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Allostasis and dysregulation of corticotropin-releasing factor and neuropeptide Y systems: implications for the development of alcoholism

Glenn R. Valdez*, George F. Koob

Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92037, USA

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Abstract

Alcoholism is a chronic relapsing disorder, accompanied by alterations in psychological and physiological functioning, which reaches an addictive state where an individual demonstrates uncontrollable compulsive alcohol drinking and impairment in social and occupational functioning. Withdrawal is one of the defining characteristics of dependence, characterized by impaired physiological function and enhanced negative affect, and is thought to be a major contributing factor to relapse. The negative emotional aspects of withdrawal appear to be more involved in continued alcohol craving because physical withdrawal symptoms are not highly correlated with relapse in alcoholics. Allostasis describes maintaining stability outside the homeostatic range by varying the internal milieu to match environmental demands. This concept has been applied to neurobiological models of drug addiction and is thought to contribute to the vulnerability of drug addicts to relapse, as addicts continue to use drugs in order to maintain their psychological state within a homeostatic range. With regard to alcohol, two neuropeptides appear to be involved in the regulation of alcohol-related stress, corticotropin-releasing factor (CRF), which is associated with an increased stress response and negative affect, and neuropeptide Y (NPY), a neuropeptide with anxiolytic properties. The hypothesis to be developed in the present review is that a dysregulation of the CRF and NPY systems significantly contributes to the motivational basis of continued alcohol-seeking behavior during alcohol dependence. It appears that increases in CRF contribute to the negative affective state that is strongly associated with alcohol withdrawal, and NPY provides a motivational basis to consume alcohol because the anxiolytic effects of alcohol, which are strongly associated with relapse, appear to be regulated in part by this neuropeptide.

Keywords: Alcohol; Corticotropin-releasing factor; Neuropeptide Y; Allostasis; Stress; Anxiety

1. Introduction

Alcoholism is a chronic relapsing disorder, accompanied by alterations in psychological and physiological functioning (McLellan et al., 2000), which reaches an addictive state where an individual demonstrates uncontrollable compulsive alcohol drinking and an intense desire for alcohol (McKinley and Moorhead, 1965, 1967; Moorhead and McKinley, 1966). One of the classic beliefs regarding alcoholism is that the continued use of alcohol is supported by the experience of positive reinforcing effects during alcohol consumption. However, the development of tolerance tends to decrease the associated euphoria of alcohol use over time (Koob, 1998). In addition to its positive reinforcing effects, alcohol takes on negative reinforcing properties when it is used to alleviate the symptoms of alcohol withdrawal.

Withdrawal is one of the defining characteristics of most definitions of drug dependence, and is often characterized by impaired physiological function and enhanced negative affect. In humans, physical withdrawal symptoms include disturbed sleep patterns, convulsions, tremor, perspiration, nausea, and vomiting (Hershon, 1973, 1977). In addition, withdrawing alcoholics experience depressed mood and

* Corresponding author. Current address: New England Primate Research Center, Harvard Medical School, 1 Pine Hill Drive, P.O. Box 9102, Southborough, MA 01772-9102, USA. Tel.: +1 508 624 8027; fax: +1 508 624 8197.

E-mail address: glenn_valdez@hms.harvard.edu (G.R. Valdez).

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negative affect that have been strongly associated with relapse in many alcoholics (Cloninger, 1987). Laboratory animals examined in animal models of alcoholism also display physical signs of withdrawal, indicated by tremors, abnormal body posture, rigidity, and enhanced susceptibility to seizures (Becker, 1994; Cooper et al., 1979; Hunter et al., 1975; Macey et al., 1996; Majchrowicz, 1981). These animals also show signs of a distinct negative affective-like state, characterized by an enhanced responsiveness to stressful stimuli (Baldwin et al., 1991; Hölter et al., 1998; Möller et al., 1997; Rassnick et al., 1993).

Allostasis is a concept that was first used to describe fluctuations in blood pressure and immune system function that were inexplicable in terms of homeostasis (Sterling and Eyer, 1981). It has since been applied to the neurobiological basis of the vulnerability of drug addicts, including alcoholics, to relapse (Koob and Le Moal, 1997, 2001). Whereas homeostasis refers to the consistency of internal parameters within a normal range, allostasis describes maintaining stability outside the normal range by varying the internal milieu to match environmental demands (Koob and Le Moal, 1997, 2001). One hypothesis regarding allostasis and alcohol relapse is that the negative affective state that accompanies alcohol-related stress becomes a new set point for mood regulation, and that alcoholics continue to drink in a failed attempt to regulate their mood within the homeostatic range. This hypothesis involves the concepts of tolerance and sensitization. The euphoric effects that once accompanied alcohol consumption become diminished because of tolerance, and the negative affect that accompanies withdrawal is enhanced due to sensitization. Alcoholics no longer consume alcohol to experience euphoria, but rather to alleviate negative mood states.

Two neuropeptides, corticotropin-releasing factor (CRF) and neuropeptide Y (NPY), appear to be involved in the regulation of alcohol-related stress. CRF is a 41-amino-acid neuropeptide involved in mediating neuroendocrine (Vale et al., 1981), autonomic (Dunn and Berridge, 1990; Vale et al., 1983), and behavioral responses to stress (Koob and Heinrichs, 1999; Koob et al., 1994). The neuroanatomical substrates associated with the mediation of behavior by CRF include the central nucleus of the amygdala (Gray and Bingaman, 1996), the locus coeruleus (Valentino and Wehby, 1988), and the hypothalamus (Menzaghi et al., 1994a). NPY is a 36 amino acid neuropeptide (Tatemoto et al., 1982) shown to have anxiolytic-like properties in animal models of anxiety (Heilig and Murison, 1987; Heilig et al., 1989). NPY neurons have a wide anatomical distribution in the brain, including the hypothalamus and amygdala (Chronwall et al., 1985). Because CRF and NPY systems have been implicated in the regulation of anxiety (Heilig et al., 1994), a hypothesis to explain the effects of the long-lasting nature of alcohol dependence on the reinforcing properties of alcohol is that CRF contributes to the aversive state associated with abstinence from alcohol, and NPY provides the motivational basis for negative reinforcement since the anxiolytic effects of alcohol that appear to be a critical factor in continued alcohol-seeking behavior appear to be regulated in part by NPY (Badia-Elder et al., 2001). It is possible that alcohol dependence can lead to long-term dysregulation of brain CRF and NPY systems, leading to the maintenance of alcohol-seeking behavior.

It should be noted that alcoholism is not a homogeneous disorder that develops and is maintained in all alcoholics in the same manner. One type of alcoholism exists in a highly heritable form that involves the interaction between polygenic and environmental influences (Goldman, 1995). In contrast, alcohol dependence can also develop slowly over time due to the neuroadaptive changes that result from chronic bouts of alcohol intoxication and repeated episodes of withdrawal. This review will focus on the latter type of alcoholism by examining the neurochemical changes that occur within stress neuropeptide systems as this disorder progresses. The hypothesis to be developed in the present review is that dysregulation of CRF and NPY systems contribute significantly to the motivational basis of continued alcohol-seeking behavior during alcohol dependence. Changes in CRF and NPY levels are observed during acute withdrawal (Ehlers et al., 1998a; Merlo-Pich et al., 1995). Dependence may lead to a long-lasting adjustment in the normal parameters of these neuropeptides and regulation outside the normal homeostatic range. Dysregulation of CRF and NPY may represent a failed attempt of alcoholics to consume alcohol in such a manner as to allow these neuropeptides to return to a homeostatic range.

2. The tension reduction hypothesis and alcohol withdrawal

The original formulation of the tension reduction hypothesis was that alcoholics are motivated to drink to alleviate symptoms associated with withdrawal (Cappell and Herman, 1972). This hypothesis has generated some controversy because withdrawal had been traditionally viewed as a physical syndrome, and evidence points toward a negative correlation between physical withdrawal severity and alcohol consumption (Metten et al., 1998). Alcoholics experience tremor, convulsions, nausea, vomiting, perspiration, and delirium tremens during the early stages of withdrawal (Hershon, 1973). However, it appears that these physical symptoms are not an important contributing factor to relapse. For example, one study examining the extent to which physical withdrawal symptoms provoked drinking in male alcoholics found a questionable relationship between the physical signs of withdrawal and motivation to drink alcohol. Less than 25% of the patients examined reported that they continued to drink in order to alleviate physical withdrawal symptoms, such as body shakes, cramps, and sweating (Hershon, 1977). Laboratory animals examined using animal models of alcoholism also display physical withdrawal signs such as tremor, stereotypy, gastrointestinal problems, and convulsions (Freund, 1969). However, much of the data regarding animal studies suggest that physical withdrawal signs are not important factors in continued alcohol-seeking behavior (Meisch and Stewart, 1994).

One problem in viewing physical withdrawal as the main measure of alcohol dependence is the discrepancy in the time course of physical signs of withdrawal versus the chronic nature of dependence. In humans, physical withdrawal symptoms typically last for 12–72 h following the cessation of drinking (Mello and Mendelson, 1972), whereas abstinent alcoholics report cravings for alcohol for months after experiencing withdrawal (Roelofs, 1985). In laboratory rodents, peak withdrawal signs are usually observed 4–24 h following chronic alcohol exposure (Freund, 1969; Majchrowicz, 1981). Animals with a history of dependence, however, continue to self-administer increased amounts of ethanol for 4–8 weeks after chronic exposure, and in the absence of obvious physical withdrawal signs (Roberts et al., 2000).

