Drug dependence: stress and dysregulation of brain reward pathways

Mary Jeanne Kreek a,*, George F. Koob b

a The Laboratory of the Biology of Addictive Diseases, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA
b Division of Psychopharmacology, Department of Neuropharmacology, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, CA 92037, USA

1. Drug dependence-conceptual framework

1.1. Motivational view of drug dependence

The motivating factors for the development, maintenance and persistence of drug addiction can be distilled to four major sources of reinforcement in drug dependence: positive reinforcement and negative reinforcement, conditioned positive reinforcement and conditioned negative reinforcement (Widler, 1973). In addition to the major sources of reinforcement in drug dependence, both the persistence of ongoing addiction, as well as the relapse to drug addiction, days, months or years after the last use of the drug may be due, in part, not only to the conditioned positive and negative reinforcement, and also to the negative reinforcement of protracted abstinence when that exists (as, for instance, has been well documented in the case of opiate addiction) but also to much more subtle factors which result from chronic changes or abnormalities in the brain following chronic exposure to a drug of abuse due to the intrinsic neuroplasticity of the brain (Dole et al., 1966; Kreek, 1987, 1992). These changes may contribute to a general, ill-defined feeling of dysphoria, anxiety or abnormality and also could be considered a form of protracted abstinence (Koob and LeMoal, 1997). In addition, genetic factors and early environmental factors may contribute to variations or abnormalities in neurobiologic function which may yield some individuals more vulnerable, both to the initial acquisition of drug addiction, as well as relapse to drug use once achieving the abstinent state. Extensive studies both in animal models of a variety of types, as well as basic clinical research in humans, using neurochemical, molecular and related behavioral technologies, as well as a variety of imaging techniques, have documented that indeed chronic exposure to drugs of abuse does cause alterations in specific aspects of brain function which are persistent over varying periods of time or in some cases, may even be permanent. Clearly, positive reinforcing effects are critical for establishing self-administration behavior, and some have argued the hypothesis that positive reinforcement is the key to drug dependence (Wise, 1988). However, while alleviation of aversive withdrawal symptoms (negative reinforcement) may not be a major motivating factor in the initiation of compulsive drug use, a compelling case can be made for dysregulation in hedonic processing associated with drug abstinence as the driving force of addiction (Solomon, 1977; Koob and LeMoal, 1997). Also, in some cases, abnormalities may be present before the first exposure to a drug of abuse, either on a genetic or early environmentally-induced basis. These abnormalities or alterations in the brain may contribute to adverse symptoms which may be actually relieved or perceived as being ameliorated by initial or early self-administration of a drug of abuse. Clearly, the construct of negative reinforcement plays an important role in the maintenance of drug use after the development of dependence. Thus, while initial drug use in most individuals may be motivated primarily by the positive affective state produced by the drug, continued use leads to adaptation to the presence of drug and to another source of reinforcement, the negative reinforcement associated with relieving negative affective and physical consequences of drug termination. Indeed, the defining feature of drug dependence has been argued to be the establishment of a negative affective state (Russell, 1976). However, an even more compelling motivational force is the hypothesis that negative affective states, even during

*Corresponding author.
protracted abstinence, can contribute to the reinforcement associated with drug taking by changing the ‘set point’ for hedonic processing or the ‘set point’ for simply relieving physical or mental discomfort (Koob and LeMoal, 1997). Much progress has been made in identifying the neuronal substrates for the acute positive reinforcing effects of drugs of abuse (see Wise this issue). More recent focus has been on the neuronal substrates for negative reinforcement and the conditioned reinforcing effects that contribute to both continued drug use and relapse after the ‘drug free’ state has been achieved.

1.2. Counteradaptation

Historically, neuronal systems have long been hypothesized to respond to chronic drug insult by counteradaptive response (Himmelsbach, 1942). The development of tolerance and the manifestation of a withdrawal syndrome in the absence of drug presumably reflected the body’s attempt to counter the chronic effects of the drug. In a homeostatic adaptive formulation, the initial acute and subacute effects of a drug of abuse is opposed or counteracted by homeostatic changes in systems that mediate the primary drug effects (Meyer and Sparber, 1977; Solomon and Corbit, 1974; Siegel, 1975; Poulos and Cappell, 1991). Recent discussions have focused on changes in hedonic processing including the manifestation of hedonic withdrawal even with a minimal contribution of physical signs (Koob and Bloom, 1988; Koob and LeMoal, 1997). In opponent process theory, both tolerance and dependence were inextricably linked (Solomon and Corbit, 1974). Affective states, pleasant or aversive, were hypothesized to be opposed by centrally-mediated mechanisms that reduce the intensity of these affective states and this counteradaptive response was hypothesized to get larger and larger with repeated exposure (Solomon and Corbit, 1974).

1.3. Sensitization

Another adaptive process, the phenomenon of sensitization, where a drug response gets progressively larger with repeated administration, has also been conceptualized as a critical adaptive process (Robinson and Berridge, 1993). With repeated drug administration, sensitization is more likely to occur with intermittent exposure to a drug, in contrast to tolerance which is more likely to occur with continuous exposure, although recent studies have shown that regular, daily ‘binge’ pattern exposure to the stimulant cocaine can result in sensitization with respect to locomotor activity (Unterwald et al., 1994a). In a recent formulation of the role of sensitization in drug dependence, a shift in an incentive-salience state described as ‘wanting’ was hypothesized to be progressively increased by repeated exposure to drugs of abuse (Robinson and Berridge, 1993), and the transition to pathologically ‘strong wanting’, ‘craving’, or ‘drug hunger’ defined compulsive use (Dole et al., 1966).

1.4. Spiralling distress cycle

A compromised reward state (sensitized, tolerant or deficit, altered, or a combination thereof) could be produced by several mechanisms. First, genetic or environmental factors could produce an increased sensitivity to the reinforcing effects of drugs or could produce a priori a neurobiological deficit that requires reversal, perhaps by drug use initiation. Alternatively, chronic drug-taking itself could produce a form of an increased sensitivity to the reinforcing effects of drugs or of a hedonic deficit state that required self-medication to reverse or ameliorate. These changes have been hypothesized to exist at the molecular, cellular and system level, and combined together form a powerful motivation for drug-seeking behavior (Kreek, 1992; Koob and LeMoal, 1997) (Fig. 1).

![Diagram showing the spiralling distress addiction cycle](image)

**Fig. 1.** Diagram describing the spiralling distress–addiction cycle from two conceptual perspectives: dysadaptational, and neurobiological. The three major components of the addiction cycle are preoccupation–anticipation, binge–intoxication, and withdrawal–negative affect. (A) The places of emphasis for the theoretical constructs of sensitization and counteradaptation. (B) The hypothetical role of different neurochemical and endocrine systems in the addiction cycle. Small arrows refer to increased functional activity. DA, dopamine; CRF, corticotropin-releasing factor. Note that the addiction cycle is conceptualized as a spiral that increases in amplitude with repeated experience, ultimately resulting in the pathological state known as addiction. Taken with permission from Koob and LeMoal (1997).
2. Neurobiology of reward dysregulation in drug dependence

2.1. Dysregulation of neuronal elements of reward

Many drugs of abuse, such as heroin and alcohol, upon chronic administration, produce a characteristic withdrawal syndrome characterized by distinct, often physical signs and symptoms. However, drugs of abuse also have several common actions associated with acute withdrawal that appear independent of physical signs of withdrawal. Acute withdrawal is associated with a negative affective state including various negative emotions, such as dysphoria, irritability, anxiety, restlessness, inability to concentrate, sleep disorders, depressive symptoms, and even depression. Cocaine withdrawal in humans in the outpatient setting is characterized by severe depressive symptoms combined with irritability, anxiety, and anhedonia, lasting several hours to several days (i.e. the ‘crash’) and may be one of the motivating factors in the maintenance of the cocaine-dependence cycle (Gawin and Kleber, 1986). Qualitatively similar changes in mood and anxiety states have been seen in inpatient studies, but they generally are much less severe, suggesting the roles of both environmental and situational stressors, as well as conditioned cues in the ‘crash’ symptomatology (Weddington et al., 1991; Cambor et al., 1992; Ho et al., 1992). Few physical abnormalities can be measured, other than rapid weight gain, secondary to hunger, enormous food consumption, along with sleep abnormalities (Smith et al., 1997). Acute opiate withdrawal is characterized by severe dysphoria, along with numerous physical signs, symptoms and objective changes, while ethanol withdrawal produces dysphoria and anxiety, in addition to profound physical signs and symptoms, some general and some specific, many of which can be objectively measured. Also, following cycles of short-acting opiate (e.g. heroin) addiction, objective signs and symptoms of protracted physical abstinence may be measured, along with dysphoria and depressive symptoms for weeks or months (Dole et al., 1966; Martin and Jasinski, 1969).

Neurobiological studies have focused on the neural substrates and neuropharmacological mechanisms of these negative affective states and the same neural systems implicated in the positive reinforcing effects of drugs of abuse have been hypothesized to be involved in these aversive motivational effects of drug withdrawal. Employing measures of reward thresholds using the technique of intracranial self-stimulation throughout the course of drug dependence in animal models, recent studies have shown that reward thresholds are increased (reflecting a decrease in reward) following chronic administration of all major drugs of abuse including opiates, psychostimulants, alcohol, and nicotine. These effects can last up to 72 h depending on the drug and dose administered (see Fig. 1). The decreased reward function may reflect changes in the activity of the same reinforcement circuitry implicated in the positive reinforcing effects of drugs and the recruitment of other systems involved in mediating aversive stressful states (Leith and Barrett, 1976; Markou and Koob, 1991; Legault and Wise, 1994; Parsons et al., 1995).

