsalis; RE, nucleus reuniens of thalamus; RF, reticular formation; RM, nucleus raphe magnus; RME, nucleus raphe medianus; SC, superior colliculus; SCP, superior cerebellar peduncle; SM, stria medullaris thalami; SNC, substantia nigra (pars compacta); SNR, substantia nigra (pars reticulata); SNT, sensory nucleus of trigeminal (main); SON, supraoptic nucleus; SPT, septal nuclei; STN, spinal nucleus of trigeminal; SUB, subiculum; VM, ventromedial nucleus of hypothalamus; VP, ventral pallidum; ZI, zona incerta.

(B) Role of nociceptin in dependence.

as a "gate" that regulates the flow of information through the intra-amygdaloidal circuits, and the fine-tuning of the GABAergic inhibitory system in the central nucleus of the amygdala may be a prerequisite for controlling both local and output neurons to downstream nuclei. Because GABAergic drugs are typically robust anxiolytics, the fact that anxiogenic-like neurotransmitters would activate GABAergic neurotransmission and anxiolytic-like neurotransmitters would depress GABAergic transmission in a brain region known to be involved in stress-related behavior may seem paradoxical. However, local GABAergic activity within the central nucleus of the amygdala may functionally influence neuronal responsivity of inhibitory central nucleus of the amygdala gating that regulates information flow through the local intra-amygdaloidal circuits (i.e., by disinhibiting the central nucleus of the amygdala), leading to increased inhibition in downstream regions that mediate the behavioral response.

In the bed nucleus of the stria terminalis, whole-cell recordings from slice preparations demonstrated that CRF enhanced GABAergic neurotransmission, and the CRF effect appeared to be via the CRF_1 receptor similar to the effects in the amygdala, and NPY inhibited GABAergic neurotransmission (Kash and Winder, 2006). The predominant noradrenergic innervation of the bed nucleus of the stria terminalis is in the ventral part, and here norepinephrine decreases glutamatergic activity measured both electrophysiologically and with in vivo microdialysis (Egli et al., 2005; Forray et al., 1999). Norepinephrine also increased $GABA_A$ IPSCs (Dumont and Williams, 2004). Thus, if one combines the data from the central nucleus of the amygdala and the bed nucleus of the stria terminalis, then certain consistencies evolve (Table 3). CRF, vasopressin, and norepinephrine increase GABAergic activity, and NPY and nociceptin decrease GABAergic activity, actions at the cellular level that are parallel to the behavioral effects described above with neuropharmacological studies (Table 3).

Other researchers have argued that increasing excitability in the basolateral nucleus of the amygdala contributes to the anxiogenic-like effects of CRF (Rainnie et al., 2004). Using whole-cell patch-clamp recordings from basolateral amygdala neurons of animals chronically administered a CRF_1/CRF_2 agonist, urocortin, showed an *N*-methyl-D-aspartate (NMDA) receptor-mediated decrease in both spontaneous and stimulation-evoked IPSPs (Rainnie et al., 2004). Ethanol withdrawal, diazepam withdrawal, and uncontrollable stress also suppress IPSCs of the cells in the basolateral amygdala using a whole-cell patch-clamp

Table 3. Effects of Brain Stress Neurotransmitters on GABAergic Activity in the Extended Amygdala

	Central Nucleus of the Amygdala	Bed Nucleus of the Stria Terminalis
Stress Neurotransmitters		
Corticotropin-releasing factor	↑	↑
Norepinephrine	—	↑
Vasopressin	↑	—
Antistress Neurotransmitters		
Neuropeptide Y	—	↓
Nociceptin	↓	—

—, not determined.

preparation (Isoardi et al., 2007). These NMDA-mediated effects are the opposite of the GABA-mediated effects observed in the central nucleus of the amygdala and suggest that an integration of the role of the central and basolateral nuclei of the amygdala in stress and dependence responses will be required.