It appears that a separate component of withdrawal, independent of physical withdrawal, is a major contributing factor to alcoholism. The major problem with the focus on physical symptoms as a measure of dependence is that it neglects the affective or psychological component of withdrawal, manifested by anxiety or depressed mood (Hershon, 1973). It has been speculated that the affective component of withdrawal is critical in the development of alcoholism because anxiety and depression, which contribute to alcohol stress, are thought to set the stage for relapse in many alcoholics (Cloninger, 1987). For example, in the abovementioned study where less than 25% of alcoholic patients reported drinking to alleviate physical withdrawal symptoms, more than 80% of these same patients reported drinking due to feelings of anxiety or depressed mood (Hershon, 1977). In addition, both male and female alcoholics report negative emotional states as the most common reason for relapse (Annis et al., 1998). Taken together, these clinical studies indicate those negative affective states likely play an important role in the maintenance of continued alcohol craving and relapse. Therefore, a revisionist tension reduction hypothesis may be more appropriately described as consuming alcohol in order to specifically alleviate negative affect and stress rather than general withdrawal.

Animal models of excessive alcohol consumption include the alcohol deprivation effect and alcohol self-administration following chronic alcohol exposure. The alcohol deprivation effect is an increase in ethanol intake, generally short in duration, following periods of abstinence from ethanol (Sinclair and Senter, 1968; Spanagel et al., 1996). The increase in ethanol intake can be seen post-detoxification when the animals do not manifest any obvious physical signs of withdrawal. A rat model of oral ethanol self-administration during both acute and prolonged periods of abstinence has been developed, which is useful in examining the effects of withdrawal on self-administration in laboratory animals (Roberts et al., 1996, 2000). Under this model, rats are trained to self-administer ethanol, exposed to chronic ethanol, and then retested in the operant procedure during various stages of withdrawal. Rats display increased oral ethanol self-administration post-detoxification in the absence of physical withdrawal signs (Roberts et al., 2000), suggesting that reinstatement of self-administration is not likely related to physical withdrawal.

In laboratory animals, measures of anxiety-like behavior are used as an indication of a change in emotionality that could contribute to a negative affect-like state. One of the most common animal models of anxiety is the elevated plus maze, which examines exploration of an unfamiliar environment as a conflict with a safe environment. Because rats prefer dark, enclosed spaces when exposed to a novel environment, open-arm preference in the elevated plus maze, measured by preferential time and entries directed at the open as opposed to the closed arms, is proposed to inversely relate to anxiety-like states (Cruz et al., 1994). Rats chronically exposed to ethanol commonly show decreased open-arm exploration when tested during withdrawal (Baldwin et al., 1991; Hölter et al., 1998; Rasmussen et al., 2001; Rassnick et al., 1993). Depression-like signs have also been observed in laboratory animals following withdrawal from chronic ethanol. Rats exposed to chronic ethanol vapor for approximately 2–3 weeks show significantly elevated intracranial self-stimulation (ICSS) thresholds compared to air-exposed controls for up to 48 h following removal from the vapor chambers (Schulteis et al., 1995). Elevated ICSS thresholds have been proposed to indicate a depression-like state in animals (Leith and Barrett, 1976). Taken together with reports of increased ethanol self-administration during withdrawal, these animal models suggest a propensity to consume ethanol to alleviate negative affect.

Many studies show a strong link between exposure to stress and the acquisition or reinstatement of ethanol self-administration in laboratory animals. Increases in ethanol consumption are seen in C57BL/6J mice, a strain of alcohol-preferring mice, during the anticipation of exposure to an intense loud noise (Mollenauer et al., 1993). In addition, conflict conditions and food deprivation, which act as stressors, can influence voluntary consumption of ethanol by rats (Caplan and Puglisi, 1986). Although at this time, no published studies have examined a direct link between alcohol self-administration and affective-like withdrawal signs in animals, rats with a history of dependence show increased anxiety-like behavior in the elevated plus maze and increased ethanol self-administration up to 6 weeks post withdrawal in the absence of physical withdrawal signs (Valdez et al., 2002b). Rats also have been shown to self-administer alcohol up to 8 weeks post withdrawal in the absence of any obvious physical withdrawal signs (Roberts et al., 2000). The time course of these models indicates that some of these changes are long lasting, persist far beyond the acute withdrawal phase, and may be linked.

3. Protracted abstinence

Relapse is likely to occur beyond the usual time period during which acute signs of alcohol withdrawal are observed, and abstinent alcoholics tend to experience protracted withdrawal symptoms, such as long-term physiological abnormalities and mood disturbance (Begleiter and Porjesz, 1979). As discussed above, one of the most critical characteristics of abstinence following chronic alcohol is anxiety, which can lead to mood disturbances and negative affect in alcoholics that can persist for months following chronic alcohol consumption (Begleiter and Porjesz, 1979; Grant et al., 1987; Roelofs, 1985). For example, abstinent alcoholics show symptoms of anxiety for months and even years following their last drink (De Soto et al., 1985; Roelofs, 1985). Such protracted symptoms are associated with an increased risk of relapse (De Soto et al., 1989). Laboratory animals with a history of dependence also exhibit an anxiety-like state during which an enhanced stress response is experienced (Hölter et al., 1998; Möller et al., 1997; Rassnick et al., 1993) that is often associated with a negative affective-like state (Begleiter and Porjesz, 1979).

Depressed mood and anxiety during long-term abstinence have been positively correlated with relapse (De Soto et al., 1989; Miller and Harris, 2000; Mossberg et al., 1985; Parsons et al., 1990). For example, one study showed a strong correlation between self-reported feelings of anxiety in male alcoholics and the intensity of cravings for alcohol,

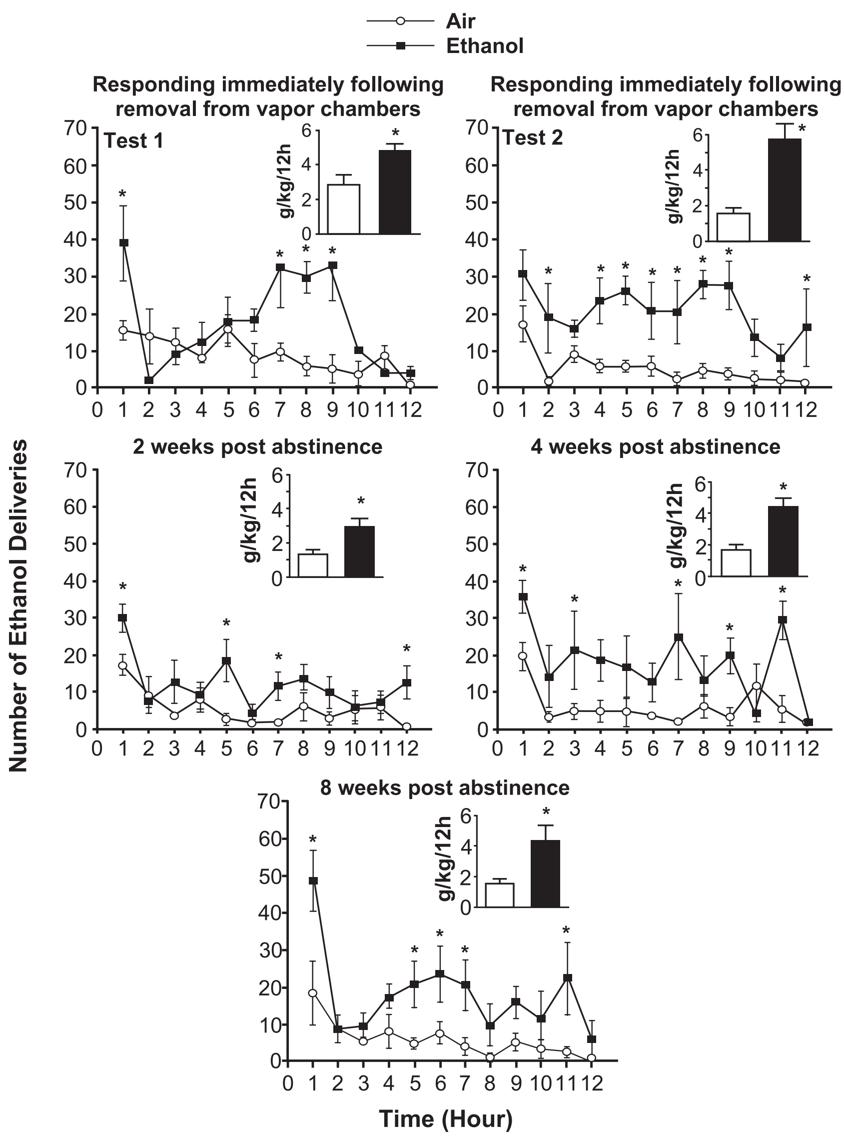

Fig. 1. Operant responding for oral ethanol across 12-h test sessions in rats exposed to ethanol vapor or air. Test 1 was conducted immediately upon removal of the ethanol vapor chambers after the initial 2 weeks of exposure. Test 2 was conducted immediately upon removal of the ethanol vapor chambers after an additional 5 days of exposure. Rats were tested again at 2, 4, and 8 weeks following removal. The insets to each panel depict total ethanol consumption relative to body weight for ethanol vapor-exposed (black bar) and air-exposed (white bar) rats. The numbers of ethanol deliveries and g/kg consumptions are represented as means±S.E.M. The symbol * indicates a significant difference between the ethanol and control groups (p<0.05). (Taken with permission from Roberts et al., 2000).

