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Development of Mechanical Hypersensitivity in Rats During Heroin and Ethanol Dependence: Alleviation by CRF₁ Receptor Antagonism

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Abstract

Animal models of drug dependence have described both reductions in brain reward processes and potentiation of stress-like (or anti-reward) mechanisms, including a recruitment of corticotropin-releasing factor (CRF) signaling. Accordingly, chronic exposure to opiates often leads to the development of mechanical hypersensitivity. We measured paw withdrawal thresholds (PWTs) in male Wistar rats allowed limited (short access group: ShA) or extended (long access group: LgA) access to heroin or cocaine self-administration, or in rats made dependent on ethanol via ethanol vapor exposure (ethanol-dependent group). In heroin self-administering animals, after transition to LgA conditions, thresholds were reduced to around 50% of levels observed at baseline, and were also significantly lower than thresholds measured in animals remaining on the ShA schedule. In contrast, thresholds in animals self-administering cocaine under either ShA (1 h) or LgA (6 h) conditions were unaltered. Similar to heroin LgA rats, ethanol-dependent rats also developed mechanical hypersensitivity after eight weeks of ethanol vapor exposure compared to non-dependent animals. Systemic administration of the CRF1R antagonist MPZP significantly alleviated the hypersensitivity observed in rats dependent on heroin or ethanol. The emergence of mechanical hypersensitivity with heroin and ethanol dependence may thus represent one critical drug-associated negative emotional state driving dependence on these substances. These results also suggest a recruitment of CRF-regulated nociceptive pathways associated with escalation of intake and dependence. A greater understanding of relationships between chronic drug exposure and pain-related states may provide insight into mechanisms underlying the transition to drug addiction, as well as reveal new treatment opportunities.

Keywords

Addiction; Ethanol; Heroin; Cocaine; Hyperalgesia; Corticotropin-Releasing Factor

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1. Introduction

1.1 Drug Addiction and Negative Motivational Symptomatology

Drug addiction (or substance dependence, DSM-IV) is a chronic relapsing disorder characterized by a persistent compulsion to seek and take drugs (Self and Nestler, 1995) and the recruitment of negative motivational (or anti-reward) mechanisms that manifest during abstinence (Koob and Le Moal, 1997). Addiction can be conceptualized as a progressive disorder whereby drugs of abuse are initially taken for their pleasurable effects, although tolerance to these effects will often develop over time. The parallel development of intense negative emotional states (e.g., anxiety, dysphoria, irritability) as the addiction timeline progresses may represent the final definitive stage of this disease. Such conditions may further drive excessive drug-seeking behavior even as tolerance to the positive rewarding effects of drugs persists as a consequence of within- and between-circuit neuroadaptations (Edwards and Koob, 2010).

1.2. Pain as a Negative Motivational Symptom Influencing Drug Addiction

Chronic pain affects approximately one third of the U.S. adult population (Johannes et al., 2010), a number that will likely increase over the next several decades given an aging population in several western countries including the United States. Effective pain management is a central aim of medical care, with opioids remaining the best resource for analgesia and overall improvement of quality of life in chronic pain patients. Unfortunately, chronic opiate exposure intended to alleviate pain often paradoxically renders individuals more sensitive to nociceptive stimuli, a condition termed opioid-induced hyperalgesia (extensively reviewed in Angst and Clark, 2006). Importantly, hyperalgesia is also prevalent in former opiate addicts maintained on methadone, further suggesting that this condition emerges with protracted opiate use itself. In accordance with this hypothesis, heroin exposure in rodents leads to a spontaneous reduction in mechanical nociceptive thresholds (Laulin et al., 1998) that is exacerbated after chronic treatment (Simonnet and Rivat, 2003), suggesting a recruitment or sensitization of pronociceptive systems (Celerier et al., 2001). Given that chronic pain is well known to cause both emotional distress and produce a sustained negative emotional state (King et al., 2009), opiate-induced hyperalgesia may constitute a condition intimately associated with the transition to drug dependence by facilitating negative reinforcement processes.