2.2. Compromised positive reinforcement neurochemical mechanisms

The neuronal basis for neuroadaptive mechanisms described by both counteradaptation and sensitization can be envisioned at the molecular, cellular and system levels. Within-system adaptations have been hypothesized wherein neurochemical changes associated with the same neurotransmitters implicated in the acute reinforcing effects of drugs are altered during the development of dependence (Koob and Bloom, 1988, p. 7615). Examples of such homeostatic, within-systems adaptive neurochemical events which persist for varying periods of time following withdrawal from chronic drug exposure, include decreases in dopaminergic and serotonergic transmission in the nucleus accumbens during drug withdrawal as measured by in vivo microdialysis (Weiss et al., 1992; Maisonneuve et al., 1995; Parsons et al., 1995), persistent reductions in both dopamine D1 and D2 receptor-binding in vivo as measured by positron emission tomography (PET) for different periods of extended time after withdrawal from ‘binge’ pattern cocaine exposure (Volkow et al., 1990, 1993; Tsukada et al., 1996; Kreuter et al., 1998; Maggos et al., 1998), increased sensitivity of opioid receptor transduction mechanisms in the nucleus accumbens during opiate withdrawal (Stinus et al., 1990), decreased GABAergic and increased NMDA glutamatergic transmission during ethanol withdrawal (Fitzgerald and Nestler, 1995; Roberts et al., 1996; Weiss et al., 1996) and differential regional changes in nicotinic receptor function (Collins et al., 1990; Dani and Heinemann, 1996). Many other adaptive changes due to the intrinsic plasticity of the brain have been identified during, or immediately following chronic drug exposure, but their possible persistence into protracted withdrawal has not yet been reported.

2.2.1. Dopamine

Many studies have shown that dopaminergic systems undergo significant changes following chronic administration of psychomotor stimulant drugs. High dose continuous access, 'binge-like', self-administration is associated with decreases in extracellular dopamine (Weiss et al., 1992) and increased sensitivity to dopamine receptor antagonists within 24 h 'post-
binge' (Baldo et al., 1998). A similar animal model was developed in which cocaine is delivered by the experimenter in a 'binge' pattern, mimicking the most common human pattern of cocaine abuse. In this procedure, three (or five) doses of cocaine are given at 1-h intervals; using the technique of microdialysis, extracellular levels of dopamine were measured both in the nucleus accumbens, as well as in the caudate putamen (Maisonneuve and Kreek, 1994; Maisonneuve et al., 1995). Following each dose of cocaine delivered by the intraperitoneal route, there was a brisk rise in both cocaine levels and also in extracellular fluid concentration of dopamine. As in other reported studies, the increments in extracellular fluid levels of dopamine following each administration of cocaine were much greater (from four- to fivefold increase) in the nucleus accumbens region than in the caudate putamen region (from two- to threefold). When cocaine was administered on a chronic 'binge' pattern basis, with microdialysis studies performed on day 14 of 'binge' pattern cocaine administration and contrasted to control animals to whom saline had been administered in the 'binge' pattern and who received cocaine on the 14th day only, several provocative findings were made. The basal dopaminergic levels were significantly lowered both in the nucleus accumbens region and in the caudate putamen of the striatum 22 h after the last dose of cocaine was administered and just before the final day of 'binge' pattern cocaine administration in the chronic treated animals, as contrasted with the saline control animals. In addition, at every time point following cocaine administration, the actual nM levels of dopamine in the extracellular fluid were significantly lower in the chronic cocaine treated animals. Also, following chronic treatment, a longer time was required for dopamine levels to return to the pretreatment baseline. Thus, in animals who had been receiving cocaine chronically in the 'binge' pattern, there was a significant decrease in the actual extracellular dopamine levels at the basal time points and following each cocaine administration both in the caudate putamen and in the nucleus accumbens regions of the striatum (Maisonneuve et al., 1995).

In parallel studies using exactly the same 'binge' pattern cocaine administration, animals were treated in home cage and locomotor activity was followed on a daily basis. Interestingly, sensitization was observed, with much greater locomotor activity following the 13th day of cocaine treatment as contrasted with the enhancement of locomotor activity following each dose of cocaine administration on day 1 (Unterwald et al., 1994b). Thus, despite a lowering in both basal and cocaine-induced dopamine concentrations, but with a preservation of the cocaine-induced brisk rise and amplitude of rise in dopamine concentrations, behavioral sensitization with respect to locomotor activity was observed in this daily 'binge' pattern cocaine administration paradigm. After 14 days of 'binge' pattern cocaine administration, significant increases in D₁ dopamine receptor densities were found in the olfactory tubercle, the nucleus accumbens and in the ventral pallidum using quantitative autoradiography (Unterwald et al., 1994a). However, after 14 days of 'binge' pattern cocaine administration, no significant changes were found in D₂ dopamine receptor density in any brain region studied. These findings were in sharp contrast to the very different findings made in the living rat using positron emission tomography (PET), but following the same 'binge' pattern cocaine administration paradigm. Although dopamine concentrations were shown by the microdialysis studies to be at lowered levels, both at basal time points and following cocaine administration, after 14 days of binge cocaine administration than after day 1 of cocaine administration, no changes in D₁ or D₂ dopamine receptor-binding were found by PET following two days of 'binge' cocaine. However, after 14 days of 'binge' pattern cocaine administration, significant reduction in binding was found in the striatum by both D₁ and D₂ dopamine receptors (Tsukada et al., 1996; Kreuter et al., 1998; Maggos et al., 1998). In further ongoing studies, protracted time periods were found to be essential for recovery, first of D₁ dopamine-type receptors and then, later, the D₂-type dopamine receptors. In other in vitro studies, chronic 14 day 'binge' pattern cocaine administration did not alter the quantitatively measured dopamine transporter (DAT) mRNA levels (Maggos et al., 1997).

In related studies, the effects of chronic 'binge' pattern cocaine administration on D₁ receptor mediated transduction has been studied. By quantitative measurement of adenylyl cyclase activity, both using dopamine and a selected dopamine D₁ receptor agonist, it was found that dopamine D₁ receptor signal transduction is significantly enhanced both in the nucleus accumbens and caudate putamen following chronic 'binge' pattern cocaine administration (Unterwald et al., 1996a). Also elevations in preproenkephalin mRNA levels were seen after acute, subacute and chronic 'binge' pattern cocaine administration (Spangler et al., 1993, 1996a, 1997a,b) and may contribute to the behavioral stereotypy observed following the 'binge' pattern cocaine administration that was blocked by the D₁ dopamine receptor antagonist, but not a D₂ dopamine receptor antagonist (Spangler et al., 1997b). In summary, intense continuous access to cocaine via intravenous self-administration or repeated 'binge-like' experimenter administered cocaine can significantly decrease extracellular dopamine and perturb receptor and second messenger function.
In contrast, chronic administration of cocaine or amphetamine under intermittent schedules rather than a binge-like or continuous pattern results in a different pattern of results. The increase in extracellular dopamine produced by indirect sympathomimetics is increased with repeated administration (Kalivas and Duffy, 1990, 1993; Pierce and Kalivas, 1997). These effects are more robust after lengthy withdrawal periods (over 14 days) and are consistently associated with the expression of behavioral sensitization, usually in the form of enhanced locomotor activity (Pierce and Kalivas, 1997a). The cellular basis for this enhanced dopamine release appears to involve calcium-dependent protein kinase control of dopamine release (Pierce and Kalivas, 1997b).

Decreases in dopamine transporter function including both binding and mRNA levels have been observed in selective brain regions, following 10 days of withdrawal from chronic cocaine administration, but not during or immediately following cocaine administration (Kuhr and Pilotte, 1996; Maggos et al., 1997). Sub-sensitivity of dopamine D$_1$ autoreceptors following chronic cocaine administration has been reported, along with a decrease in binding at both D$_1$ and D$_2$-type dopamine receptors in vivo (Antelman and Chiodo, 1981; Volkow et al., 1990, 1993; Tsukada et al., 1996; Maggos et al., 1998; Kreuter et al., 1998). In contrast (as discussed above) in other studies, immediately following chronic cocaine administration, with in vitro receptor density measurements by quantitative autoradiography, increase in D$_1$ dopamine receptor density, coupled with increases in D$_1$ signal transduction has been observed (Unterwald et al., 1994a, 1996). After a period of 7–14 days of withdrawal following chronic cocaine administration, in other in vitro studies, a decrease in D$_1$ dopamine receptor-binding has been found (Kleven et al., 1990; Farfel et al., 1992; Laurier et al., 1994).

2.2.2. Serotonin

Serotonergic systems have also been shown to be altered by chronic administration of stimulant drugs. Twelve hours of continuous access to cocaine via intravenous self-administration produces decreases in extracellular serotonin in the nucleus accumbens (Parsons et al., 1995, 1996). These effects are consistent with changes in serotonin receptor function and transport which may contribute to a post-cocaine deficiency in extracellular serotonin. Repeated cocaine produces changes in the inhibitory serotonin-1 receptor family (Cunningham et al., 1992; King et al., 1993) and the serotonin-2 receptor family when intermittent cocaine administration is used (Levy et al., 1992). Also, increases in the density of serotonin uptake sites have been observed following repeated cocaine exposure (Cunningham et al., 1992). However, following chronic ‘binge’ pattern cocaine administration, a significant decrease in binding of a selective 5HT1A receptor agonist has been found in the dorsal dentate gyrus and also, in the ventromedial hypothalamus, but with no significant changes in 5HT2A receptor agonist binding observed in any brain region (Perret et al., 1998).

2.2.3. GABA

Changes in GABAergic function have largely been associated with chronic administration of sedative-hypnotics, such as ethanol or barbiturates. Chronic ethanol is associated with decreases in the ability of ethanol to potentiate GABA-stimulated Cl$^{-}$ flux (Frank et al., 1972; Morrow et al., 1988). Ethanol-dependent rats trained to self-administer ethanol during withdrawal showed a significant increase in sensitivity to the GABA agonist muscimol. Muscimol injected into the central nucleus of the amygdala decreased responding for ethanol intake at doses that did not alter responding in non-dependent rats (Roberts and Koob, 1996). One hypothesis to explain these results suggests that during withdrawal from chronic ethanol, the GABAergic system has become more sensitive to agonists because agonists in effect remove the motivation to self-medicate.