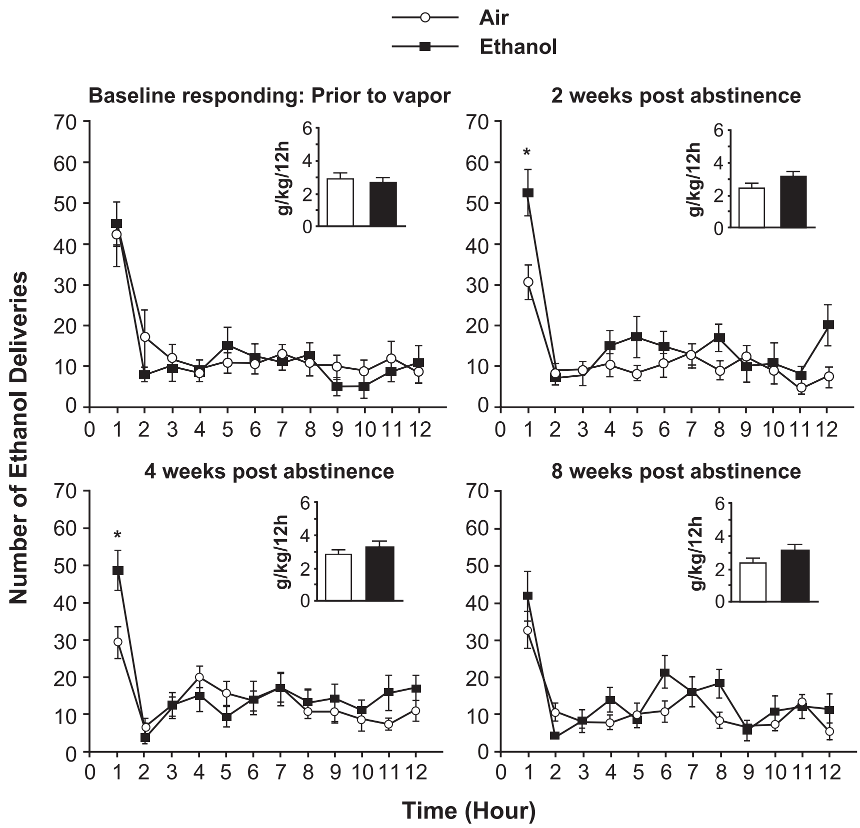

Fig. 2. Operant responding for oral ethanol across 12-h test sessions in rats exposed to 2 weeks of ethanol vapor or air. Rats were not tested immediately following removal from the vapor chambers, but were tested at 2, 4, and 8 weeks following removal from the vapor chambers. The insets to each panel depict total ethanol consumption relative to body weight for ethanol vapor-exposed (black bar) and air-exposed (white bar) rats. The numbers of ethanol deliveries and g/kg consumptions are represented as means±S.E.M. The symbol * indicates a significant difference between the ethanol and control groups ($p<0.05$). (Taken with permission from Roberts et al., 2000).

which lasted for up to 9 months following their last drink (Roelofs, 1985). In addition, a group of male alcoholics reported symptoms of anxiety and depression that lasted for 6 months to 2 years following withdrawal (De Soto et al., 1985). A follow-up study on this same group of alcoholics showed a strong correlation between these negative affective symptoms and relapse up to 2 years following withdrawal (De Soto et al., 1989). These data underscore the chronic nature of alcoholism and suggest that long-term disturbances in mood are strongly related to relapse.

To determine the neuropharmacological mechanisms involved in protracted abstinence, animal models examining long-term behavioral changes in animals with a history of alcohol dependence have been developed. Rats maintained on an ethanol-containing liquid diet over a 4-week period display significantly less gnawing behavior when examined in the novel cork gnawing test compared to rats maintained on an isocaloric sucrose-containing control diet for up to 4 weeks following withdrawal (Rasmussen et al., 2001). Decreased cork gnawing in laboratory rodents is indicative of an increased anxiety-like state (Pollard and Howard, 1990). In addition, rats with a history of dependence show decreased exploration of the open arms of the elevated plus maze up to 4 weeks after removal of the diet compared to those maintained on an isocaloric sucrose-containing control diet (Rasmussen et al., 2001). These rats also show increased locomotor activity when placed in a novel environment, which has been proposed as a risk factor for ethanol drinking in rats (Rasmussen et al., 2001). This observation appears to have face validity with the increased novelty-seeking behavior characteristic of a specific subgroup of alcoholics (Cloninger, 1987).

In addition to the anxiety-like behaviors examined in animal models of protracted abstinence, ethanol self-administration also has been studied. As noted earlier, the alcohol deprivation effect is a long established animal model of excessive alcohol consumption (Sinclair and Senter, 1968; Spanagel et al., 1996). Roberts et al. (2000) have shown a more robust alcohol-deprivation effect and a persistent increase in baseline ethanol self-administration in rats with a history of dependence for days after the acute withdrawal phase. In this study, baseline levels for ethanol self-administration were determined, and then rats were exposed to ethanol vapor or air for 2 weeks. The rats then were allowed to self-administer ethanol 2 weeks after removal from the chambers. Rats exposed to ethanol vapor showed a much more robust increase in self-administration at initial exposure to alcohol compared to air-exposed controls. This increase persisted for up to 6 days, whereas control animals returned to baseline levels of responding. These data suggest that excessive ethanol consumption can continue for an extended period of time following the acute withdrawal phase.

In addition, rats also have shown increased alcohol self-administration up to 8 weeks post-withdrawal (Roberts et al., 2000). In the first experiment of this study, rats were trained to self-administer ethanol, exposed to 2 weeks of ethanol vapor and then allowed to self-administer alcohol for 12 h following this exposure. Following this procedure, the rats were re-exposed to ethanol for 5 days and subsequently tested for ethanol self-administration 2 h and 2, 4, and 8 weeks later. At each of these time periods, rats exposed to ethanol vapor self-administered significantly more ethanol than air-exposed control rats (Fig. 1). A similar experiment was also conducted in which rats were only allowed to self-administer ethanol prior to vapor exposure and 2, 4, and 8 weeks following removal from the vapor chambers. These rats did not experience ethanol during the acute withdrawal phase. Interestingly, ethanol vapor-exposed rats in this experiment did not self-administer more alcohol compared to controls at any time point (Fig. 2). These experiments suggest that rats will self-administer ethanol long after experiencing acute withdrawal. However, a learned association must be made that ethanol can alleviate the hypothesized long-term negative affective-like state. It is possible that rats that were allowed to self-administer ethanol during acute withdrawal associated this experience with an alleviation of its characteristic anxiety-like state. Rats tested in the second experiment were unable to make this association.

These studies examining protracted abstinence underscore the chronic nature of alcohol dependence. When considered together, the studies involving human alcoholics and the corresponding animal models suggest that negative affect and mood disturbances are a driving factor in relapse following long-term abstention from alcohol. The learned association between alcohol and the alleviation of negative affect appears to be critical to excessive drinking during dependence.

4. Kindling and repeated withdrawal episodes

One important factor that must be considered in the experiment by Roberts et al. (2000) described above is that in the first experiment, the rats experienced an additional withdrawal episode compared to those tested in the second experiment. It has been hypothesized that repeated episodes of alcohol intoxication and withdrawal lead to a progressive intensification of the withdrawal syndrome (Ballenger and Post, 1978). This intensification may indicate a mechanism involved in kindling, a phenomenon by which repeated stimulation of specific brain areas leads to intensification of seizures. During the kindling process, low levels of electrical stimulation in the brain produce no initial behavioral effects (Goddard et al., 1969). However, repeated stimulation produces progressive increases in pre-seizure behaviors until convulsions are produced by levels of electrical activity that normally fail to elicit seizure activity (Ballenger and Post, 1978; Carrington et al., 1984). It has been hypothesized that the hyperexcitabilty of the central nervous system that is characteristic of alcohol withdrawal may act as a stimulus that eventually elicits a kindling-like response in neuronal excitability (Becker et al., 1997).

Studies involving human alcoholics have indicated that withdrawal episodes following repeated instances of relapse during detoxification become progressively more severe. One study examining a large cohort of alcoholic men found that patients who experienced seizures and reported more severe withdrawal episodes had more prior detoxifications and alcohol-related hospitalizations compared to others who did not experience seizures (Booth and Blow, 1993). However, it is unclear if the severity of withdrawal and the subsequent seizures are directly related to the number of detoxifications or if they are the result of heavier alcohol use by these patients, especially since they were hospitalized more often for alcohol-related problems. In addition, many withdrawal episodes occur outside of formal detoxification treatments. Therefore, general alcohol use patterns should be evaluated more carefully in this context. Another study examining male alcoholics who suffered from withdrawal seizures found that 48% of them had five or more detoxifications prior to the study compared to only 12% in the control group that did not experience withdrawal seizures (Brown et al., 1988). Examination of the alcohol use patterns of these patients showed that the amount of alcohol use reported did not differ between groups, indicating a positive relationship between withdrawal seizures and the number of detoxifications rather than general use of alcohol.

In order to study the effects of repeated withdrawal episodes more closely, animal models have been developed under which this phenomenon can be examined using a more controlled environment. Becker (1994) has shown a positive relationship between the number of withdrawal episodes and the severity of handling-induced convulsions following the final withdrawal episode in mice. Using a between-subjects design, mice were exposed to either one, two, three, or four cycles of 16 h of ethanol vapor or air followed by 8 h of withdrawal. Handling-induced seizures became progressively more severe as the number of withdrawal cycles increased in the ethanol-exposed mice but not in air-exposed controls. One shortcoming of this study, however, is that the total amount of exposure to ethanol increased as the number of withdrawal cycles increased, making it unclear if the results were specifically due to the number of withdrawals. Another study controlled for this problem by comparing mice receiving three cycles of 16 h of ethanol vapor followed by 8 h of withdrawal and those receiving 48 h of continuous ethanol vapor (Becker and Hale, 1993). Using this method, all ethanol-exposed mice received the same total amount of ethanol. Mice experiencing three withdrawal cycles had significantly higher handling-induced seizure scores compared to those in the single withdrawal condition. Since the total amount of ethanol exposure was equal between the two groups, it appears that the increased seizure severity is likely correlated with the increased number of withdrawal episodes. The increased severity of withdrawal seizures is unlikely due to increased blood alcohol levels, as this measure was equivalent between groups. In addition, rats exposed to multiple withdrawal episodes and injected with phenobarbital in order to prevent a withdrawal reaction show significantly less seizure activity compared to those not pretreated with phenobarbital (Ulrichsen et al., 1992). Alcohol withdrawal seizures due to repeated intoxication and withdrawal episodes also appear to be long lasting in nature. Ulrichsen et al. (1998) showed that rats re-exposed to repeated episodes of ethanol withdrawal following 26 ethanol-free days showed displayed seizure activity comparable to that observed during the initial period of intermittent ethanol exposure. The above data clearly demonstrate that repeated alcohol withdrawal episodes can intensify the physical signs of withdrawal in people and laboratory animals.