With the exception of recent studies with ethanol dependence, little work has been done at the cellular level in the extended amygdala on the changes in neurotransmission in the brain stress systems with the development of dependence. Chronic ethanol-induced changes in neuronal activity of GABA interneurons in the central nucleus of the amygdala have been linked to actions of CRF and nociceptin. Acute administration of doses of alcohol in the intoxicating range increased $GABA_A$ receptor-mediated IPSCs in central nucleus of the amygdala neurons, and this effect has been hypothesized to be attributable to an increase in presynaptic GABA release (Roberto et al., 2003; Nie et al., 2004). Even more striking is that the augmented GABA release is increased even further in dependent animals, shown both by electrophysiological and in vivo microdialysis measures (Roberto et al., 2004). The ethanol-induced enhancement of GABAergic IPSCs was blocked by CRF_1 antagonists (Nie et al., 2004; Roberto et al., 2004) and was not observed in CRF_1 knockout mice (Nie et al., 2004). Nociceptin-induced inhibition of IPSCs was increased in dependent animals, suggesting an increased sensitivity to nociceptin (Roberto and Siggins, 2006). Thus, not only do the brain stress/antistress systems interact systematically with the hypothesized GABAergic interneurons of the central nucleus of the amygdala, but ethanol dependence also sensitizes these neurons to the actions of the brain stress/antistress systems.

5. Neurocircuitry of the Brain Stress Systems in Dependence

Five potential arousal-stress neurotransmitter systems (CRF, norepinephrine, vasopressin, orexin, dynorphin) and two potential antistress neurotransmitter systems (NPY, nociceptin) have been explored in the present review from the perspective of a role in the neuroadaptation associated with the development of negative emotional states associated with drug dependence and addiction. The most compelling data are in the domain of CRF, where, for virtually all major drugs of abuse, (1) CRF is released during acute withdrawal, (2) CRF antagonists block the anxiogenic-like effects of acute withdrawal, (3) CRF antagonists block the excessive drug intake associated with dependence, and (4) CRF antagonists block stress-induced reinstatement. The focal point for most of these effects is the central nucleus of the amygdala and the bed nucleus of the stria terminalis (see Figure 1).

Although less extensive, similar data exist for some noradrenergic antagonists that block the anxiogenic-like effects of opiate withdrawal, block excessive drug intake associated with dependence on ethanol, cocaine, and opioids, and block stress-induced reinstatement to cocaine, opioids, ethanol, and nicotine (see Figure 4). Again, the focal point for many of these effects is the central nucleus of the amygdala and the bed nucleus of the stria terminalis.

Much evidence has been marshaled to show that dynorphin is increased in the nucleus accumbens in response to dopaminergic activation and, in turn, that overactivity of the dynorphin systems can decrease dopaminergic function. κ antagonists have been shown to block the aversive effects of drug withdrawal and the excessive drinking associated with ethanol dependence and stress-induced reinstatement of drug seeking (see Figure 5). Evidence suggests that κ receptor activation can produce CRF release (Song and Takemori, 1992), but recently some have argued that the effects of dynorphin in producing negative emotional states are mediated via activation of CRF systems (Land et al., 2008).

Much less evidence to date has demonstrated a direct role for vasopressin and orexin in the negative emotional states associated with drug dependence (see Figures 6 and 7). A vasopressin antagonist blocked stress-induced reinstatement of heroin-seeking behavior and withdrawal-induced ethanol drinking, and an orexin antagonist blocked stress-induced reinstatement of cocaine seeking. Much more work will be required to explore the role of these systems and their interactions with other major players, such as CRF.

Significant evidence suggests that activation of NPY in the central nucleus of the amygdala can block the motivational aspects of dependence associated with chronic ethanol administration. NPY administered intracerebroventricularly blocked the anxiogenic-like effects of withdrawal from ethanol and blocked the increased drug intake associated with ethanol dependence (see Figure 8). Direct administration or viral vector-enhanced expression of NPY into the central nucleus of the amygdala also blocked the increased drug intake associated with ethanol dependence. Few or no studies have examined the effects of NPY on motivational aspects of dependence with other drugs of abuse.