2.2.4. Opioid peptide systems

Chronic administration of opiate drugs produces profound changes in the sensitivity to the aversive effects of opiate antagonists in rodents. There is a shift to the left of the dose effect function for opiate antagonists to produce place aversions (Hand et al., 1988), and this increased sensitivity to the aversive effects appears to be localized to the nucleus accumbens and central nucleus of the amygdala (Stinus et al., 1990). While such enhanced sensitivity is very obvious in animals that are physically dependent on opiates, it is clear that the increased sensitivity to aversive-like effects can even be seen after a single injection of opiate in the rat (Schulteis et al., 1997). Much earlier studies in mice demonstrated the development of acute tolerance to the analgesic effects of morphine after a single dose and also, the development of acute physical dependence, with the appearance of a weak abstinence syndrome of increased sniffing, exploration, hyper excitability and jumping following opioid antagonist administration (Huidobro et al., 1976). Termed acute dependence or sensitization to opiate antagonists, the enhanced sensitivity to opiate antagonists after a limited experience with opiates has been seen in a variety of studies in rodents (Meyer and Sparber, 1977; Bickel et al., 1988; Adams and Holtzman, 1990; Schulteis et al., 1997). In humans, there is a marked enhanced sensitivity of recently or former opiate-dependent individuals to
antagonist challenge (Bickel et al., 1988; Heishman et al., 1989a,b; Culpepper-Morgan et al., 1992a; Culpepper-Morgan and Kreek, 1997; Higgins et al., 1992; Rosen et al., 1995, 1996) and there is one report of dependence in non-addict humans after a single dose of morphine (Jones, 1979). This phenomenon may reflect the initial adaptive changes that lead to the state known as chronic opiate dependence. Supporting this hypothesis is the observation that repeated intermittent exposure to moderate doses of morphine can result in a progressive increase in the potency of opiate agonists to disrupt behavior (Young, 1986; Adams and Holtzman, 1990; Schulteis et al., 1997). The potential motivational significance of dependence with opiates has been well explored in rodent and primate models. For example, conditioned withdrawal has been repeatedly observed in opiate-dependent animals and humans. In the early, classic studies by Wikler and colleagues (Wikler and Pescor, 1967; Wikler, 1973), rats made dependent by gradually increasing daily doses of morphine were exposed to a particular environment while experiencing morphine abstinence. After 6 weeks of such pairings, rats exposed to this same distinct environment showed physical withdrawal signs up to 155 days after the last morphine injection. In morphine-dependent rhesus monkeys trained to lever-press for food on a fixed ratio FR-10 schedule, injection of the opiate mixed agonist/antagonist nalorphine-produced suppression of food-maintained responding and physical signs of withdrawal [reviewed in Goldberg (1976)]. After repeated pairings of a light or tone with the nalorphine injection, these conditioned stimuli alone could completely suppress responding. Similar results have also been obtained in morphine-dependent rats trained on an FR-15 schedule for food (Baldwin and Koob, 1993). Here, responses to the conditioned stimulus persisted for 1 month after morphine termination. More importantly for the present treatise, motivational signs of withdrawal have been conditioned in animals (see below).

2.3. Recruitment of stress/aversive systems

2.3.1. Hypothalamic–pituitary–adrenal (HPA) axis

2.3.1.1. Human studies of the HPA axis. Recruitment of neurotransmitter systems not linked to the acute reinforcing effects of the drug in the adaptive responses to drugs of abuse, has been termed a ‘between-system’ adaptation (Koob and Bloom, 1988). A common between-system adaptation to repeated administration of drugs of abuse may be activation of brain, hypothalamic and pituitary stress systems. Pituitary adrenal function is activated during some types of drug dependence and during acute withdrawal from drugs of abuse in humans; dysregulation can persist even past acute withdrawal (Kreek et al., 1984; Kreek, 1987; Schluger et al., 1998). Hypothalamic–pituitary–adrenal function is either depressed (heroin and other short-acting narcotics used acutely or on a chronic basis in humans, and when administered on a chronic basis, but not an acute basis, in rodent models) or activated (cocaïne and ethanol in humans and in rodent models and nicotine in humans) during chronic use, drug dependence and addiction (Kreek, 1972, 1973a,b, 1996b). During acute withdrawal from essentially all drugs of abuse which have been studied, activation of hypothalamic-pituitary-adrenal axis is seen in both humans and in rodent models. In parallel, but normally under different feedback regulatory control at extra hypothalamic sites, the major hypothalamic hormone of the hypothalamic–pituitary–adrenal axis, corticotropin-releasing factor (CRF), is also deranged in many regions by drugs of abuse and in the setting of withdrawal from drug dependence. This dysregulation of the hypothalamic–pituitary–adrenal axis has been shown to persist long past the acute withdrawal period. In very early studies of the acute and chronic effects of opiates in humans and in the early studies of cycles of opiate addiction, one of the abnormalities observed both during addiction to heroin or morphine and following withdrawal of the drug were abnormalities of the hypothalamic–pituitary–adrenal axis and hypothalamic–pituitary–gonadal axis. These included early observations from USPHS Service in Lexington, Kentucky of reduced levels of both urinary 17-keto-steroids, representing an index of both adrenal and gonadal function in morphine-dependent former heroin addicts and later, similarly, reduced 24-h urinary excretion of 17-hydroxycorticosteroids reflecting adrenocortical function, as well as reduced plasma levels of 17-hydroxycorticosteroids (Eisenman et al., 1958, 1961). Much later subsequent studies also showed reduced plasma levels of ACTH and cortisol in active heroin addicts not receiving treatment (Ho et al., 1977). In the early study in Lexington, it was also shown that there were increments in plasma and urinary 17-hydroxysteroids following withdrawal and that longer periods of addiction were associated with higher elevations in glucocorticoid levels, suggesting that the elevation in plasma and urinary glucocorticoids during withdrawal reflected the duration of addiction. In these early toxicological studies, there was apparently no contemplation that these findings might be mechanistically related both to the perpetuation of addiction and the propensity to relapse after reaching an opiate-free state.

From the 1964 initial studies which led promptly to the development of methadone maintenance treatment, the hypothesis underlying the work was that heroin addiction is a ‘metabolic disease’, with neu-
robiologic changes either caused by chronic use by the short-acting opiate drug, heroin, or alternatively, by some intrinsic abnormality, either genetically or environmentally induced, in the individual yielding an enhanced vulnerability to develop an addiction once exposed to the illicit drug of abuse. It was suggested that the 'euphoric effect' of heroin appeared to be a 'learned phenomena, like pleasure from smoking' since the majority of the studied subjects stated that the first experiences with heroin were usually aversive and caused nausea and vomiting, rather than pleasure and that only later did the euphoric experience 'become central to the addict's life' (Dole et al., 1966). Early studies were initiated to determine the physiologic effects of opiates which might contribute to the development of addiction. Treatment with the long-acting opiate methadone was found to exert very different effects as compared with heroin, with persistent physiologic abnormalities observed only during ascending dose and then, with normalization of heroin-induced abnormalities during stabilization on methadone treatment (Kreek, 1972; Inturrisi and Verebey, 1972a,b; Kreek, 1973a,b,c, 1978; Kreek et al., 1979; Nakamura et al., 1982; Inturrisi et al., 1984). Methadone has a profoundly different pharmacokinetic and pharmacodynamic profile than heroin in humans, with a half-life of the racemic mixture used in treatment of 24 h and the half-life of its active enantiomer of over 48 h, as contrasted to the half-life of heroin of 3 min, its first active metabolite 6-acetyl morphine of 30 min and its major metabolite, morphine, of 4–6 h in humans. Specific aspects of neuroendocrine function were studied which are known to be central to survival mechanisms, including the hypothalamic–pituitary–adrenal stress responsive axis and the reproductive biology regulating hypothalamic–pituitary–gonadal function, as well as aspects of thyroid function, and also prolactin release, which was then known to be important in lactation and is now known to be important also for immune function and other aspects of physiology. Since prolactin release in humans is essentially completely under tonic inhibitory modulation through action of the tuberoinfundibular dopaminergic system, studies of prolactin levels and responsivity to changes may be used as a reflection of central dopaminergic activity, at least of the tuberoinfundibular dopaminergic system (Cushman et al., 1970; Kreek, 1972, 1973a,b,c; Cushman and Kreek, 1974a,b; Kreek, 1975; Stimmel and Kreek, 1975a,b; Kreek, 1978). In these early studies, the Lexington findings were confirmed and extended. Of interest, it was found that the diurnal rhythm of plasma levels of cortisol was flattened or actually reversed in heroin addicts, an abnormality frequently seen primarily in disorders of adrenocortical neuroendocrine function. Lower basal levels, but within normal range values, of cortisol were observed in most heroin addicts entering methadone treatment.

The provocative test of hypothalamic–pituitary reserve, the metyrapone test, was performed, in these early studies, a test in which the final step of 11-β hydroxylation in the adrenal cortex, which is essential for the synthesis of cortisol, is temporarily blocked. The result is an excessive outpouring of hypothalamic and pituitary stimulatory hormones, both CRF and ACTH, with an increase of levels of those hormones measured, in the early tests, as increases in the urinary excretion of the precursors of the glucocorticoids ('Porter–Silber chromogens') (see Fig. 2). Abnormal (lower than normal) results, or a 'reduced hypothalamic–pituitary reserve', were found in active heroin addicts. However, adrenal function was shown not to be impaired, since ACTH stimulation tests in heroin addicts and former heroin addicts during methadone maintenance treatment were found to be normal. As part of the early prospective studies, repeated challenge tests were conducted in the same subject and it was found that the inadequate hypothalamic-pituitary reserve, as seen in the metyrapone test, became normal after 2 months or more of stable moderate to high dose (60–120 mg/day) methadone maintenance treatment. These studies, including metyrapone challenge tests, have been replicated using modern techniques, with plasma measurements of ACTH, β-endorphin and cortisol. Again, it has been found that during chronic moderate to high dose methadone maintenance treatment, plasma levels and diurnal rhythm of levels of the stress-responsive HPA axis become normalized, with normal diurnal variation of these levels. It has again been shown that ACTH stimulation tests are normal. It also has been found that metyrapone challenge tests become normalized after the first 2 months or more of stabilization, with the expected magnitude of rise of ACTH and β-endorphin plasma levels.

These studies, which were conducted with the hy-

![Fig. 2. Schematic design of metyrapone challenge test of hypothalamic pituitary function in human subjects.](image-url)
hypothesis that disruption of the critical stress-responsive axis may contribute to the dysphoric and abnormal feelings that both contribute to the perpetuation of addiction, as well as play a powerful role in the ‘drug craving’ or ‘drug hunger’ which leads to relapse, made several important findings. During cycles of heroin addiction, suppression of adrenocortical function was subsequently found to be counter-balanced by spontaneous hyperactivity of the stress-responsive axis in the setting of opiate withdrawal, a hyperactivity that could be immediately and specifically reversed by the reapplication of a short-acting opiate, such as heroin, thus potentially contributing to the perpetuation of administration of opiates in that setting. Both laboratory and clinical studies have documented the long-standing clinical observation of the highly significant enhanced sensitivity of opiate-dependent persons, to the application of a specific opioid antagonist whether persons with addictive disease or receiving opiates on a chronic basis for the relief of pain, showing the disregulation of this important stress-responsive system (Cushman et al., 1970; Kreek, 1972, 1973a,b, 1975, 1978, 1990a, 1997; Cushman and Kreek, 1974a,b; Stimmel and Kreek, 1975a,b; Kreek and Hartman, 1982; Kreek et al., 1981, 1983; Bickel et al., 1988; Kennedy et al., 1990; Culpepper-Morgan et al., 1992b; Culpepper-Morgan and Kreek, 1997).