In addition to increasing the severity of physical signs of withdrawal, multiple withdrawal episodes also can influence the affective and motivational components of withdrawal. In the social interaction test, male and female rats receiving multiple withdrawal episodes from an ethanol liquid diet display decreased social interaction, which is indicative of an increased anxiety-like state, compared to rats receiving an equal amount of total ethanol exposure but only one deprivation episode (Overstreet et al., 2002, 2004). Repeated withdrawals from an ethanol liquid diet can also decrease open arm exploration in the elevated plus maze compared to a single withdrawal episode (Overstreet et al., 2004). Another study, using a between-subjects design, demonstrated that repeated ethanol deprivation episodes in rats trained to freely drink 5–20% alcohol solutions have been shown to further decrease open-arm exploration in the elevated plus maze compared to rats receiving a single deprivation episode (Hölter et al., 1998). In this same study, when given free access to tap water and ethanol solutions ranging from 5% to 20%, rats preferentially consumed the higher concentrations as they experienced repeated episodes of deprivation (Hölter et al., 1998). Taken together, these results indicate that repeated episodes of deprivation can lead to an anxiety-like state, thereby increasing motivation to consume ethanol. The preferential consumption of the highest concentrations suggests that intake is likely due to the pharmacological effects of ethanol.

The alcohol deprivation effect also has been examined with regard to repeated deprivation episodes. In these studies, both adult female alcohol-preferring rats and adult male high alcohol drinking rats were given cycles of 2 weeks of free access to ethanol followed by 2 weeks of deprivation. Both sets of animals showed a prolonged alcohol deprivation effect as the number of deprivations increased (Rodd-Henricks et al., 2000a,b). In addition, preference for ethanol increases following repeated episodes of withdrawal. Rats exposed to intermittent episodes of ethanol vapor for seven weeks display a marked increase in ethanol self-administration (Rimondini et al., 2003). In order to determine motivation to self-administer ethanol, the break point at which the number of lever presses a rat would perform in order to receive an ethanol reinforcer before ceasing to respond has been measured (Brown et al., 1998). Rats were given four cycles of 9 days of maintenance on an ethanol-containing liquid diet followed by 2–7 days of withdrawal during this study. Multiple withdrawal episodes increased the number of lever presses a rat with a history of dependence would perform to receive ethanol compared to controls, indicating an increased motivation to self-administer ethanol. Physical withdrawal signs were examined prior to each operant responding session, and interestingly, no physical signs of withdrawal were observed prior to testing, indicating that the affective-like component of withdrawal was most likely responsible for this increased motivation to self-administer ethanol.

5. Corticotropin-releasing factor, the behavioral stress response, and alcohol

Although the hypothalamic–pituitary–adrenal (HPA) axis stress response mediated by CRF is important in the regulation of physiological responses to stress, a behavioral response to stress also mediated by CRF occurs independently of the HPA axis. For instance, hypophysectomy and blockade of the HPA axis response via dexamethasone suppression do not alter the activational and behavioral responses to stress produced by central administration of CRF (Britton et al., 1986; Eaves et al., 1985). Thus, it appears that a central site of action is responsible for coordinating stress-related behavior.

Central administration of CRF mimics the behavioral response to stress in laboratory animals, although the types of behavior elicited appear dependent on the baseline state of the animal. CRF injected into unstressed animals under familiar conditions leads to increased locomotor activation (Koob et al., 1984; Sutton et al., 1982). In contrast, CRF administered to animals in unfamiliar settings can lead to behavioral suppression, decreasing exploration of the open field (Sutton et al., 1982), multicompartment chambers (Berridge and Dunn, 1986), and the elevated plus maze (Baldwin et al., 1991). In addition, CRF administration will suppress behavior during the conflict test, as animals will show an even greater reduction in responding when a response is accompanied by a shock (Britton et al., 1985). Other examples of anxiety-like behavior induced by central administration of CRF include an enhanced acoustic startle response (Swerdlow et al., 1986), increases in the conditioned fear response (Cole and Koob, 1988), and decreased appetite (Arase et al., 1988; Krahn et al., 1986).

Further evidence that brain CRF systems play an important role in the regulation of stress-like behaviors comes from studies using competitive CRF receptor antagonists. α-helical $CRF_{9–41}$, a $CRF_{1/2}$ receptor antagonist, attenuates the CRF-enhanced acoustic startle response in rats when centrally injected. In addition, this same study showed that α-helical $CRF_{9–41}$ could attenuate an acoustic startle response that was enhanced by pairing the sound with an electric shock (Swerdlow et al., 1989). In the elevated plus maze, animals centrally injected with α-helical $CRF_{9–41}$ show increased exploration of the open arms when subjected to restraint stress, swim stress, or social conflict stress compared to those receiving vehicle injections (Heinrichs et al., 1994). A second $CRF_{1/2}$ receptor antagonist, D-Phe-$CRF_{12–41}$, reduces both CRF- and stress-induced increases in locomotor activation, as well as attenuates stress-induced decreases in exploration of the open arms of the elevated plus maze (Menzaghi et al., 1994b). Astressin, a third $CRF_{1/2}$ receptor antagonist, decreases CRF-induced locomotor activation and closed arm exploration in the elevated plus maze (Spina et al., 2000). This same antagonist also increases open arm exploration in the elevated plus maze in rats subjected to social conflict stress (Spina et al., 2000). These studies indicate that CRF receptors not only regulate stress-like behaviors that are induced by CRF administration, but also anxiety-like behaviors influenced by external stressors.

Two genes encoding distinct G-protein-coupled CRF receptors have been identified. The CRF_1 receptor is found mainly in the pituitary, amygdala, hippocampus, cerebellum, and cortex, and is generally associated with increases in anxiety-like behavior (Koob and Heinrichs, 1999). For example, mice lacking a functional CRF_1 receptor show decreased anxiety-like behavior in various animal models of anxiety (Contarino et al., 1999; Smith et al., 1998; Timpl et al., 1998) and decreased spontaneous motor activity (Contarino et al., 2000). In addition, the CRF_1 receptor-selective antagonist CP-154,526 has been shown to reduce defensive behaviors in mice when confronted with a rat as a stressor (Griebel et al., 1998). A second CRF_1 receptor selective antagonist, antalarmin, reduces freezing behavior in rats when confronted with a foot shock, an indication of conditioned fear (Deak et al., 1999). Inhibition of CRF_1 receptors via central administration of CRF_1 receptor antisense has been shown to reduce stress-induced (Heinrichs et al., 1997; Liebsch et al., 1999) and CRF-induced anxiety-like behavior in the elevated plus maze and open field (Skutella et al., 1998).

The CRF_2 receptor is found mainly in the lateral septum, ventromedial hypothalamus, and choriod plexus (Chalmers et al., 1995; Perrin et al., 1995), and is most strongly associated with appetite suppression (Pelleymounter et al., 2000; Spina et al., 1996). For example, central infusion of urocortin 1, a CRF-related peptide with high affinity for the CRF_2 receptor, significantly suppresses feeding in food-deprived rats (Spina et al., 1996). There is some controversy regarding the role of the CRF_2 receptor in anxiety-like behaviors. CRF_2 receptor knockout mice show an anxiogenic-like phenotype (Bale et al., 2000; Kishimoto et al., 2000), and central infusions of urocortin 2 and urocortin 3, neuropeptides that are selective CRF_2 receptor agonists, result in increased exploration of the open arms in the elevated plus maze, indicative of an anxiolytic-like effect (Valdez et al., 2002a, 2003a). However, antisense inhibition of CRF_2 receptors in the lateral septum (Ho et al., 2001) and injections of antisauvagine-30 (Takahashi et al., 2001), a preferential CRF_2 receptor antagonist, have produced reductions in the conditioned fear response and increased exploration of the open arms in the elevated plus maze, respectively, in rats. These apparently conflicting findings may be the result of site-specific actions. Astressin, but not antisauvagine-30, attenuates CRF-enhanced fear conditioning when injected in the hypothalamus. Both of these antagonists, however, reduce fear conditioning when injected into the lateral septum (Radulovic et al., 1999). Clearly, further research is needed to fully determine the function of the CRF_2 receptor in anxious states.

As discussed, alcohol withdrawal can act as a stressor, leading to many of the anxiety-like responses induced by CRF administration and attenuated by CRF receptor antagonism in laboratory animals. Thus, it appears that CRF may be an important regulator of the anxious state characteristic of alcohol withdrawal. For example, centrally administered CRF potentiates the locomotor-activating effects of chronic ethanol exposure (Ehlers and Chaplin, 1987), and enhances electroencephalogram (EEG) parameters in ethanol-withdrawing rats (Slawecki et al., 1999). In addition, rats display a significant increase in anxiety-like behavior in the elevated plus maze during withdrawal, an effect attenuated by injections of the CRF antagonist D-Phe-$CRF_{12–41}$ into the lateral ventricles (Baldwin et al., 1991) and the amygdala (Rassnick et al., 1993). In vivo microdialysis studies also implicate CRF as a mechanism underlying the anxiety-like symptoms of acute withdrawal, as withdrawing rats show increases in extracellular CRF concentrations in the amygdala up to 12 h following withdrawal (Merlo-Pich et al., 1995). Finally, CRF_1 receptor knockout mice fail to show the traditional decrease in open arm exploration in the elevated plus maze during acute withdrawal (Timpl et al., 1998).