The role for nociceptin in dependence suggests interactions both with the rewarding effects of drugs of abuse and in the motivational aspects of dependence, mainly with ethanol. Nociceptin blocks the rewarding effects of most major drugs of abuse measured by place preference (see Supplemental Data). Nociceptin decreased ethanol self-administration in msP rats known to have a constitutive increase in CRF activity and a stress-like phenotype. msP rats are known to have a high basal stress response, to show decreased ethanol intake similar to dependent rats with administration of a CRF_1 antagonist, and to carry a genetic polymorphism of the CRF_1 promoter, resulting in increased CRF_1 density in several brain regions (Hansson et al., 2006) (see

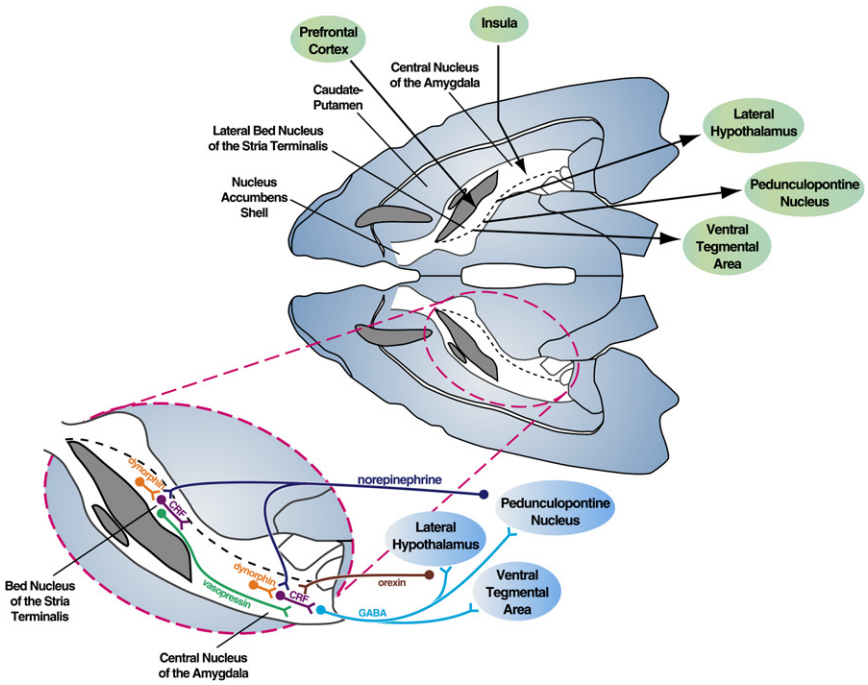


Figure 10. The Extended Amygdala and Its Afferent and Major Efferent Connections and Modulation via Brain Arousal-Stress Systems

Horizontal section through a rat brain depicting the extended amygdala and its afferent and major efferent connections and modulation via brain arousal-stress systems. (Top) Central division of the extended amygdala with the central nucleus of the amygdala and lateral bed nucleus of the stria terminalis and a transition area in the shell of the nucleus accumbens highlighted. (Bottom) Enlargement of the hypothesized interaction of the brain stress systems and the extended amygdala. Note that dynorphin may activate CRF neurons or be activated by CRF neurons, that norepinephrine and CRF are hypothesized to be involved in a feed-forward circuit, and that vasopressin for the central nucleus of the amygdala is hypothesized to derive from the bed nucleus of the stria terminalis. NPY and nociceptin are not depicted in this figure but may act either via modulation of the CRF system or independently, directly on the output of the central nucleus of the amygdala (to be determined).

Figure 9). Nociceptin also significantly reduced stress-induced reinstatement of ethanol. Future studies should explore the role of both of these antistress systems (NPY, nociceptin) in the negative emotional responses associated with dependence on other drugs of abuse.

A pronounced interaction exists between central nervous system CRF and norepinephrine systems. Conceptualized as a feed-forward system at multiple levels of the pons and basal forebrain, CRF activates norepinephrine, and norepinephrine in turn activates CRF (Koob, 1999; see Supplemental Data).