The rise in prescription analgesic abuse and dependence (Maxwell, 2011) further suggests a foreboding link between pain modulation and addiction. Indeed, a long-standing concern for medical practitioners is how to administer chronic opioid analgesics to pain patients without generating an unwanted dependence or addiction (Fields, 2011). Fortunately, opioid pharmacotherapy rarely leads to abuse following discontinuation of therapy; however, the risk of drug addiction is enhanced in patients with a history of illicit opioid use or abuse (Fishbain et al., 2008; Turk et al., 2008). Thus, hyperalgesia may exacerbate or maintain addicted states (drug dependence and compulsive drug-seeking behaviors) in this more compromised population. Shurman, Koob, and Gutstein (2010) further hypothesize that effective pain management with doses of opioids that are strictly titrated to the relief of pain represents the best treatment strategy, whereas any over-exposure to opioids in excessive amounts (i.e., above physiological needs) may lead to the recruitment of opponent motivational processes in terms of both pain (hyperalgesia) and negative affect (termed hyperkatifeia). Such opponent neuroadaptations are apparent at even the cellular level following opiate exposure (Bederson et al., 1990; Kaplan and Fields, 1991; Nestler and Aghajanian, 1997), but may also progress to the recruitment of multiple brain stress circuits during dependence (Koob, 2008). Targeting neuroadaptations at either or both of these

levels may represent viable anti-dependence therapeutic strategies in the future (McClung, 2006; Koob et al. 2009; Heilig et al., 2010).

Similar to dependence on opiates, alcoholism (or ethanol dependence) is a chronically relapsing mental disorder involving profound motivational disturbances and a loss of control over drinking. Alcoholism is also often accompanied by the emergence of negative emotional states that constitute a motivational withdrawal syndrome when access to alcohol is disrupted (Gilpin and Koob, 2008). The limited, recreational use of alcohol seen in a majority of users is clinically distinct from the escalated drinking, loss of control, and emergence of compulsive ethanol-seeking behaviors that characterize alcoholism. Chronic alcohol use impacts several peripheral and central nervous system actions, and while it has long been observed that oral alcohol administration increases human pain thresholds (Wolff et al., 1942), withdrawal from chronic use often results in increased pain sensitivity as one component of a larger alcohol withdrawal syndrome (Jochum et al., 2010). In fact, polyneuropathy, characterized by axonal degeneration and demyelination, is the most common neurological complication in alcoholics (Diamond and Messing, 1994). Such data suggest that drinking in alcoholics may in part be motivated by a desire to alleviate ethanol withdrawal-induced hyperalgesia. Indeed, self-reports of alcohol use specifically for pain management are common, even in young adults (e.g., Riley and King, 2009). In one study (Brennan et al., 2005), problem drinkers not only described more severe pain symptoms compared to non-drinkers, they also reported a higher incidence of using alcohol to manage their pain. Moreover, follow-up studies indicated that the use of alcohol to manage pain resulted in a worsening of drinking and other health-related problems three years later, indicating that negative reinforcement mechanisms related to pain-targeted drinking could underlie the persistence and exacerbation of both alcohol-related disorders and overall life morbidity.

1.3. Animal Models of Drug Dependence: Recruitment of CRF₁R Signaling

A critical breakthrough in the ability to examine negative reinforcement theories of drug addiction has been the development and refinement of reliable animal models of excessive drug (Ahmed and Koob, 1998) and alcohol (Gilpin et al., 2008) self-administration. For example, prolonged access to heroin self-administration (6-23 h per day) leads to escalation of heroin intake and dependence in rats, while animals given restricted access (1 h per day) fail to escalate intake (Edwards et al., 2009; Vendruscolo et al., 2011). Escalated animals go on to display a prolonged latency to extinguish heroin self-administration (a measure of drug seeking) as well as enhanced sensitivity to stress- and heroin-induced reinstatement (Ahmed et al., 2000; Lenoir and Ahmed, 2007). Similarly, in one model of ethanol dependence, rats trained to self-administer ethanol exhibit substantial increases in operant self-administration following chronic intermittent ethanol vapor exposure (Rogers et al., 1979; Gilpin et al., 2008). Ethanol vapor exposure allows for the reliable titration of blood alcohol levels (BALs) that are sufficient for inducing ethanol dependence, as indicated by both somatic withdrawal signs (Roberts et al., 2000) and negative motivational symptoms (Schulteis et al., 1995) during acute withdrawal. As a cardinal mediator of the magnified stress response that manifests during drug withdrawal, the recruitment of corticotropin-releasing factor (CRF), a neuropeptide critical to stress signaling, represents a central pharmacological system contributing to the establishment and maintenance of both heroin and alcohol dependence (Logrip et al., 2011). Chronic administration of opiates or ethanol leads to withdrawal-induced increases in brain CRF levels (Weiss et al., 2001; Funk et al., 2006; Merlo-Pich et al., 1995; Olive et al., 2002). Moreover, pharmacological blockade of CRF₁ receptors reduces both ethanol self-administration in ethanol-dependent animals (Funk et al., 2007; Richardson et al., 2008) and escalated heroin self-administration in heroin-dependent rats (Greenwell et al., 2009). CRF signaling mediates the expression of various withdrawal-