Studies examining the anxiolytic-like characteristics of alcohol in laboratory animals also indicate that CRF systems play an important role in the behavioral effects of alcohol. Rats selectively bred to have a high preference for alcohol have lower concentrations of CRF in the amygdala and the cortex and show an increased EEG response when centrally injected with CRF, indicating a possible up-regulation of CRF receptors in this rat strain (Ehlers et al., 1992). When examined using the conflict test, the increases in punished

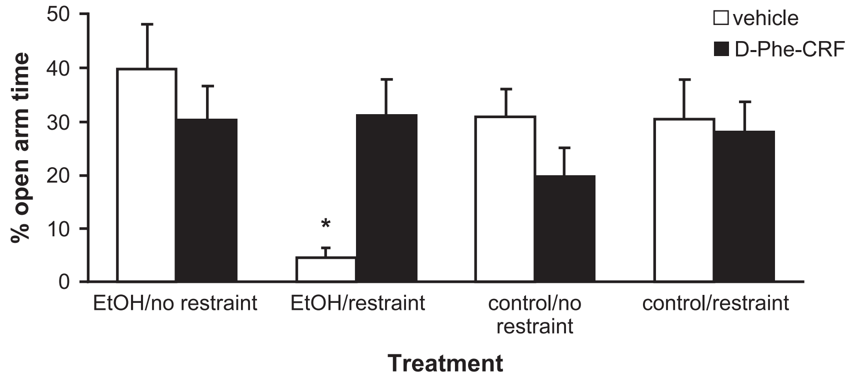

Fig. 3. Effect of restraint stress on exploratory behavior in the elevated plus maze 6 weeks after exposure to an ethanol liquid diet over a 3 week period. Control rats received a sucrose-containing liquid diet. Rats were injected intracerebroventricularly with 10 μg of D-Phe-$CRF_{12–41}$ or vehicle and subsequently placed in restraint tubes or returned to their home cages for 15 min. The mean percentage of time spent on the open arms of the elevated plus maze±S.E.M. was measured. *p<0.05 compared to all other groups. (Taken with permission from Valdez et al., 2003b).

responding produced by ethanol are reversed by central CRF injection (Thatcher-Britton and Koob, 1986). These studies suggest that the anti-anxiety properties of alcohol may involve a suppression of brain CRF systems. Conversely, the anxiety-like effects that accompany withdrawal may be the result of a hyperactivity of CRF systems. For example, rats maintained on an ethanol liquid diet for approximately 3 weeks show an increased anxiety-like response in the elevated plus maze following 15 min of restraint stress (Valdez et al., 2003b). This level of exposure to restraint, however, did not induce a stress-like response in rats that were fed a control diet, suggesting that chronic ethanol exposure can lead to hypersensitivity to stress.

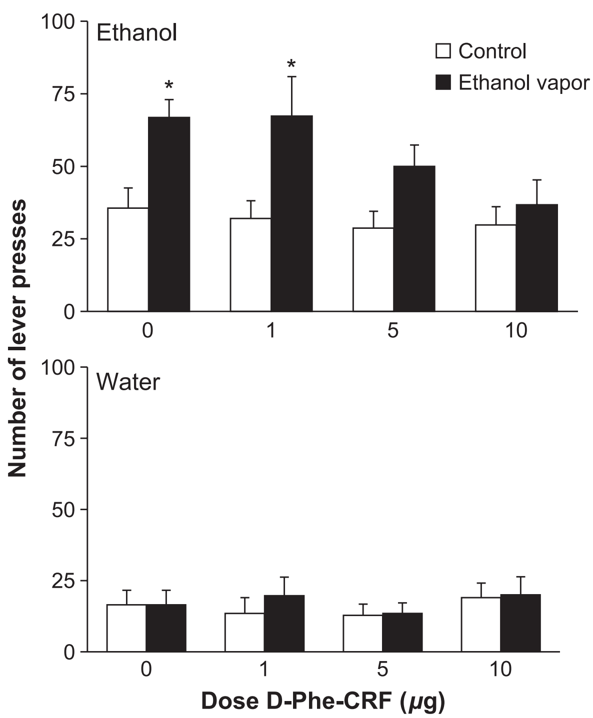

Fig. 4. Effects of D-Phe-$CRF_{12–41}$ on responding for ethanol and water 2 h after chronic ethanol vapor exposure. Control rats were exposed to air vapor. Rats received an intracerebroventricular microinjection of 0–10 μg of D-Phe-$CRF_{12–41}$ using a within subjects Latin square design 2 h after removal from the vapor chambers. The number of lever presses for ethanol and water±S.E.M. were measured 10 min after injection. After the initial test session, rats were re-exposed to ethanol vapor or air, and the procedures were repeated until the Latin square design was completed. *p<0.05 compared to controls. (Taken with permission from Valdez et al., 2002b).

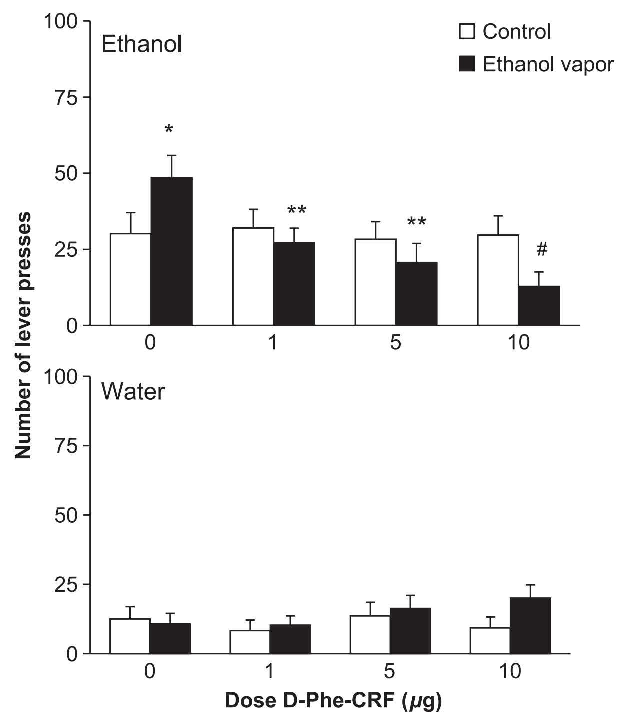

Fig. 5. Effects of D-Phe-$CRF_{12–41}$ on responding for ethanol and water 2–5 weeks after chronic ethanol vapor exposure. Control rats were exposed to air vapor. Rats received an intracerebroventricular microinjection of 0–10 μg of D-Phe-$CRF_{12–41}$ using a within subjects Latin square design 2 weeks after removal from the vapor chambers. The number of lever presses for ethanol and water±S.E.M. were measured 10 min after injection. After the initial test session, rats were returned to their home cages and left undisturbed. The testing procedures were repeated over the next 3 weeks until the Latin square design was completed. *p<0.05 compared to controls; **p<0.05 compared to ethanol-exposed rats that received an injection of 10 μg of D-Phe-$CRF_{12–41}$; #p<0.05 compared to ethanol-exposed rats that received and injection of 0 μg of D-Phe-$CRF_{12–41}$ and controls. (Taken with permission from Valdez et al., 2002b).

Reversal of the increase in anxiety-like behavior seen in the ethanol-exposed rats by central injections of D-Phe-CRF_{12-41} implies that CRF is a likely mechanism underlying the enhanced responsiveness to stress (Fig. 3). Heightened CRF activity may also contribute to increased ethanol self-administration during withdrawal and protracted abstinence (Valdez et al., 2002b). Injections of D-Phe-CRF_{12-41} dose dependently reverse the increase in lever pressing for ethanol that is observed in rats 2 h after removal from the ethanol vapor chambers following approximately 2 weeks of exposure (Fig. 4). This increase in CRF activity also appears to be long lasting in nature because a similar dose-dependent decrease in responding for ethanol is observed in rats between 2 and 5 weeks following ethanol vapor exposure (Fig. 5).

These studies demonstrate an important role for CRF in the behavioral response to stress, and implicate CRF as a key mediator of the anxious state experienced by alcoholics that accompanies abstinence and subsequently, the associated susceptibility to relapse. Therefore, understanding the role of CRF in the negative affective states associated with withdrawal appears to be critical in understanding the factors associated with relapse.

6. Neuropeptide Y, stress, and alcohol

Alcohol consumption is accompanied by anti-anxiety effects, as seen in the use of alcohol to alleviate anxiety associated with withdrawal in alcoholics (Hershon, 1977) and the increased punished drinking observed during the conflict test in laboratory animals (Thatcher-Britton and Koob, 1986). In addition to the hypothesized suppression in brain CRF systems that may contribute to this anti-anxiety effect, it is likely that activation of a second brain system, involving NPY, is also a contributing factor. NPY is a 36-amino-acid neuropeptide (Tatemoto et al., 1982) that acts as an anxiolytic and sedative agent (Heilig and Murison, 1987; Heilig et al., 1989), and has a wide and distinct anatomical distribution in the central nervous system. In post mortem human tissue, high amounts of NPY-like immunoreactivity have been found in the basal ganglia, nucleus accumbens, and amygdala, whereas moderate amounts were detected in the hypothalamus, septum, cortex, and periaqueductal gray (Adrian et al., 1983). Although not identical, the distribution of NPY in the rat brain parallels that of the human. In the rat, NPY-like immunoreactivity was highest in the periaqueductal gray, nucleus accumbens, hypothalamus, septum, and amygdala, whereas lower concentrations were detected in the basal ganglia, hippocampus, and cortex (Adrian et al., 1983). NPY receptors also have been found in the hippocampus, olfactory bulb, and cerebellum (Dumont et al., 1993), as well as in the spinal cord (Rowan et al., 1993).