The common neurocircuitry actions of drugs of abuse on the brain stress systems and the change in plasticity of these circuits (see above) may involve molecular neuroadaptations that either differentially drive the circuits or result from the changes in activity of the circuits or both. Repeated perturbation of intracellular signal transduction pathways may cause changes in neuronal function and/or changes in nuclear function and altered rates of transcription of particular target genes. Altered expression of such genes would lead to presumably long-term altered activity of the neurons where such changes occur and ultimately to changes in neural circuits in which those neurons operate. Much work in addiction has shown that chronic exposure to opioids and cocaine leads to activation of CREB in the nucleus accumbens and central nucleus of the amygdala (Shaw-Lutchman et al., 2002; Edwards et al., 2007). Although acute administration of drugs of abuse can cause a rapid (within hours) activation of members of the Fos protein family, such as FosB, Fra-1, and Fra-2 in the nucleus accumbens, other transcription factors, isoforms of ΔFosB, have been shown to accumulate over longer periods of time (days) with repeated drug administration (Nestler, 2005). Animals with activated ΔFosB have exaggerated sensitivity to the rewarding effects of drugs of abuse, and ΔFosB may be a sustained molecular "switch" that helps to initiate and maintain a state of addiction (McClung et al., 2004). Whether (and how) such transcription factors influence the function of the brain stress systems, such as CRF and those described above, remains to be determined.

A focus of this review has been on the connections of the brain arousal-stress systems with the extended amygdala, particularly the central nucleus of the amygdala and the bed nucleus of the stria terminalis. Three of the seven systems (norepinephrine, orexin, NPY) are widely distributed in the brain but with a heavy innervation of the extended amygdala. Four of the systems (CRF, vasopressin, nociceptin, dynorphin) are more localized to local circuits throughout the forebrain but also with a heavy innervation of the extended amygdala (Figure 10). However, the convergence of these neurotransmitter systems in the region of the extended amygdala suggests key roles in the processing of emotional stimuli potentially triggered by neurons deriving from the brainstem (norepinephrine), hypothalamus (nociceptin, NPY), and within the extended amygdala itself (CRF, vasopressin, nociceptin, dynorphin). The extended amygdala receives afferents from the prefrontal cortex and insula and sends efferents to the lateral hypothalamus, ventral tegmental area, and pedunculopontine nucleus (Figure 10). Which parts of this neurocircuitry play a key role in the negative emotional states of drug dependence and how they interact with the brain stress systems remain to be elucidated. What is known is that most of the cells in the lateral division of the central nucleus of the amygdala and bed nucleus of the stria terminalis (extended amygdala) are GABAergic and that a distinct subpopulation colocalizes with either enkephalin or CRF, but they virtually never colocalize together on the same GABAergic cell (Day et al., 1999). Only enkephalin, and not CRF, colabeled neurons were activated by interleukin-1β, suggesting that discrete neural circuits exist within the extended amygdala (Day et al., 1999). Additionally, the

electrophysiological anatomical studies outlined above suggest that these GABAergic neurons in the central nucleus of the amygdala respond to arousal-stress neurotransmitters with increased firing and respond to antistress neurotransmitters with decreased firing. These GABAergic neurons that are intrinsic to the central nucleus of the amygdala may be interneurons that inhibit another GABAergic link in the efferent pathway (Day et al., 1999; Davis et al., 1994).

The hypothesis that the central nucleus of the amygdala forms a focal point for a convergence of emotional stimuli to produce emotional responses has long been formulated for conditioned fear and pain. A *cortex→lateral amygdala→central nucleus of the amygdala* circuit has been shown to be critical for the expression of fear conditioning (Phelps and Le Doux, 2005). A conditioned acoustic stimulus activated the lateral nucleus of the lateral amygdala via auditory processing areas in the medial division of the medial geniculate body and auditory association cortex. The lateral amygdala, in turn, projects to the central amygdala, which controls the expression of fear responses through projections to the brainstem (Phelps and Le Doux, 2005).