Provocatively, in other studies, it has been found, without precedent at the time, that in medication-free, illicit opiate-free former heroin-dependent persons, there is a hyper responsivity to the metyrapone challenge test, with excessive levels of ACTH and β-endorphin released in response to the abrupt cut-off in the normal, negative feedback control by cortisol at both the hypothalamic sites of CRF production and the anterior pituitary sites of proopiomelanocortin (POMC) peptide production (Kreek et al., 1984; Kreek, 1987, 1992). Very recent studies have also shown a similar hyper responsivity to metyrapone challenge in recently abstinent cocaine-dependent persons (Schluger et al., 1998). Also, unexpectedly, in the earliest studies of heroin addicts entering and during methadone maintenance treatment, normal responses to the standard (1 or 2 mg) dexamethasone suppression tests were uniformly found, with all subjects suppressing on usual test doses (Kreek, 1972, 1973a). Similarly, in more recent studies, normal responses to the standard dexamethasone tests have been found in methadone maintained patients, with or without ongoing cocaine addiction (Aouizerate et al., 1999). This was surprising since 20–50% of heroin addicts entering and during methadone maintenance treatment have depressive symptoms and a lesser number have diagnosis of major depression. It would, therefore, be anticipated that some would have an abnormal dexamethasone suppression test with failure of this synthetic glucocorticoid to suppress the HPA axis as measured by plasma cortisol levels. Studies have shown that although baseline levels of cortisol are normal in active cocaine addicts, just as they are in most former heroin addicts in methadone maintenance treatment and also, in methadone maintained patients who can have a co-dependency with cocaine, nevertheless, cortisol levels are at the lower limit of normal (Aouizerate et al., 1999). Therefore the question has been raised of whether or not there may be excessive suppression or super-sensitivity at hypothalamic or pituitary sites of feedback control by glucocorticoids, which could explain, in part, the uniform ‘normal’ response to the standard dexamethasone suppression tests.

Activation of the HPA function has been found in active cocaine addicts, with enhancement of levels of ACTH and cortisol immediately following cocaine administration, although normal baseline cortisol, ACTH and β-endorphin levels have been found (Mello and Mendelson, 1997; Schluger et al., 1998). Similarly, both alcohol, in extensive studies, and nicotine, in limited studies, have been shown to enhance levels of ACTH and β-endorphin, as well as the peripheral stress responsive steroid, cortisol, in humans.

Thus, both acute and chronic administration of opiates in humans suppress the hormones of the HPA axis. They also suppress hormones of the hypothalamic-pituitary-gonadal axis. In the setting of acute, spontaneous or precipitated, opiate withdrawal, activation of the stress responsive axis is seen, with increased levels of ACTH, β-endorphin and cortisol. After stabilization in moderate to high dose treatment with the long-acting opioid, methadone, normalization of this axis occurs, with normal levels and diurnal rhythm of the levels of ACTH, β-endorphin and cortisol. Also, normalization of hormone levels and function of the hypothalamic-pituitary-gonadal axis occurs (Kreek, 1978). Prolactin levels which are elevated after each acute or chronic dose of short-acting opiate, such as heroin are no longer elevated during chronic methadone treatment, although responsibility of prolactin release to administration of this long-acting opioid is found, with peak serum prolactin levels occurring at time of peak plasma levels of methadone, suggesting a continuing effect of methadone on tuberoinfundibular dopaminergic tone (Kreek, 1978, 1997). Recent studies have found that in the long-term opioid abstinence state and during chronic methadone treatment, both early and late abstinence from cocaine (although basal levels of HPA hormones are normal), there is a hyper responsivity to a chemically induced stressor, i.e. metyrapone, challenge. In contrast, cocaine, nicotine and alcohol in humans acutely activate the HPA axis immediately following drug administration with
increased levels of ACTH, β-endorphin and cortisol. Basal levels of these hormones are normal between self-administrations of the drug of abuse and remain normal into the withdrawal period.

The early findings of profound disruption of the HPA axis in heroin addicts, coupled with the finding of normalization of that axis, along with normalization of behaviors, and rates coupled with reduction of 'drug craving' or 'drug hunger', and a significant reduction in in most patients, elimination, of self-administration of illicit opiates in methadone-maintained former heroin addicts, led to the early hypothesis that an atypical responsivity to stress and stressors contributes to the acquisition and persistence of and relapse to cycles of heroin addiction (Kreek, 1972, 1973a,b, 1987, 1992). More recently, the hypothesis of the role of the altered stress responsivity in the neurobiology of addictive diseases has been extended to include its role in cocaine addiction, alcoholism and possibly, nicotine dependency (Kreek, 1972, 1973a,b, 1987, 1992, 1996a,b, 1997; Piazza and LeMoal, 1995, 1996; Culpepper-Morgan and Kreek, 1997; Koob and LeMoal, 1997).

2.3.1.2. Animal model studies of HPA axis integrity during opiate and cocaine exposure. Over the past several years, the peptides have been defined and the genes cloned for CRF, the major regulator of POMC peptide release in humans and other mammals and more recently, CRF receptors of two different types. Using riboprobes constructed from cDNAs from several of these genes and modified techniques of solution hybridization RNAase protection assay, the normal regulation of the HPA axis has been studied (Zhou et al., 1996a). These studies have elucidated both actual mRNA levels as well as the localization of gene expression of CRF and CRF-R1-type receptors and the POMC gene, in different brain and anterior vs. intermediate–posterior lobe pituitary regions. In these studies, it was shown quantitatively that a glucocorticoid, such as dexamethasone will cause decreased gene expression of CRF in the hypothalamus and also, decreased mRNA levels for CRF-R1 receptor and POMC in the anterior pituitary. However, neither CRF or CRF-R1 gene expression or POMC gene expression was altered in other brain regions where they were found, including no glucocorticoid regulation of POMC mRNA in the hypothalamus or in the amygdala and no regulation of CRF mRNA in the amygdala or frontal cortex. Also, these studies confirmed many earlier studies that CRF administration results in increased levels of POMC mRNA in the anterior pituitary (Zhou et al., 1996a). It has been shown that opiates, such as morphine when delivered on an intermittent basis cause acute activation of the HPA axis in rats and that during chronic intermittent administration, tolerance or habituation of these effects occur, with ultimate suppression of the HPA axis. These findings, in part, contrast with the findings in humans where suppression of the HPA axis by short range opiates is found both in naive subjects and in persons with defined long-term addictive diseases. However, it also has been recently shown that when methadone, which is short-acting in rats (half-life of 90 min) as contrasted to long-acting in humans (half-life over 24 h) is delivered in a steady-state by pump, such administration on a chronic basis does not change mRNA levels of CRF, CRF-R1 receptor or POMC, nor does it change plasma levels of ACTH or corticosterone (Zhou et al., 1996b). These findings thus parallel the findings in humans where normalization of the stress responsive HPA axis occurs during steady state modest to high dose methadone maintenance treatment (Kreek, 1972, 1973a, 1978, 1996a,b, 1997; Kreek et al., 1984). Also, steady state pump administration of methadone did not alter CRF mRNA levels in other regions important for other potential anxiogenic effects of CRF; CRF mRNA levels were not altered in the frontal cortex, the olfactory bulb or the amygdala during steady state methadone administration in the rodent model (Zhou et al., 1996b).

In contrast, several groups have shown that cocaine profoundly activates the HPA axis in rodent models, as it has been shown to do in humans; more recent studies have shown that these changes are paralleled by the changes in gene expression induced by acute cocaine administration (Rivier and Vale, 1987; Sarnyai et al., 1992, 1993; Rivier and Lee, 1994; Zhou et al., 1996b). Acute 'binge' pattern cocaine administration delivered on the first day has been shown to significantly increase CRF mRNA levels in the hypothalamus, as well as another area of potential great importance for the effects of drugs of abuse and the persistent changes following chronic exposure (see below), the amygdala. However, on the first day of 'binge' pattern cocaine administration, no changes in CRF-R1 or POMC mRNA levels were found in the anterior lobe of the pituitary. Large increases in corticosterone levels were seen after the first day of 'binge' pattern cocaine administration and these persisted following the second day of cocaine administration. However, by the second day of cocaine administration, CRF gene expression in the hypothalamus and in the amygdala had returned to normal. Although there were no changes in mRNA levels in the frontal cortex following one or two days of cocaine administration, a significant increase in CRF mRNA levels was found in the frontal cortex after three days of chronic cocaine administration. Since CRF mRNA has been shown to be regulated by glucocorticoids only in the hypothalamus and not in the frontal cortex, nor in limbic-related areas, such as the amygdala.
dala or olfactory bulb, these findings may be of potential importance for the more persistent anxiogenic effects of cocaine (Zhou et al., 1996a,c). By 14 days of ‘binge’ pattern cocaine administration, CRF mRNA levels were significantly lower in the hypothalamus than in saline controls. Following further subacute and chronic administration of cocaine in the ‘binge’ pattern, it was found that plasma corticosterone levels remained elevated after 14 days of ‘binge’ pattern cocaine administration, as compared with saline controls, but by day 14, they were significantly lower than during the first 3 days of ‘binge’ pattern cocaine administration. Following 10 days of withdrawal after 14 days of chronic ‘binge’ pattern cocaine administration, plasma corticosterone levels had returned to normal (Zhou et al., 1996c). It is possible that this chronic 14 day ‘binge’ pattern cocaine administration which mimics, a common pattern of cocaine abuse and begins to act as an unremitting stress. Such unavoidable stress of a variety of types studied in both human and animal models, including post-traumatic stress disorder and chronic spinal cord injury, is characterized by a depressed state of the HPA axis, rather than activation of this axis (Culpepper-Morgan et al., 1992b). All of these findings suggest that an atypical responsivity to stress may pertain with different observations made in different stages of drug exposure and withdrawal.