To date, six NPY receptor subtypes have been identified. The Y_1 receptor appears to have a wide distribution throughout the rat brain, where it is most abundantly found in the amygdala, cortex, olfactory tubercle, hippocampus, hypothalamus, and thalamus (Parker and Herzog, 1999). The distribution of the Y_2 receptor is similar to that of the Y_1 receptor, although Y_2 receptor expression is less abundant in the cortex and thalamus and more abundant in the hippocampus (Parker and Herzog, 1999). Little is known about the Y_3 receptor, as it has not been cloned or well characterized (Lee and Miller, 1998). The Y_4 receptor, which has a low affinity for binding NPY, appears to have a moderate distribution in the cortex, olfactory tubercle, and hypothalamus (Parker and Herzog, 1999). The highest distribution of Y_5 receptors is found in the cortex, olfactory tubercle, and hippocampus, whereas moderate amounts have been detected in the hypothalamus and thalamus (Parker and Herzog, 1999). The Y_6 receptor appears to be nonfunctional in primates and absent from the rat genome, although it has been found in the hypothalamus of the mouse (Gregor et al., 1996; Mullins et al., 2000). In addition to NPY, these receptors also can be activated by structurally related peptides, peptide YY (PYY) and pancreatic polypeptide (PP). Short C-terminal fragments of NPY preferentially activate the Y_2 receptor and NPY_{2-36} selectively binds the Y_5 receptor. Additionally, PP has a high affinity in binding the Y_4 receptor.

NPY affects a large range of behaviors in laboratory animals (Table 1). NPY and its related peptides are highly

Table 1
Behavioral effects of NPY and related peptides in laboratory animals

Behavioral effects	Neuropeptides	
↑ Food intake	NPY	Clark et al., 1984; Levine and Morley, 1984; Stanley et al., 1986
↓ Locomotor activity	NPY	Heilig and Murison, 1987
↑ Open arm exploration in the elevated plus maze	NPY; NPY_{13-36}	Heilig et al., 1989; Kask et al., 1998a,b
↑ Punished responding in the conflict test	NPY; NPY_{13-36}; peptide YY; [Leu^{31},Pro^{34}]-NPY; [Gly^{6},Glu^{26},Lys^{29},Pro^{34}]-NPY	Heilig et al., 1989; Britton et al., 1997
↑ Social interaction between unfamiliar rats in the social interaction test	NPY	Sajdyk et al., 1999
↓ Oral ethanol self-administration	NPY	Badia-Elder et al., 2001

implicated in the regulation of appetite, as central injections of NPY have been shown to increase food intake in laboratory animals (Clark et al., 1984; Levine and Morley, 1984; Stanley et al., 1986). The hypothalamus seems to be largely implicated in the regulation of feeding behavior by NPY (Morley et al., 1987), especially the paraventricular nucleus (Chronwall et al., 1985; Gray and Morley, 1986) and caudolateral perifornical region (Stanley et al., 1989). In addition, NPY may have a role in regulating basic vegetative functions, such as respiration and cardiac regulation (Fuxe et al., 1983), and circadian rhythms (Albers and Ferris, 1984). Centrally administered NPY also can lead to sedation, resulting in dose-dependent decreases in locomotor behavior (Heilig and Murison, 1987).

Animal models of anxiety have revealed an anxiolytic-like role for NPY in the regulation of stress-related behaviors. Centrally administered NPY can increase open arm preference in rats tested in the elevated plus maze (Heilig et al., 1989). In the conflict test, NPY injections increased the number of shocks that rats would accept while drinking a glucose-sweetened solution (Heilig et al., 1989). Control experiments suggested that this effect was specific to anxiety-like behavior because the same doses of NPY failed to alter shock threshold or motivation to drink in the absence of shock (Heilig et al., 1989). Centrally administered PYY also increases punished responding in the conflict test (Britton et al., 1997). It appears that the Y_1 receptor may have an important role in mediating the anxiolytic-like actions of NPY because central injections of the specific Y_1 agonists [Leu^{31},Pro^{34}]NPY and [Gly^{6}, Glu^{26}, Lys^{29}, Pro^{34}]-NPY into the central nucleus of the amygdala also increased punished responding in the conflict test (Britton et al., 1997).

In addition, NPY overexpressing and knockout mice also have been developed to examine the effects of endogenous NPY on behavior. NPY-overexpressing rats appear to be less sensitive to stress. Exposure to restraint stress failed to elicit an anxiogenic-like response in the elevated plus maze and punished drinking failed to induce fear suppression (Thorsell et al., 2000). In contrast, NPY knockout mice displayed an anxiogenic-like phenotype when tested in various animal models of anxiety (Bannon et al., 2000).

The amygdala has been implicated as a possible mechanism of action in the mediation of anxiety-like behavior via NPY. Whereas intracerebroventricular injections of NPY can elicit both anxiolytic-like effects in animal models of anxiety and increases in appetite, microinjections into the central nucleus of the amygdala produce anxiolytic-like effects in the conflict test without increasing appetite (Heilig et al., 1993). This effect seems to be mediated by Y_1 receptors because the selective Y_1 receptor agonist p[Leu^{31},Pro^{34}]NPY was equipotent to NPY in producing this response, but significantly more potent than the selective Y_2 agonist $NPY_{13–36}$ (Heilig et al., 1993). In addition, injections of NPY into the amygdala also decrease anxiety-like behavior in the social interaction test, indicated by an increase in social interaction time between two unfamiliar rats (Sajdyk et al., 1999). This effect is reversed by co-administration of the Y_1 receptor antagonist ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-*N*2-(diphenylacetyl)-argininamide trifluoroacetate) 3304 (Sajdyk et al., 1999). In addition, antisense inhibition of Y_1 receptor expression blocks the anxiolytic-like effects seen in the elevated plus maze following NPY injections into the amygdala (Heilig, 1995). Another site of action that has been implicated in the anxiolytic-like actions of NPY is the dorsal periaqueductal gray matter. Injections of the Y_1 receptor antagonist BIBP3226 resulted in increased anxiogenic-like behavior in the elevated plus maze (Kask et al., 1998a). Finally, Y_2 receptors in the locus coeruleus also appear to be involved in mediating decreases in anxiety-like behavior. Both NPY and the Y_2 receptor agonist $NPY_{13–36}$ increased anxiolytic-like behavior in the elevated plus maze (Kask et al., 1998b). These studies indicate that although the Y_1 receptor in the amygdala is important in the regulation of the anti-anxiety-like effects of NPY, this system also may interact with other brain regions and receptor subtypes to produce these effects.

Given the behavioral profile of NPY, it is possible that the negative reinforcing effects produced by alcohol when consumed during withdrawal may be related to the anxiolytic-like properties associated with NPY systems. For example, Wistar rats exposed to chronic ethanol vapor show significantly higher levels of NPY 1 month following withdrawal compared to air-exposed controls (Ehlers et al., 1998a). In addition, central administration of NPY and intraperitoneal administration of ethanol have been shown to produce identical electrophysiological profiles in the brain (Ehlers et al., 1998b). Furthermore, selectively bred alcohol-preferring rats and alcohol-nonpreferring rats displayed opposite electrophysiological profiles in response to NPY (Ehlers et al., 1998b). Central injections of NPY also decrease oral ethanol self-administration in alcohol-preferring rats compared to alcohol-nonpreferring rats (Badia-Elder et al., 2001). Badia-Elder et al. (2003), however, have also shown that NPY increases open arm exploration in the elevated plus maze in both high-alcohol-drinking and low-alcohol-drinking rats, but only decreases ethanol intake in the high-alcohol-drinking rats. These data suggest that the ability of NPY to decrease ethanol drinking may not be directly related to its anxiolytic-like properties. However, this effect may be due to a lower baseline level of ethanol intake in the low-alcohol-drinking rats. Ethanol intake in the high-alcohol-drinking rats following NPY injections was similar to that of the low-alcohol-drinking rats following injections of vehicle or NPY. The inability of NPY to decrease ethanol intake in low-alcohol-drinking rats may have simply been a floor effect. Nonetheless, further research is needed to clarify the relationship between the anti-anxiety effects of NPY and the ability of this neuropeptide to alleviate increased alcohol intake.

Studies involving NPY-deficient mice also implicate NPY as a mechanism in the regulation of alcohol consumption. NPY receptor knockout mice self-administer significantly higher amounts of ethanol compared to wild-type controls (Thiele et al., 1998). These mice are also able to recover from ethanol-induced inactivity faster than wild-type controls with similar blood alcohol concentrations, implying a decreased sensitivity to the sedative effects of ethanol (Thiele et al., 1998). In contrast, NPY overexpressing mice show a lower preference for ethanol and are more sensitive to the sedative effects of ethanol compared to controls (Thiele et al., 1998). In addition, a study using C57BL/6J mice has shown that peripheral injection with L-152,804, a Y_5 receptor antagonist, delays the onset of ethanol-reinforced responding in these mice without altering locomotor activity (Schroeder et al., 2003a). This same strain of mice also has been shown to express lower levels of NPY in the shell of the nucleus accumbens compared to alcohol-nonpreferring DBA2/J mice (Misra and Pandey, 2003).