Substantial evidence implicates the amygdala in both pain modulation and emotional responses to pain. In addition to receiving well-processed affective and cognitive inputs, pain-related information is conveyed to the lateral, basolateral, and central nuclei of the amygdala via both the spinothalamic and spinohypothalamic pain pathways but also via projections from the spino-parabrachial-amygdaloid pain pathway (spinal cord and trigeminal nucleus to the parabrachial nucleus and then to the central nucleus of the amygdala) (Bernard and Besson, 1990). Both of these pathways have been implicated in mediating the affective dimension of pain (Neugebauer et al., 2004). Numerous parallels may exist in amygdala mediation of the emotional dysregulation of addiction outlined above and the emotional component of pain mediated by the amygdala. These parallels include interactions between stress, depression, and pain (Neugebauer et al., 2004), the relationship between tolerance and sensitization to pain (Celerier et al., 2001), and the glucocorticoid modulation of pain (Greenwood-Van Meerveld et al., 2001). How the brain stress neurotransmitters outlined above play a role in both processes is a challenge for future research.

6. Hedonic Homeostatic Dysregulation as a Conceptual Framework for Linking Stress Systems and Addiction

6.1. Hypothalamic-Pituitary-Adrenal Axis as a Facilitator

As noted above, all drugs of abuse engage the HPA axis during acquisition of drug taking and again during acute withdrawal from the drug, and both CRF and vasopressin in the paraventricular nucleus of the hypothalamus control these responses. However, as the cycle of drug taking and withdrawal continues, the HPA axis response shows tolerance, but the repeated exposure of the brain to high levels of glucorticoids can continue to have profound effects on the extrahypothalamic brain stress systems. 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, engagement of the brain stress systems may contribute to the negative emotional state that dissipates with time following a single injection of a drug, but with repeated administration of drug grows larger with time (or fails to return to normal homeostatic baseline), in contrast to the HPA axis, setting up a negative reinforcement mechanism (see also "Allostasis and Addiction" section below). Thus, the HPA axis and glucocorticoids are linked to high responsivity to novelty and facilitation of reward in initial drug use and also may be involved in potentiating adaptations in many parts of the neuraxis, particularly in extended amygdala systems where they contribute to the shift from homeostasis to pathophysiology associated with drug abuse. These results suggest that activation of the HPA component of stress can play an important role in facilitating both reward and brain stress neurochemical systems implicated in the development of addiction.

6.2. Opponent Process/Between-System Neuroadaptations

As defined above, opponent process, between-system neuroadaptations (Table 1) are hypothesized to involve activation of the neurotransmitter systems grouped together in this review as the brain arousal-stress systems. Thus, recruitment of the CRF system occurs during the development of dependence for all drugs of abuse that has motivational significance (Figure 1B above), but additional between-system neuroadaptations associated with motivational withdrawal include activation of the dynorphin/κ opioid system, norepinephrine brain stress system, extrahypothalamic vasopressin system, and possibly the orexin system. 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 vulnerability to stressors observed during protracted abstinence in humans. However, brain antistress systems, such as NPY and nociceptin, also may be compromised during the development of dependence, thus removing a mechanism for restoring homeostasis (Koob and Le Moal, 2008). 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 GABA, but also recruitment of the brain stress systems and/or disruption of the brain antistress systems (Koob and Le Moal, 2005).

The neuroanatomical entity integrating these brain arousal-stress and antistress systems may be the extended amygdala. Thus, the extended amygdala may represent a neuroanatomical substrate for the negative effects on reward function produced by stress that help drive compulsive drug administration (Koob and Le Moal, 2008) (Figure 10). The extended amygdala has a role in integrating emotional states such as the expression of the conditioned fear response in the central nucleus of the amygdala (Phelps and Le Doux, 2005) and emotional pain processing (Neugebauer et al., 2004) (see above). The integration of data from addiction neurobiology and from behavioral neuroscience of fear and pain point to a rich substrate for the integration of emotional stimuli related to the arousal-stress continuum (Pfaff, 2006) and provides insights not only into the mechanisms of emotional dysregulation in addiction but also into the mechanisms of emotions themselves.