2.3.1.3. Implications of disruption of the HPA axis on acquisition and persistence of addictions. The possible involvement of the endogenous opioids and opioid receptors in three addictive diseases, opiate addiction, cocaine addiction and alcoholism, during chronic exposure and during periods of abstinence, both potentially contributing to perpetuation of addictions and relapse to drug use when abstinent have been suggested and supported in part by diverse studies.

Again, parallel studies have been conducted in a variety of animal models, including studies utilizing the most recent techniques of modified solution hybridization RNAase protection assay to quantitate levels of mRNA of genes of importance to the stress responsive axis, both in HPA function, as well as in other brain regions, along with measurements of resultant peptides and steroids.

Both stress and repeated administration of glucocorticoids can increase behavioral effects of psychostimulants and it has been hypothesized that circulating glucocorticoids can function to maintain the sensitized state (Piazza and LeMoal, 1996, 1997) and as such, may represent a within system adaptation (Piazza and LeMoal, 1996). In numerous studies conducted in Sprague–Dawley rats and more recently, studies conducted in a very different strain, the Long–Evans rat, pronounced individual variation with respect to response to a novel environment, as measured by locomotor activity has been identified. In several sequences of studies, it has been shown that animals divided at the median into ‘higher’ and ‘lower’ responders to a novel environment and then studied in a variety of cocaine administration paradigms, including self-administration and ‘binge’ pattern administration, differences in both plasma corticosterone levels and locomotor activity were found to parallel each other and also, in self-administration studies, were positively correlated with self-administration behaviors (Piazza et al., 1989, 1990; Piazza and LeMoal, 1995; Lucas et al., 1998). In the study in Long–Evans rats, it was found that chronic 14-day ‘binge’ pattern cocaine administration resulted in converting the locomotor activity behavior of the novel environment ‘low responders’ to a level similar to that of the potentially more vulnerable ‘high responders’ phenotype, suggesting that chronic exposure to this stimulant could actually alter responses to a novel environment and thus, potentially, vulnerability to cocaine or other psychostimulant self-administration. There were no significant differences between the ‘high’ and ‘low’ responding rats with respect to corticosterone levels in the Long–Evans rats, however, either before or following 14-day ‘binge’ pattern cocaine administration induced increases in corticosterone (Lucas et al., 1998).

In other studies, the question of whether or not glucocorticoids are essential for the expression of sensitized behaviors following chronic cocaine administration has been challenged. Using the ‘binge’ pattern cocaine administration model, it was found that both D₁ and D₂ dopamine receptor antagonists would block the elevation of plasma corticosterone levels caused by cocaine. However, only the D₁ dopamine receptor antagonist blocked the behavioral stereotypy observed in that setting; behavioral stereotypy continued despite blockade of elevation in corticosterone levels following D₂ receptor antagonist administration (Badiani et al., 1995; Spangler et al., 1997b).

However, clearly activation of release of the peptides, ACTH and β-endorphin and cortisol, has been repeatedly shown in humans, as well as in animal models, to occur following administration of a specific opioid antagonist, acting primarily at the µ, but also possibly at the K opioid receptors. All of these findings provide evidence that the stress responsive axis in humans and in animal models, is at least, in part, under negative feedback control modulation both by glucocorticoids and by the endogenous opioids directed at the µ and K system. Also, these findings suggest that super imposition of an exogenous opioid acutely, or following intermittent administration of a short-acting opioid on a chronic basis, such as occurs during cycles of heroin addiction, may attenuate HPA axis function by enhanced negative feedback mecha-
nism by the endogenous plus exogenous opioids. Removal of all exogenous opioids may leave a persistent, relative opioid deficiency with inability for the endogenous opioids to fully counter the impact of removal of glucocorticoid regulation (e.g. the metyrapone test) and possibly, also such an inability or defective counter-regulation in the setting of environmental stressors (Kreek, 1987, 1992, 1997). The relationship of this opioid receptor system and the other components of the classical HPA stress responsive axis and the acquisition and persistence of addictive diseases and the relapse to addiction continues under study.

2.3.2. Central nervous system stress systems

Much recent evidence suggests that brain stress systems independent of the pituitary-adrenal axis can contribute to the aversive state associated with discontinuation of chronic drug administration. Corticotropin-releasing factor in addition to being a major mediator of the effects of stress on the pituitary adrenal axis is widely distributed in the limbic systems and CRF mRNA levels have been quantitated in a variety of brain regions beyond the hypothalamus; CRF is known to contribute significantly to behavioral responses to stressors (Koob et al., 1994). Corticotropin-releasing factor function, outside of the pituitary adrenal axis, appears to be activated during acute withdrawal from cocaine, alcohol, opiates, and THC and thus may mediate behavioral aspects of stress associated with abstinence (Koob et al., 1994; Heinrichs et al., 1995; Merlo-Pich et al., 1995; Richter and Weiss, 1997; Rodriguez de Fonseca et al., 1997) (see Fig. 3). Rats treated repeatedly with cocaine, nicotine and ethanol show significant anxiogenic-like responses following cessation of chronic drug administration which are reversed with intracerebroventricular administration of a CRF antagonist (Rassnick et al., 1993; Sarnyai et al., 1995). Microinjections into the central nucleus of the amygdala of lower doses of a CRF antagonist also reversed the anxiogenic-like effects of ethanol withdrawal (Rassnick et al., 1993) and similar doses of the CRF antagonist injected into the amygdala were active in reversing the aversive effects of opiate withdrawal (Heinrichs et al., 1995). Since other studies have shown that glucocorticoids including the synthetic steroid dexamethasone, regulate CRF gene expression in the hypothalamus, but not in the other brain regions including the amygdala, olfactory bulb and frontal cortex, the enhancement of CRF activity in these brain regions may not benefit from the normal counter-regulatory negative feedback effects of glucocorticoids on CRF gene expression and peptides of the HPA axis in ameliorating the stress responsivity, such as undoubtedly occurs during withdrawal (Zhou et al., 1996a).

2.3.3. Anti-reward systems

Evidence for other between systems adaptations following chronic use of drugs of abuse can be found in studies exploring the role of the neuropeptides including dynorphin, neuropeptide FF, and more recently orphanin FQ. Acute, subacute and chronic self-administration and ‘binge’ pattern administration of cocaine or amphetamine have been shown to increase preprodynorphin gene expression as measured by either in situ hybridization or modified solution hybridization RNAase protection assay; these changes have been found to occur in the caudate putamen region of the striatum (Hurd and Herkenham, 1992; Hurd et al., 1992; Daunais et al., 1993; Spangler et al., 1993; Daunais and McGinty, 1995; Spangler et al., 1996a, 1997a). Increases in prodynorphin gene expression after acute or chronic cocaine administration result in increased dynorphin peptides, both in the substantia nigra, where this increase may lead to the decrease in κ opioid receptor gene expression which has been recently identified, as well as increases of dynorphin peptides in other regions of the extrapyramidal and limbic dynorphin systems (Sivam, 1989; Smiley et al., 1990; Spangler et al., 1996b). Dynorphin peptides in the nucleus accumbens may regulate the dopamine system via a presynaptic action on κ opioid receptors which have been shown to be abundant in density in the nucleus accumbens region and increased in density after ‘binge’ pattern cocaine administration (Unterwald et al., 1994b; Hyman, 1996).
Studies using techniques of microdialysis in freely moving animals have shown that synthetic \( \kappa \) agonists may decrease basal dopaminergic levels, as well as dopamine release in the nucleus accumbens (Spanagel et al., 1990, 1992). Other recent studies have shown that local perfusion of the natural \( \kappa \) opioid peptide dynorphin A1–17 reduces extracellular dopamine levels in the nucleus accumbens in freely moving rats (Claye et al., 1997). Synthetic \( \kappa \) agonists have been shown to produce aversive effects in both rodents and humans; however, the natural peptide, dynorphin A1–13, has not been found to cause adverse effects when administered both to normal, healthy volunteer subjects, as well as patients with defined addictive diseases (King et al., 1998; Speckler et al., 1998). Similarly, a synthetic dynorphin A1–8-like peptide, Elsai 2078, which has been shown to cross the blood–brain barrier in non-human primates, has not been found to cause any dysphoric effects in human subjects (Ohnishi et al., 1994; Yu et al., 1997).

Hypothetically, dynorphin gene expression and peptides released in excess following cocaine or amphetamine stimulation could have opposite or opposing effects to cocaine on reward mechanisms by lowering dopaminergic tone. Although synthetic receptor agonists have aversive effects, possibly \( \kappa \) agonists, such as natural or synthetic peptides without aversive or dysphoric effects in humans could be used in the management of cocaine dependency (Kreek, 1997). Thus, chronic administration of cocaine or amphetamine may induce prodynorphin gene expression that hypothetically could have the opposite or opposing effects of cocaine on reward mechanisms.

Activities which seem to oppose opiate and opioid effects have been hypothesized for the neuropeptide FP (NPFF), previously called F8Fa, based on the effects of intracerebroventricular (I.C.V.) injection of NPFF-related peptides. NPFF attenuates morphine- and stress-induced analgesia (Kavaliers, 1990) and precipitates morphine withdrawal (Malin et al., 1990). An NPFF antagonist can increase both morphine- and stress-induced analgesia, reverse morphine tolerance (Lake et al., 1992), and attenuate naloxone-precipitated withdrawal syndrome in morphine-dependent rats. An NPFF antagonist also blocks some aspects of nicotine withdrawal (Malin et al., 1996). Effects which antagonize opiates have also been reported with administration of the orphan receptor binding peptide orphanin FQ (Mogil et al., 1996). Other natural neuropeptides including cholecystokinin and shortened forms of ACTH have also been reported to antagonize opiate effects. However, it remains to be determined whether these peptides, which appear to attenuate or oppose opiates and opioids, have motivational significance and contribute to the negative affective state produced during drug abstinence.