In addition to the nucleus accumbens, another possible site of action for these NPY-mediated effects of alcohol is the amygdala. Selectively bred alcohol-preferring rats show lower concentrations of NPY-like immunoreactivity in the amygdala, hippocampus, and frontal cortex compared to selectively bred alcohol-nonpreferring rats (Ehlers et al., 1998a). Additionally, alcohol-preferring rats and high alcohol drinking rats show similar concentrations of NPY-like immunoreactivity in the central nucleus of the amygdala, which is significantly lower compared to both alcohol-nonpreferring rats and low-alcohol-drinking rats (Hwang et al., 1999). Wistar rats also show a blunted electrophysiological response to central injections of NPY in the amygdala following chronic alcohol exposure (Slawecki et al., 1999). Finally, injections of the Y_1 receptor antagonist BIBP 3226 into the amygdala attenuate operant responding for ethanol without significantly altering water responding (Schroeder et al., 2003b). Although this finding appears to contradict the findings of other studies in which NPY injections suppress ethanol intake, an important issue to consider is that rats in this study were not subjected to any type of chronic ethanol exposure and were therefore not dependent on ethanol. It is possible that BIBP 3226 was suppressing the acute reinforcing effects of endogenous NPY that may have been activated by ethanol self-administration. Alternatively, BIBP 3226 injections may have also had appetite suppressive effects given the caloric value of ethanol.

The studies described above demonstrate the importance of NPY in the alleviation of the stress response, and implicate a role for NPY in the anti-anxiety properties of alcohol. Therefore, it appears that NPY may provide a motivational basis for alcohol self-administration during the anxious state that is experienced during alcohol withdrawal. Fully understanding the role of the NPY system in alcohol dependence may lead to further insights in the treatment of this disorder.

7. Allostasis and dysregulation of CRF and NPY in the development of alcohol dependence

Two significant concepts that are important in understanding the long-term significance of alcoholism are homeostasis and allostasis. Homeostasis refers to the ability of the body to remain stable within its physiological systems and to maintain the normal internal parameters that are necessary for the survival of an organism (Sterling and Eyer, 1981). In order for an organism to maintain homeostasis, it must be able to correct deviations from these parameters and return them to their normal range. Homeostasis also can refer to the process by which physiological systems are maintained within a range optimal for the survival of an organism (McEwen, 2000). This process appears to be particularly important with regard to the stress system because the elevated stress hormone levels outside the homeostatic range can lead to compromised immune, gastrointestinal, cardiovascular, and metabolic function (Brown, 1991; Fisher, 1993; Irwin et al., 1990; Taché et al., 1990). In addition, chronic stress can lead to long-term negative affect and subsequent behavioral pathology (Koob and Le Moal, 2001).

Chronic exposure to stress may place demands on an organism to the point where it is unable to maintain its physiological and psychological systems within a normal homeostatic range. Allostasis is a concept that refers to the regulation of physiological systems outside the normal homeostatic range in which the body varies the parameters of its physiological systems to adapt to any perceived or anticipated environmental demands (Sterling and Eyer, 1981). Chronically varying internal parameters can lead to allostatic load, which are the changes the body must enlist to face these environmental challenges (McEwen, 1998). Constant exposure to allostatic load can lead to inefficient operation of physiological systems, and may result in an inability of these systems to return to a homeostatic range (McEwen, 2000). At this point, the organism begins to regulate itself at a dysregulated state, during which physiological systems are maintained at a baseline state outside the homeostatic range. Although this altered set point may seem appropriate to the perceived conditions that are endured, it may be within a range that can lead to pathological behavior with little additional demand.

Chronic drug and alcohol intake may be perceived as stressors that place an allostatic load on the reward systems of the brain. As a consequence, these systems may begin to regulate themselves at an altered set point to maintain stability when faced with this chronic demand. This dysregulated state has been hypothesized to be long lasting and to be involved in vulnerability to dependence and relapse (Koob, 2003; Koob and Le Moal, 1997, 2001). This model of drug dependence proposed by Koob and Le Moal (2001) is an extension of the opponent process model of motivation (Solomon and Corbit, 1974) and takes into account the concepts of tolerance and sensitization. With regard to drug-taking behavior, tolerance can be defined as diminished effects following repeated administration of a drug, whereas sensitization involves increasing effects following repeated administrations. Under this proposed model, when a drug is taken, a positive mood state is experienced followed by an equally powerful negative affective state. Following this negative affect, the mood of the drug user would return to a normal homeostatic baseline state. Following repeated instances of drug taking, however, the positive mood state experienced following acute intake would diminish due to tolerance, whereas the subsequent negative affective state becomes greater due to sensitization. As the drug user moves toward an addictive state, allostatic load is endured due to the more powerful negative affective states experienced, and as a result, the mood state is unable to return to a homeostatic range and becomes dysregulated. This baseline state of negative affect leads the drug user to self-administer more drug to return to a homeostatic range. Under this model, the transition from casual drug use to addiction occurs when the drug user's reason for self-administering the drug moves from positive reinforcement to negative reinforcement (Koob, 2004). In other words, the drug taker is no longer self-administering a drug to experience euphoria, but rather to alleviate negative affect and regulate mood at a normal homeostatic range.

With regard to alcoholism, this transition from positive to negative reinforcement would likely occur following long periods of alcohol intake that lead to blood alcohol levels sufficient to produce dependence. Given the nature of clinical studies, it is difficult to determine a precise period of exposure and blood alcohol level necessary for an individual to become dependent because many of these experiments involve alcoholics who have been drinking for years prior to their participation. Animal models, however, demonstrate that dependence can occur with a matter of weeks if the experimental animals are exposed to sufficiently high levels of ethanol. For example, rats maintained on an ethanol liquid diet for approximately 4 weeks or exposed to ethanol vapor for approximately 2 weeks at blood ethanol concentrations ranging from 100 to 200 mg% show significant signs of physical withdrawal (Macey et al., 1996). Rats maintained under similar conditions also show increased ethanol self-administration (Valdez et al., 2002b) and a heightened stress response (Baldwin et al., 1991; Rassnick et al., 1993; Valdez et al., 2003b). One hypothesis to explain the development of alcohol dependence is that chronic exposure to alcohol at levels sufficient to produce intoxication may lead to a disruption of homeostasis within the stress systems due to allostatic load.

Maintaining a balance between CRF and NPY appears critical in the regulation of stress, anxiety, and depression. For example, central administration of CRF reduces the duration of sodium pentobarbital-induced sleep, an effect that can be reversed by the injection of NPY (Yamada et al., 1996). In a study examining the effects of CRF and NPY on brain activity related to arousal, CRF significantly increased brain waves related to auditory responding in rats (Ehlers et al., 1997). Co-administration of NPY significantly decreased this measure. Additionally, co-administration of NPY attenuates the ability of CRF to increase sleep latency and reduce the duration of nonrapid eye movement sleep (Ehlers et al., 1997). These studies indicate that maintaining a balance between CRF and NPY systems is critical for normal physiological function. Because disturbance in physiological function also can lead to disruptions in mood states, maintaining a balance between CRF and NPY may be vital to maintaining a homeostatic psychological state and avoiding psychopathology.

Both chronic and acute ethanol exposure has been shown to alter the long-term function of CRF and NPY systems. Long-term alterations in these two systems appear to be critical in contributing to the chronic nature of alcoholism. Studies involving human alcoholics have demonstrated conflicting results regarding the long-term effects of chronic alcohol consumption on the HPA axis. One study that examined the circadian patterns of cortisol secretion in alcoholic men during acute and long-term abstinence found disruptions in normal cortisol secretions during the acute phase, which returned to normal with prolonged abstinence (Iranmanesh et al., 1989). In this study, the timing of the circadian patterns of cortisol secretion was significantly delayed in alcoholic men compared to controls during the first 3 to 16 days of abstinence. Follow-up measures on these patients showed that circadian patterns returned to normal at 29–39 days of abstinence. Although the hormone levels appear to have returned to normal, other studies have shown that the functioning of this system may be compromised. For example, detoxified alcoholics and controls show similar baseline levels in cortisol levels when examined at 4 weeks of abstinence (Bernardy et al., 1996). However, when forced to endure both a mental (mental arithmetic test) and physical (isometric handgrip test) stressor, alcoholics show a blunted cortisol response compared to controls (Bernardy et al., 1996), likely due to an exhaustion of the system. It appears that this compromise in function also may be due to an altered response to CRF. For example, a study in which the HPA axis function in male alcoholics was examined following the first 2 weeks of abstinence showed a compromised function of the neuroendocrine response to stress following intravenous CRF injection (Costa et al., 1996). Although mean pre-injection levels of adrenocorticotropic hormone (ACTH) and cortisol were comparable in the alcoholic patients and non-alcoholic controls, alcoholic patients showed a significantly blunted ACTH and cortisol response following injections of CRF. This blunted response to CRF injection has been shown to last for up to 6 weeks (Von Bardeleben et al., 1989).

Long-term changes also have been observed in CRF systems in animal models of alcohol dependence. For example, rats that have been maintained on an ethanol-containing liquid diet for about 4 weeks have shown modest increases in plasma corticosterone levels associated with disruptions in circadian function of the HPA axis compared to pair-fed controls (Rasmussen et al., 2000). Three weeks following the removal of the liquid diet, rats receiving ethanol had significantly suppressed plasma corticosterone levels compared to controls (Rasmussen et al., 2000). A second study in which rats were maintained on an ethanol liquid diet for 3–4 weeks yielded similar results. Plasma corticosterone was significantly suppressed 1 day and 3 weeks following removal of the diet in rats receiving alcohol compared to controls (Zorrilla et al., 2001). Although plasma corticosterone levels returned to control levels 6 weeks following the removal of the liquid diet, brain CRF-like immunoreactivity remained altered. One day following the removal of the liquid diet, there was a significant suppression of CRF-like immunoreactivity in the amygdala, frontal cortex, and hippocampus, suggesting a depletion of these systems following acute withdrawal (Zorrilla et al., 2001). In addition, whereas CRF-like immunoreactivity in all other brain regions examined returned to control levels at 3 weeks following removal of the liquid diet, CRF-like immunoreactivity was significantly increased in the amygdala 6 weeks post-withdrawal (Zorrilla et al., 2001). The apparent hyperactivity of the CRF system in the amygdala suggests a possible increased sensitivity to stress, as the amygdala has been implicated in the behavioral stress response associated with alcohol withdrawal (Möller et al., 1997). Rats maintained on an ethanol-containing liquid diet in a manner similar to the experiment described above show increased anxiety-like behavior in the elevated plus maze when exposed to restraint stress for 15 min (Valdez et al., 2003b). This response appears to be specific to a stressor because rats that did not receive restraint stress did not differ, regardless of whether they had a history of dependence. In addition, this increased stress response was blocked by central injection of the CRF antagonist D-Phe CRF_{12-41}. These results indicate the possibility of independent but concurrent mechanisms for the regulation of the behavioral and physiological responses to stress during withdrawal.