The development of the aversive emotional state that drives the negative reinforcement of addiction is hypothesized to involve a long-term, persistent plasticity in the activity of neural circuits mediating motivational systems that derive from recruitment of antireward systems that drive aversive states. 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. Antireward is a concept based on the hypothesis that brain systems are in place to limit reward (Koob and Le Moal, 1997, 2005, 2008). As dependence and withdrawal develop, brain antireward systems such as CRF, norepinephrine, dynorphin, vasopressin, and possibly orexin are hypothesized to be recruited to produce stress-like aversive states (Koob and Le Moal, 2001; Nestler, 2005; Aston-Jones et al., 1999) (Figure 10). The present thesis also argues that antistress systems, such as NPY and orexin that presumably buffer the stress response, also may be compromised. At the same time, decreases in reward function occur within the motivational circuits of the ventral striatum-extended amygdala (Figure 10). The combination of decreases in reward neurotransmitter function, recruitment of antireward systems, and compromised antistress systems provides a powerful source of negative reinforcement that contributes to compulsive drug-seeking behavior and addiction.

6.3. Stress Systems in Relapse

Although less developed except in studies with CRF and norepinephrine, the brain stress systems also may contribute to the critical problem in drug addiction of chronic relapse, where addicts return to compulsive drug taking long after acute withdrawal. The *preoccupation/anticipation* (craving) stage consists of two processes: protracted abstinence and stress-induced relapse. In animals, protracted abstinence can include increased sensitivity to a stressor or increased drug seeking long after acute withdrawal, both of which having been observed in alcohol studies (Valdez and Koob, 2004). Using CRF as an example in protracted abstinence, CRF is hypothesized to contribute to a residual negative emotional state that provides a basis for drug seeking (Valdez et al., 2002; Valdez and Koob, 2004).

Stress-induced reinstatement is robust and mediated by different elements of the same brain stress systems implicated in drug dependence, as noted above (for review, see Shaham et al., 2000, 2003). In stress-induced reinstatement, CRF systems in the bed nucleus of the stria terminalis are activated when acute stressors induce relapse (Shaham et al., 2003). CRF antagonists block stress-induced reinstatement of cocaine, alcohol, and opioid self-administration (Erb et al., 1998; Liu and Weiss, 2002; Shaham et al., 1998; Zislis et al., 2007). However, stress-induced reinstatement occurs independently of stress-induced activation of the HPA axis (Erb et al., 1998; Le et al., 2000; Shaham et al., 1997). Other brain stress systems implicated in stress-induced reinstatement include norepinephrine, orexin, vasopressin, and nociceptin (see above). 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 versus 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'' by activation of the drug-, cue-, and stress-induced reinstatement neurocircuits now driven by a hypofunctioning, and possibly reorganized, prefrontal system (Volkow and Fowler, 2000).

6.4. Allostasis and Addiction

An overall conceptual framework throughout this review is that drug dependence represents a break with homeostatic brain regulatory mechanisms that regulate the emotional state of the animal. However, the nature of engagement of the brain stress and antistress systems produced by repeated self-administration of drugs of abuse argues that the view of drug addiction representing a simple break with homeostasis is not sufficient to explain a number of key elements of addiction. 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 ''readdiction'' 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 a dynamic break with homeostasis of these systems, termed *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. Thus, the interaction between CRF and norepinephrine in the brainstem and basal forebrain, the interaction between orexin and CRF in the hypothalamus and basal forebrain, and the interaction between CRF and vasopressin and/or orexin could lead to chronically dysregulated emotional states (Koob, 1999). Similar allostatic mechanisms can be hypothesized to be involved in driving the pathology associated with the brain stress and antistress systems in addiction (Koob and Le Moal, 2001). Repeated challenges (e.g., with drugs of abuse) lead to attempts of the brain via molecular, cellular, and neurocircuitry changes to maintain stability, but at a cost. For the drug addiction framework elaborated here, the residual deviation from normal brain reward threshold regulation is termed an *allostatic state*. This state represents a combination of chronic elevation of reward set point fueled by numerous neurobiological changes, including decreased function of reward circuits, loss of executive control, and facilitation of stimulus-response associations, but also recruitment of the brain stress systems and compromises

to the brain antistress systems. All of these effects contribute to the compulsivity of drug seeking and drug taking known as addiction (Koob and Le Moal, 2008).

SUPPLEMENTAL DATA

The Supplemental Data can be found with this article online at http://www.neuron.org/cgi/content/full/59/1/11/DC1/.

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