2.4. Extended amygdala: a common substrate for drug reinforcement

A separate entity within the basal forebrain, termed the 'extended amygdala' has been hypothesized to be a common neural circuitry for the reinforcing actions of drugs based on recent neuroanatomical data and new functional observations (Alheid and Heimer, 1988). Originally described by Johnston (1923), the term 'extended amygdala' represents a macrostructure that is composed of several basal forebrain structures: the bed nucleus of the stria terminalis, the central medial amygdala, the medial part of the nucleus accumbens, i.e. shell (Heimer and Alheid, 1991) and the area termed the sublenticular substantia innominata. Similarities in morphology, immunohistochemistry and connectivity characterize these structures (Alheid and Heimer, 1988); 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, medial ventral tegmental area, various brainstem projections, and a considerable projection to the lateral hypothalamus (Heimer et al., 1991). The observation of a major connection to the lateral hypothalamus is intriguing from a functional point of view since this observation effectively links a potential major site of drug reinforcement to classical substrates for 'natural' reinforcers.

Selective neurochemical and neuropharmacological actions in specific components of the extended amygdala both for the acute reinforcing effects of drugs of abuse and in the negative reinforcement associated with drug dependence have recently been demonstrated. Dopamine \( D_1 \) dopamine antagonists are effective in blocking cocaine self-administration, when the antagonist is administered directly into the shell of the nucleus accumbens, the central nucleus of the amygdala (Caine et al., 1995) and the bed nucleus of the stria terminalis (Epping-Jordan et al., 1997). In addition, selective activation of dopaminergic transmission occurs in the shell of the nucleus accumbens in response to acute administration of virtually all major drugs of abuse (Pontieri et al., 1995, 1996; Tanda et al., 1997). In addition, the central nucleus of the amygdala has been implicated in the GABAergic and opioidergic influences on the acute reinforcing effects of ethanol (Heyser et al., 1995; Hyttia and Koob, 1995).

Evidence for parts of the extended amygdala being involved in the aversive stimulus effects of drug with-
drawal includes the observation of activation of CRF systems in the central nucleus of the amygdala during acute withdrawal (see above) (Merlo-Pich et al., 1995). Also there is the enhanced sensitivity to opiate antagonists in opiate-dependent rats in the nucleus accumbens and central nucleus of the amygdala (see above) (Koob et al., 1989; Stinus et al., 1990). The link of recent developments in the neurobiology of drug reinforcement with existing knowledge of the substrates for emotional behavior (Davis, 1997), may ultimately bridge what has been largely independent research pursuits and provide critical insights into the neurobiology of the addiction process. Perhaps more importantly this neuronal circuit is well situated to form a heuristic model for exploring the mechanisms associated with vulnerability to relapse and concepts, such as craving, both of which may involve secondary conditioned reinforcement constructs.

2.5. Molecular and cellular adaptations

Insights into the molecular and cellular mechanisms of drug dependence have begun to be focused on mechanisms of motivational aspects of dependence. Molecular actions that could represent motivationally important neuroadaptations to chronic cocaine or amphetamine administration include activation of second and third messenger systems associated with the activation of dopamine receptors. Dopamine action at D1-like receptors stimulates a cascade of events, including activation of stimulatory G proteins and increased intracellular cyclic AMP formation that ultimately lead to the transcription factor CREB phosphorylation and immediate early gene expression (see Self and Nestler, 1995; Hyman, 1996). Chronic ‘binge’ pattern cocaine administration has been shown to significantly increase dopamine D1 receptor-mediated signal transduction as measured by both dopamine and selected dopamine D1 agonist stimulation (see above) and this enhanced signal transduction has been documented to occur in both the nucleus accumbens and in the caudate putamen (Unterwald et al., 1996). Recent evidence of effective actions from dopamine D1 antagonists in blocking cocaine self-administration when the antagonist is administered directly into the shell of the nucleus accumbens, the central nucleus of the amygdala, or the bed nucleus of the stria terminalis (see above) and also effective actions of D1 agonists in decreasing the priming effects of cocaine, support a role for the D1 receptor-cAMP-CREB pathway in animals with a history of cocaine self-administration (Self et al., 1996). Activation of inhibitory G proteins, linked to D1 receptors, also appears to be involved in the acute effects of cocaine since pertussis toxin, which inactivates inhibitory G proteins, produces a dopamine receptor antag-
onist-like effect on cocaine self-administration (Self et al., 1994). This presents the apparent paradox of acute cocaine, activating both D1-like and D2-like receptor transduction systems, which act in opposite directions. There is further molecular biological support for acute or subacute cocaine administration activating both D1 and D2 systems, yet chronic cocaine administration activating only D1-type systems. It has been shown that 3 days of ‘binge’ pattern cocaine administration results in an increase in the enkephalin gene whose expression has been linked to D2-type dopamine receptors, whereas it has been shown that preproenkephalin mRNA is not altered by cocaine after 14 days of ‘binge’ pattern administration (Branch et al., 1992; Spangler et al., 1997a). In contrast, prodynorphin gene expression, which has been linked by many studies to D1 dopamine receptor activation, is enhanced after 1, 2, 3 and up through 14 days of acute, subacute and chronic ‘binge’ pattern cocaine administration (Spangler et al., 1993, 1997a). These findings suggest that D1 dopamine receptor activation may be important during acute and subacute cocaine administration, but that during chronic cocaine administration, the effects of D1-type dopamine receptors activation predominate.

In fact, repeated administration of cocaine in the ventral tegmental area produces transient decreases in inhibitory G proteins that may lead to D2 receptor subsensitivity (Nestler et al., 1990; Striplin and Kalivas, 1992) and chronic cocaine transiently increases levels of tyrosine hydroxylase (Beitner-Johnson and Nestler, 1991). Effects of chronic cocaine in the nucleus accumbens that are more prolonged, persisting up to 1 month, include a supersensitivity to D1-mediated responses (Henry and White, 1991), increased levels of adenylyl cyclase and protein kinase A (PKA) (Terwilliger et al., 1991; Unterwald et al., 1996), decreased levels of inhibitory G proteins (Terwilliger et al., 1991), a decrease in the ability of cocaine to induce the immediate early gene c-fos, followed by the sustained expression of AP-1 transcription factor complexes with altered composition and persistent elevations of preprodynorphin mRNA levels in the caudate putamen after each administration of cocaine (Spangler et al., 1993).

Other changes in the mesocorticolimbic dopamine system induced by chronic administration of cocaine include a decrease in the levels of neurofilament proteins in the ventral tegmental area (VTA) (Beitner-Johnson et al., 1992). Lower levels of neurofilament proteins are associated with decreased axonal transport and this could decrease the amount of tyrosine hydroxylase transported from the VTA to the dopamine nerve terminals in the nucleus accumbens (Self and Nestler, 1995). One could hypothesize that such changes could be responsible for short-term re-
ductions in extracellular levels of dopamine release during drug withdrawal (Weiss et al., 1992; Maisonneuve et al., 1995), which then could trigger up-regulation of the cyclic AMP system for more long-term changes (Self and Nestler, 1995; Untewald et al., 1996) (see above). Of interest has been the recent finding of DAT gene expression with quantification of measurable amounts of DAT mRNA in both the caudate putamen and in the nucleus accumbens — areas where DAT mRNA had not been previously observed (Maggos et al., 1997). These areas are that are known to contain abundant DAT protein which is thought to be transported via axons from the cell bodies in the substantia nigra and the ventral tegmental area, respectively. It is not known whether the DAT mRNA in the caudate putamen or nucleus accumbens also came by way of axonal transport of mRNA from the midbrain, as has been previously proposed to explain the presence of tyrosine hydroxylase mRNA in the caudate putamen, or whether there are indeed some dopaminergic neurons in both the nucleus accumbens and the caudate putamen (Melia et al., 1994; Maggos et al., 1997). The effects of chronic cocaine administration on DAT mRNA in these regions could be studied to determine whether or not axonal transport is from the VTA to the nucleus accumbens and whether this transport is impaired following chronic cocaine administration.

With chronic opiate administration, there is little evidence of changes in opioid peptide activity or changes in the number of opioid receptors. However, the recent cloning of the opioid receptors (reviewed by Mansour et al., 1995) and the discovery of new endogenous opioid ligands (Zadina et al., 1997) may provide new potential avenues for exploration. Using probes available from the recently cloned μ receptor, the effects of both opioids and opioid antagonists on μ receptor gene expression have been studied. Whereas previous studies have shown that acute and chronic administration of a variety of opioid agonists have no effects on the density or the binding at μ opioid receptors, numerous studies have shown that chronic administration of specific opioid antagonists which are primarily μ opioid receptor directed, such as naltrexone, significantly increases both the density of μ opioid receptor in diverse regions of the brain, as well as increases binding at those receptors, as studied in vitro. However, recent studies have shown that chronic administration of the specific opioid antagonist, naltrexone, has no effect on quantitatively measured μ opioid receptor mRNA in any brain region studied (Untewald et al., 1995). Very recent studies have also shown that sub acute cocaine administration can increase μ opioid receptor mRNA levels in the striatum; such an increase in μ receptor mRNA levels may preceede chronic cocaine-induced development of increased density of μ opioid receptor in both the rat and in humans as measured by quantitative autoradiography and positron emission tomograph (PET), respectively (Unterwald et al., 1992, 1994a; Zubieta et al., 1996; Azaryan et al., 1996a,b; Yuferov et al., 1999).

With chronic opiate administration, there is strong evidence that a dramatic change in sensitivity to the action of opioid antagonists can occur in brain areas implicated in the acute reinforcing effects of opiates (Nestler, 1992) (see above) and opiate receptors in the region of the nucleus accumbens are much more sensitive to the aversive effects of opiate antagonists in dependent rats. Highly significantly increased sensitivity to opioid antagonists has been documented in opioid-dependent humans (see above), with very low doses of specific opioid antagonists required to precipitate objective and subjective signs, symptoms and biochemical changes related to opiate withdrawal (Stinus et al., 1990; Culpepper-Morgan et al., 1992a; Rosen et al., 1995, 1996; Culpepper-Morgan and Kreek, 1997). The molecular basis for this effect may again be at the signal transduction level.

Acute morphine decreases adenylyl cyclase activity in the nucleus accumbens, whereas chronic morphine treatment has long been associated with increases in second messenger systems including adenylyl cyclase activity (Nestler, 1996) and PKA (Nestler, 1996). Support for the hypothesis that these effects contribute to the motivational effects of opiates (e.g. tolerance and dependence) can be found in studies showing that direct administration into the nucleus accumbens of agents that inhibit G_{i0} or activate protein kinase increase the reinforcing effects of opiates (Self et al., 1994; Self and Nestler, 1995). Chronic morphine has also been shown to decrease the level of the transcription factor cyclic AMP response element-binding protein (CREB) in the nucleus accumbens. This provides a possible mechanism for the long-term changes in motivational systems associated with opioid dependence, possibly through alterations in gene expression which are yet to be identified (Widnell et al., 1996).