Chronic ethanol vapor exposure also has been shown to alter the long-term neurophysiological response to both NPY and CRF injections (Slawecki et al., 1999). In rats that were exposed to ethanol vapor for 6 weeks, CRF injections led to an increase in cortical activity 10–15 weeks post withdrawal, whereas CRF injections decreased this measure in air-exposed controls. Central injections of NPY decreased activity in the amygdala in ethanol-exposed rats but not in control rats over this same time period. With regard to the long-term effects of chronic alcohol on NPY systems, Ehlers et al. (1998a) found increases in NPY-like immunoreactivity levels in the hypothalamus 1 month after the cessation of seven weeks of chronic ethanol exposure in rats. In addition, rats fed an ethanol liquid diet showed decreases in NPY immunoreactivity in the hippocampus during the initial stages of withdrawal, followed by a dramatic increase in NPY immunoreactivity in this same region (Bison and Crews, 2003). These observed increases likely represent a compensatory mechanism to oppose the effects of initially decreased levels of NPY, as well as increases in CRF. This view of increased NPY-like immunoreactivity as a compensatory mechanism may seem incompatible with the current model. However, the hypothesis proposed contends that increased alcohol consumption leads to a tolerance to the anti-anxiety effects of NPY, not simply a decrease in NPY levels. Although the brain may be producing more NPY in order to counteract the anxiety-like effects associated with withdrawal, a developed tolerance would diminish the effectiveness of NPY to produce an anxiolytic-like effect. In addition, these increased NPY levels may require a prompt, such as drinking alcohol, in order for them to become behaviorally active. A similar situation is observed with increased CRF immunoreactivity during withdrawal. At 6 weeks post-withdrawal, rats show increased CRF-like immunoreactivity in the amygdala (Zorrilla et al., 2001), but do not show an anxiety-like response unless faced with a mild stressor (Valdez et al., 2003b).

As indicated above, alterations in CRF (Berridge and Dunn, 1986; Sutton et al., 1982) and NPY (Bannon et al., 2000) systems can lead to anxiety-like behavior in laboratory animals. It is possible that the imbalance between these two systems can lead to continued drinking to return the systems to a homeostatic range because the alleviation of anxiety is hypothesized to be a major factor involved in relapse (Cloninger, 1987). One hypothesis is that increased CRF leads to the negative affective state that is strongly associated with alcohol withdrawal, whereas NPY provides the motivational basis to consume alcohol because the anxiolytic-like affects of alcohol appear to be regulated in part by NPY. The amygdala appears to be a likely site of action. Y_1 and Y_2 receptor mRNA is expressed throughout the amygdaloid area, especially in the basolateral amygdala and amygdalohippocampal area (Parker and Herzog, 1999). CRF receptor mRNA is also highly expressed within these same regions (Potter et al., 1994). Heilig et al. (1994) have proposed that the amygdala integrates stressful sensory inputs, leading to an initiation of the stress response. Under this model, a rapid activation of CRF occurs in the central nucleus of the amygdala during the initial phase followed by a slower activation of NPY to oppose the maladaptive consequences of excessive CRF activation.

Long-term changes have been observed in both CRF and NPY levels and activity in the amygdala (Ehlers et al., 1998a; Slawecki et al., 1999; Zorrilla et al., 2001). The increases in CRF-like immunoreactivity indicate that chronic alcohol likely can lead to an increased responsiveness to stress, which in turn can lead to enhanced negative affect. This enhanced negative affect provides a motivational basis for the negative reinforcing properties of alcohol. The underlying neural substrate for this negative reinforcement is likely NPY because alcohol and NPY have similar electrophysiological (Ehlers et al., 1998b) and behavioral (Badia-Elder et al., 2001) effects. The increases in NPY-like immunoreactivity observed following chronic ethanol exposure (Ehlers et al., 1998b) may be a compensatory mechanism, activated by alcohol consumption, designed to balance the effects of increased CRF levels. Indeed, NPY can act as a functional antagonist of CRF (Ehlers et al., 1997; Heilig et al., 1994).

Repeated episodes of alcohol deprivation also appear to contribute to the allostatic load endured by CRF and NPY systems. Repeated episodes of deprivation contribute to increased anxiety-like behavior and alcohol consumption in laboratory animals (Hölter et al., 1998). Rats also will increase the amount of work performed to receive alcohol, indicating increases in alcohol craving (Brown et al., 1998). In addition, repeated withdrawal episodes lead to alterations in gene transcription in the cortex and amygdala (Rimondini et al., 2002). CRF is the likely underlying neural mechanism involved in regulating the increased anxiety-like response because CRF receptor antagonism has been shown to attenuate the anxiogenic-like effects of withdrawal in rats (Baldwin et al., 1991; Rassnick et al., 1993). It is possible that repeated episodes of withdrawal lead to a sensitization to CRF in the behavioral stress response (Fig. 6). Although changes in brain CRF levels have yet to be examined following repeated withdrawal episodes, Zorrilla et al. (2001) have observed long-term significant increases in CRF-like immunoreactivity in the amygdala following a single withdrawal episode in rats with a history of dependence. This increase in CRF-like immunoreactivity may point to an underlying sensitization of brain CRF systems.

The increased alcohol consumption and motivation to self-administer alcohol observed following repeated deprivation episodes may be due to a tolerance to behavioral effects of NPY (Fig. 6). The behavioral effects of alcohol are similar to those of NPY (Badia-Elder et al., 2001). However, just as tolerance can occur to the euphoric effects of alcohol (Koob, 1998), it is also likely that tolerance may develop to the behavioral effects of NPY. A decrease in NPY-like immunoreactivity that has been observed in the amygdala in rats with a history of ethanol dependence (Ehlers et al., 1998a) may point to evidence of NPY tolerance in the brain. Although an increase in NPY-like immunoreactivity has been observed in the hypothalamus in rats with a history of alcohol dependence (Ehlers et al., 1998a), its effects may be countered by similar increases in CRF-like immunoreactivity (Zorrilla et al., 2001). In addition, the amygdala appears more involved in regulating the anti-anxiety effects of NPY (Heilig et al., 1993; Heilig, 1995), whereas the hypothalamus has been linked more to NPY's regulation of appetite (Morley et al., 1987).

The studies described above indicate that chronic alcohol can impair the homeostatic balance between CRF and NPY systems. These studies indicate that long-term alterations in the function of these two systems may lead to an allostatic load and regulation of these systems outside their homeostatic range. This dysregulation can lead to behavioral pathologies associated with alcoholism, which may eventually contribute to relapse. Further examination and empirical testing of the hypothesis that an imbalance

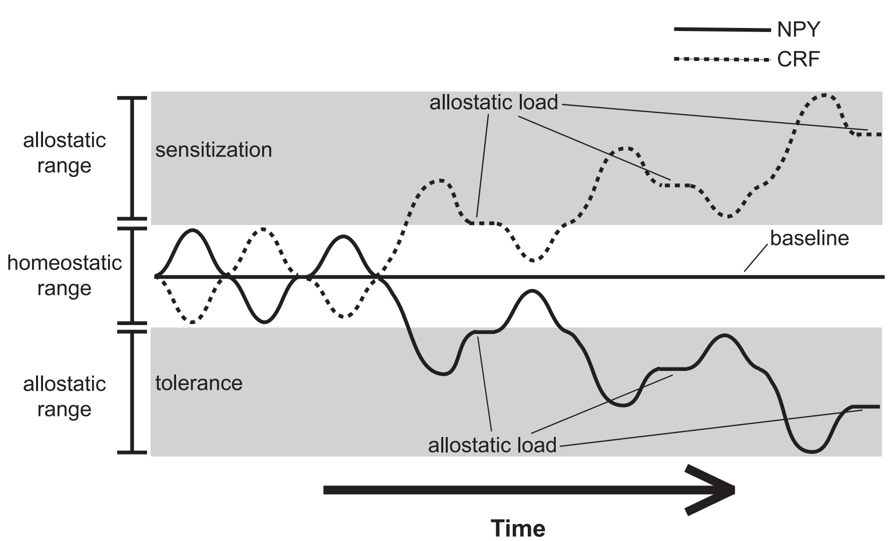

Fig. 6. Dysregulation of NPY and CRF systems following chronic alcohol use. When alcohol is first consumed, NPY increases and subsequently decreases before returning to baseline, all within the homeostatic range. CRF levels show an opposing pattern, with an initial decrease followed by an increase and then a return to baseline within the homeostatic range. As time progresses with further alcohol use, the initial increase in NPY and decrease in CRF become blunted, followed by a greater decrease in NPY and a greater increase in CRF levels. Allostatic load is then placed on these systems, and they are unable to return to their original baselines. During subsequent drinking episodes, the initial increase in NPY levels becomes even more blunted followed by a greater decrease, suggesting a tolerance to the behavioral effects of NPY. In addition, the initial decrease in CRF levels is attenuated and followed by greater increases, leading to a sensitization to the effects of CRF.

between CRF and NPY contributes to continued alcohol-seeking behavior and relapse may lead to further insights regarding the factors that are critical in the development of alcoholism.

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