Other potential molecular interactions of motivational significance with chronic opiate exposure include similar changes in cyclic AMP and PKA in neurons of the noradrenergic locus coeruleus, although curiously CREB activity actually increases in the locus coeruleus (Nestler, 1996) after 5 days of morphine exposure, whereas at this time point, there are no changes in CREB levels in the striatum or cortex and decreased CREB levels in the nucleus accumbens (Nestler, 1996; Widnell et al., 1996). In the ventral tegmental area, chronic opiate exposure, including self-administration of heroin, decreases levels of neurofilament proteins and increases levels of glial fibrillary acidic protein. This could result in impaired
axoplasmic transport and may be viewed as a compensatory response to acute opiate activation of the dopaminergic cells in the mesocorticolimbic dopamine system.

Insights into the molecular and cellular mechanisms of alcohol dependence have begun to be focused on changes in neurochemical systems known to be highly sensitive to the acute effects of alcohol. It is well documented that chronic alcohol administration decreases GABAergic neurotransmission (see above). However, there is no evidence of a decreased number of GABA receptor sites (Karobath et al., 1980) suggesting that the decreases in GABAergic function may involve the composition or function of GABAergic receptors. Chronic alcohol has been shown to decrease expression of the α1-5 subunits of the GABA complex in the cerebral cortex (Mhatre et al., 1993; Devaud et al., 1995) and other subunits (Tabakoff and Hoffman, 1996; Devaud et al., 1997). Curiously, chronic intermittent exposure to alcohol results in a long-lasting kindling effect where alcohol withdrawal increases in severity and this is paralleled by an increase in the α4 subunit in the cerebral cortex (Mahmoudi et al., 1997). An intriguing hypothesis to explain alcohol’s effects on GABAergic neurotransmission is that the expression of GABA receptor subunits is altered by chronic treatment and such changes are brain-region specific. A recent study has extended these earlier studies in the cortex to the hippocampus and perhaps more interestingly, the ventral tegmental area (the source of cell bodies for the mesolimbic dopamine system) (Charlton et al., 1997). Prolonged 12-week exposure to chronic alcohol, but not 4-week exposure, decreased α1 subunit immunoreactivity in the ventral tegmental area and hippocampus suggesting potential changes in the two brain structures implicated in the rewarding and cognitive effects of alcohol, respectively.

Chronic ethanol exposure is also associated with increases in specific subunits (NMDAR1 and NMDAR2A) of glutamate receptors (Trevisan et al., 1994). Chronic ethanol exposure upregulated NMDA receptor function in cortical cultured neurons (Hu and Ticku, 1995) and chronic ethanol increased the NMDAR2A and NMDAR2B mRNA subunits during withdrawal, but not prior to withdrawal (Follesa and Ticku, 1995). Consistent with these observations, chronic ethanol also enhanced NMDA-stimulated nitric oxide formation suggesting additional sites for ethanol actions in enhancing glutamate receptor function (Chandler et al., 1997). Ethanol withdrawal also results in increased extracellular glutamate in the striatum using microdialysis measurements (Rossetti and Carboni, 1995). Upregulation of the NMDAR1 and GluR1 subunits of the glutamate receptor complex have also been observed, linking the neuroadap-

tive changes in the glutamate complex to the motivational systems implicated by pharmacological and neurochemical studies (Ortiz et al., 1995). The relationship of these molecular changes in ethanol receptive elements to specific aspects of the motivation for excessive alcohol consumption (outlined above) will be a challenge for future work.

Other molecular changes in receptor function with chronic ethanol exposure include changes in calcium channels. Calcium channel antagonists can attenuate ethanol withdrawal signs, particularly those associated with physical signs and seizures (Colombo et al., 1995; Watson and Little, 1997). Also, alcohol withdrawal excitability in the hippocampal slice preparation appears to involve increased activity of calcium channels (Shindou et al., 1994). Chronic ethanol produces increases in protein kinase C activity that could regulate calcium channels and expression of various genes (Messing et al., 1990, 1991). Consistent with neuropharmacological studies, ethanol withdrawal results in decreases in the firing rate and firing pattern of dopaminergic cells in the VTA area of the mesolimbic dopamine system (Diana et al., 1995). Finally, acute moderate doses of ethanol, induced c-fos protein in the extended amygdala and tolerance developed to this effect with repeated dosing (Ryabinin et al., 1997).

3. Neurobiology of protracted abstinence

3.1. Continued vulnerability of reward system

Animal models of protracted abstinence with motivational significance have been developed in primate models but have yet to be extended in any major way to rodent models. In one set of studies, monkeys were allowed to intravenously self-administer morphine 24 h per day and were challenged once per day with repeated pairings of nalorphine and a light (reviewed in Goldberg, 1976). Presentation of the light and injection of saline eventually resulted in a conditioned increase in responding for morphine, presumably to avert the onset of withdrawal. In a related study, lever-pressing terminated a light that preceded infusion of an opiate antagonist and delayed the antagonist injection for 60 s. Most of the responding eventually began to occur during the period when the light cue was illuminated, but before the antagonist infusion. These results are a powerful demonstration of the negative reinforcing properties of drug withdrawal.

Conditioned opiate withdrawal has also been observed clinically. Detoxified former heroin addicts frequently report symptoms like opiate abstinence when returning to environments similar to those associated with drug experiences (O’Brien, 1975). Opiate addicts administered nalorphine on an irregular basis
showed precipitated opiate abstinence (Wikler et al., 1953) and eventually placebo injections came to elicit similar withdrawal symptoms. In an experimental study in former heroin addicts maintained on methadone, naloxone injections were repeatedly paired with a tone and peppermint smell (O'Brien et al., 1977). Subsequent presentation of only the tone and odor elicited both subjective reports of discomfort and objective physical signs of withdrawal. From a theoretical perspective such conditioned effects may act as cues for relapse in untreated heroin addicts, but may also contribute to a change in hedonic set point that would effectively enhance the efficacy of positive reinforcement when a short-acting opiate drug was taken (Koob and LeMoal, 1997).

3.2. Continued vulnerability of stress system

In a sequence of studies to evaluate the status of the stress-responsive HPA axis in patients during long-term steady moderate to high dose methadone-maintenance treatment, particularly in patients with no further on-going drug or alcohol abuse, studied after at least 6 months of stabilization in treatment, a very provocative set of findings were made. In a course of metyrapone testing in long-term, stabilized methadone-maintained patients, it was found that classical signs and symptoms of opioid withdrawal occurred within 30–60 min after metyrapone administration, which lasted only up to 2 h, much shorter than that of the pharmacokinetic profile of metyrapone itself or its effect on the HPA axis. Methadone plasma levels were found not to change due to metyrapone administration. Therefore it was hypothesized that one possible explanation for this onset of withdrawal symptoms of limited duration might be due to an internal cue of opiate withdrawal, the cue being the activation of the HPA axis, probably entailing both enhanced levels of CRF, as well as the measured increases in peripheral levels of ACTH and β-endorphin, all of which accompany the abrupt blockade of cortisol synthesis by metyrapone and resultant cut-off of the normal feedback control by cortisol (see Fig. 2). These surges in CRF, ACTH and β-endorphin might act as internal cues to drive the onset of withdrawal symptoms, since these are very major correlates of both the spontaneous and naloxone-precipitated opioid withdrawal and since most of these patients have experienced many episodes of opioid withdrawal prior to entering methadone maintenance (Kennedy et al., 1990).

Therefore these findings, in otherwise stabilized patients who had not been using any illicit opiates or other drugs for many months, were interpreted as possibly being due to a physiologic cue of the surge of either CRF in the hypothalamus (where CRF is under negative feedback control of cortisol) or the surge of ACTH and β-endorphin peptides in the anterior pituitary, since POMC gene expression and peptide production in other regions of the brain, such as the hypothalamus have not been shown to be under regulation by glucocorticoids. It has been well established by many studies that activation of the HPA axis occurs in the setting of opiate withdrawal. In recent studies conducted both in pain patients and in methadone-maintained patients and in active heroin addicts undergoing conventional detoxification, the sensitivity to opioid antagonists has been found to be enhanced (Culpepper-Morgan et al., 1992a; Rosen et al., 1995, 1996; Culpepper-Morgan and Kreek, 1997). Moreover, it has recently been found that activation of a HPA axis, as documented by increased levels of ACTH or β-endorphin, along with cortisol, actually precedes the onset of other signs and symptoms and objective measures of opiate withdrawal and in fact, activation of this axis has been documented when the brain has been exposed to extremely small amounts of opiate antagonists, which do not ever result in the appearance of subjective and objective measures of narcotic abstinence (Culpepper-Morgan and Kreek, 1997). Thus the vulnerability of the stress responsive axis to cueing due to surges in several regions of the brain and in the anterior pituitary of peptide hormones which have been documented to be involved in and possibly contribute to the cause of the narcotic abstinence symptoms, is present even in long-term, well stabilized methadone-maintained patients, who usually experience no response to environmental cues and who are otherwise stabilized, with no continuing drug-seeking behavior.

In other studies conducted in long-term heroin addicts who were treated with a specific opioid antagonist, naltrexone, it was found that even during modest to moderate-term naltrexone treatment (mean time in treatment, 5 months) activation of the HPA axis was found after every naltrexone administration with no habituation or tolerance to this effect (Kosten et al., 1986a,b). Specifically, morning levels of β-endorphin were elevated over normal control subjects and morning and afternoon levels of cortisol were also elevated. In a small subset of subjects, both HPA axis function during naltrexone treatment and then, afterwards, cessation of naltrexone treatment, was studied, thus allowing use of each subject as their own control; it was found that there is an activation of the HPA axis during naltrexone treatment which disappears when naltrexone medication has been discontinued and a medication-free interval had elapsed patients in a basal state (Kosten et al., 1986a). It should be noted that these same type of subjects, that is, medication-free illicit opiate-free former heroin addicts, have been shown to often exhibit a hyper-
responsivity to stressors when challenged with the metyrapone test (Kreek et al., 1984). The low retention rates found for unselected heroin addicts in naltrexone treatment and the high relapse rate of this group to opiate use may be in part due to the HPA axis effects of the opioid antagonist.

More recently, to compare the effects of buprenorphine, naloxone and methadone in a group of former heroin addicts in early treatment, it was found again that HPA axis function is abnormal during cycles of heroin addiction and is normalized during methadone maintenance treatment; this normalization persisted during buprenorphine treatment if it was preceded by methadone treatment, but if buprenorphine was the primary treatment, during a short period of management with buprenorphine, complete normalization of the HPA axis was not observed (Kosten et al., 1992).

3.3. Molecular/cellular mechanisms

The possibility of persistence of changes in set point associated with drug reinforcement mechanisms in drug addiction suggests that the underlying molecular mechanisms are long-lasting and considerable attention is being directed at drug regulation of gene expression both at the molecular and at the peptide levels following chronic administration of drugs of abuse. In these ongoing studies and in the future, it will be increasingly important to determine the time course of changes, both following acute or initial exposure and during the subacute exposure, which can be considered parallel to the period of acquisition of dependence and addiction and finally, during chronic exposure, analogous to cycles of addiction. Increasing numbers of studies will need to be focused on the changes that occur at varying times following cessation of exposure to the drug, that is, into the acute and ‘protracted abstinence’ period. To date, a variety of changes have been found of considerable interest following chronic cocaine exposure. In addition to the persistent changes in D₁ and D₂ receptor density binding in vitro and binding in vivo (discussed above), and the DAT binding and mRNA level changes and changes in levels of dopamine itself in extracellular fluid, a variety of signal transduction system changes, a variety of changes had been seen.

It has been shown that chronic, but not acute, cocaine administration in a ‘binge’ pattern causes a very significant increase in density the μ opioid receptors as measured by quantitative autoradiography and very specifically, in those regions where there are abundant dopaminergic terminals, including both the caudate putamen fields of the dopaminergic neurons in the substantia nigra which project to the striatum, as well as in the nucleus accumbens, the amygdala and the anterior cingulate regions, containing projec-

Tions from dopaminergic neurons from the ventral tegmental area (Unterwald et al., 1992, 1994a). At least transient with or without persistent changes in μ opioid gene mRNA levels have been reported recently (Azaryan et al., 1996a;b; Yuferov et al., 1999). A different mode of cocaine administration, administered in steady state, gave very different findings with respect to the changes in μ opioid receptor density, underscoring the impact of different modes and patterns of administration, as well as dose, routes and time course of administration (Hammer, 1989). However, in a series of studies conducted in recently abstinent cocaine addicts, similar findings were made with increased μ opioid receptor-binding as measured by [11C] carfentanil using PET. Also, in these studies it was found that these changes are persistent for extended periods of time (Zubieta et al., 1996).

Several groups have found acute changes in preproenkephalin gene expression (discussed above) which disappear with continuing exposure, whereas preprodynorphin gene expression coupled with a resultant increase in dynorphin peptides has been found following several different modes of cocaine administration or self-administration over varying periods of time and thus, this is a chronic persistent recurrent phenomenon (Branch et al., 1992; Spangler et al., 1993, 1997a). δ opioid receptor density, like μ opioid receptor density, has been found to increase in the terminal fields of the nigrostriatal and mesolimbic-mesocortical dopamine systems (Unterwald et al., 1994a). In contrast, δ opioid gene expression has been found to be reduced in the substantia nigra region, possibly the region of most abundant exposure of dopamine neurons to the excessive dynorphin peptides delivered by way of the striatoni gral pathway (Sivam, 1989; Spangler et al., 1996b). Also there are highly significant changes in levels of gene expression and as measured by mRNA levels, peptide and receptor levels and hormone changes of the stress receptor and responsive HPA axis, as well as CRF and POMC gene expression in other brain regions (discussed above). These have been shown to occur at very different time points during acute, subacute and chronic cocaine administration and probably even different patterns will be seen during withdrawal (Rivier and Lee, 1994; Zhou et al., 1996a). Similarly the mRNA levels of the specific dopamine transporter and the levels of binding to that transporter have been shown to be significantly altered following withdrawal from chronic cocaine exposure, but not during cocaine exposure (Kuhar and Pilotte, 1996; Maggos et al., 1997).

Further work is ongoing in many laboratories to determine effects of acute and chronic opiate administration, as well as the effects of other drugs of abuse on gene expression and the resultant peptides with
particular emphasis on the time course of the changes, as well as the types of exposure required to effect such changes.

Two types of transcription factors, CREB and novel fos-like proteins (termed chronic FRAs or fos-related antigens), have been hypothesized to be possible mediators of chronic drug action (Hope et al., 1994; Hyman, 1996; Steiner and Gerfen, 1993; Widnell et al., 1996) (see above). The challenge for the future will be to relate regulation of a specific transcription factor to specific features of drug reinforcement associated with specific histories of drug administration (sensitization of acute challenges vs. changes in set point associated with protracted abstinence).

4. Neurobiology of relapse and vulnerability to relapse

The study of neurobiological mechanisms associated with relapse awaits further development and refinement of animal models (Koob, 1995). In a reinstatement model, neuropharmacological probes that activate the mesocorticolimbic dopamine system have been shown to rapidly reanimate self-administration in animals trained and then extinguished on intravenous drug self-administration (deWit and Stewart, 1981; Stewart and deWit, 1987). For example, agonists selective for D1 dopamine receptors, but not for D2-like receptors, can block reinstatement of lever-pressing hypothesized to reflect cocaine-seeking behavior (Self et al., 1996). The interaction of dependence or a history of dependence on cocaine with the reinstatement model remains a challenge for future studies.

Animal models of relapse using oral ethanol self-administration have incorporated not only models of reinstatement after extinction but also more recently models involving a history of dependence and deprivation from ethanol. Using the alcohol deprivation model in non-dependent rats, acamprosate and naltrexone block the increase in drinking observed in rodents after a forced abstinence (Heyser et al., 1998; Heyser and Koob, unpublished results). Similarly, opioid antagonists were shown to prevent the increase in drinking of ethanol in animals post-stress (Volpicelli et al., 1986). Naltrexone and the longer acting nalme-fene, specific opioid antagonists, have both been shown to have efficacy in preventing relapse in detoxified human alcoholics (O'Malley et al., 1992; Volpicelli et al., 1992; Chou et al., 1993; Mason et al., 1994; Schluger et al., 1997; Unterwald et al., 1997) and acamprosate is a drug with potential glutamate modulatory action that has shown efficacy in Europe to prevent relapse in alcoholics (O'Brien et al., 1995; Sass et al., 1996).

4.1. Individual differences

The vulnerability to relapse will have both genetic and environmental bases and animal studies have begun to address both these contributions. While genetic vulnerability is beyond the scope of this review, there are rodent strains that show preferences for drinking ethanol and there is mounting evidence of alterations in the same reward neurotransmitters that may form the basis of such preferences (Murphy et al., 1987). In addition, new techniques, such as quantitative trait loci analysis and the study of knock-out and transgenic mice may reveal potential genetic sites of vulnerability not only for the acute reinforcing effects of drugs but also for vulnerability to other aspects of addiction (Cranke and Belknap, 1992).

4.2. Stress

A major component of the environmental factors involved in vulnerability is the role of stress. An atypical responsivity to stress in former opiate- and cocaine-dependent subjects has been well-documented and hypothesized to be linked to chronic relapse (Kreek, 1987). Challenge studies using metyrapone, the blocker of 6-β hydroxylation (discussed above) has been shown to result in an exaggerated hypotalamic-pituitary response in illicit drug-free, medication-free former long-term heroin addicts. This hyper responsivity to a chemically induced stress would be immediately reversed by administration of a short-acting opiate, such as heroin which would yield a hypo responsivity to this provocative test (Kreek and Hartman, 1982; Kreek et al., 1984; Kreek, 1987, 1992). Recent studies have shown that a similar hyper-responsivity to the metyrapone challenge test occurs in early abstinent cocaine addicts during early abstinence and that this response seems to persist for extended periods of time over several weeks or months in some subjects studied at more distant time points (Schluger et al., 1998). Thus, both former heroin addicts who have been opiate-free for an extended period of time and recently abstinent cocaine addicts illustrate a hyper-responsivity to a chemically-induced stressor. Further studies will reveal whether or not these findings are paralleled by hyper responsivity to environmental stressors. However, it has been observed clinically that stress and stressors of a variety of types may contribute to chronic self-administration of drugs of abuse. In animal models more definite support of this hypothesis has been and probably will continue to be forthcoming (Piazza et al., 1989; Piazza and LeMoal, 1996; Koob and LeMoal, 1997; Piazza and LeMoal, 1997). Exposure to repeated
stressors also facilitates the development of initial intravenous drug self-administration (acquisition) (Piazza and Le Moal, 1996) and can facilitate reinstatement of drug self-administration after extinction (relapse) (Shaham et al., 1996). Some of these effects may be linked to activation of the hypothalamic-pituitary-adrenal axis in that suppression of stress-induced corticosterone secretion abolishes the enhanced behavioral responsiveness to amphetamine and morphine produced by different stressors (Deroche et al., 1992) and repeated administration of corticosterone can substitute for stress and increase the behavioral effects of psychostimulants (Deroche et al., 1992). Thus, glucocorticoid hormones and also, the state of responsivity of both CRF and POMC sites of regulation by glucocorticoids, may function in the long-term maintenance of the sensitized state and may even represent a within-system mechanism (Koob and Le Moal, 1997; Kreek 1973a, 1992, 1997). What remains largely unknown is how these genetic and environmental factors combine to contribute to the development of what constitutes substance dependence (addiction) in humans. Comparison of rats that show a high and low locomotor response to forced exposure in a novel environment have revealed that high responders (HRs) show a greater propensity to develop intravenous drug self-administration compared to low responders, LRs (Piazza et al., 1989). Identification of the vulnerability for other different parts of the addiction cycle using animal models will provide clues to relapse vulnerability in human addicts. Using animal models, studies of the interaction of genetics, stress and the initial responses, as well as chronic adaptations on drug effects on various aspects of the addiction cycle other than drug-taking will be informative.

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References


stable isotope-labeled $^2$H$_3$, $^2$H$_2$, $^3$H$_2$ methadone. J. Pharm. Sci. 71, 39–43.


Unterwald, E.M., Ho, A., Rubenfeld, J.M., Kreek, M.J., 1994b. Time course of the development of behavioral sensitization and