related behaviors associated with both heroin (Heinrichs et al., 1995; Stinus et al., 2005) and alcohol (Valdez et al., 2002; Valdez et al., 2003; Huang et al., 2010) dependence, although the regulation of drug withdrawal-induced modulation of pain-related processes by CRF₁R signaling has never been examined.

1.4. Development of Mechanical Hypersensitivity During the Transition from Drug Use to Dependence

The current study tracked changes in paw withdrawal thresholds (PWTs) across an extended timeline designed to model the natural development of, or progression to, drug dependence. Tests were conducted before and after exposure to three common drugs of abuse, and more importantly, before and after transition to extended access conditions modeling the escalating intake patterns associated with drug addiction. Finally, given the role of CRF signaling in the establishment and maintenance of dependence-related symptomatology, the ability of CRF₁R antagonism to modify altered mechanosensitivity in dependent vs. non-dependent animals was also determined.

2. Materials and Methods

2.1. Animals

Male Wistar rats (n = 98, Charles River) initially weighting 275–325g were communally housed (2–3/cage) with food and water available *ad libitum*. The animals were housed in a temperature-controlled (21.5°C) vivarium and maintained on a 12 h light/dark cycle (lights on at 0800). Animals were regularly handled for one week before surgery or the onset of operant training. All experiments adhered to the guidelines provided in the NIH *Guide for the Care and Use of Laboratory Animals* and protocols were reviewed and approved by The Scripps Research Institute's Institutional Care and Use Committee.

2.2. Ethanol Self-Administration and Ethanol Vapor Chamber Procedures

Animals were first trained to self-administer 10% (w/v) ethanol and water solutions until a stable response was maintained. To facilitate acquisition of operant self-administration, rats were initially provided 2-bottle choice access to ethanol (10% w/v) and water for 48 hours in the home cage. Rats then received 1 h 2-bottle choice access, time-matched to the future operant sessions, for 4 days. Rats acquired operant self-administration behavior during a single 12-h session, in which responses on the active lever yielded delivery of 0.1 ml water while responses on the inactive lever generated no scheduled consequences. The training session spanned one dark cycle with chow available *ad libitum* and yielded rapid acquisition of operant self-administration behavior. Following stable acquisition of operant ethanol self-administration (twelve sessions), animals were then split into two groups that were matched for ethanol self-administration over their last three sessions, with one group designated as “ethanol-dependent” (vapor-exposed, n = 18) and the other as “non-dependent” (air-exposed, n = 12). Ethanol vapor exposure has been demonstrated to be a safe, reliable method to titrate blood alcohol levels (BALs) for the induction of ethanol dependence and has significant construct validity (Gilpin et al., 2008). In this procedure, ethanol exposure adjusted by the experimenter to maintain dependence-inducing BALs without jeopardizing animal health, and BALs were regulated between 150–250 mg%. Tail blood samples were taken and analyzed 1–2x per week for BAL determination as previously described (Gilpin et al., 2008). Animals underwent cycles of 14 h on vapor and 10 h off. During withdrawal, vapor-exposed animals exhibit a host of symptoms related to dependence, including both somatic withdrawal signs (Roberts et al., 2000) and negative motivational symptoms (Schulteis et al., 1995), and show excessive alcohol drinking compared to air-exposed animals at a time when their BALs approach zero (Edwards et al., 2011).

2.3. Intravenous Drug Self-Administration Procedures

After acclimation to the housing environment, rats were implanted with a silastic catheter into the right external jugular vein as described (Orio et al., 2009) and allowed to recover 1 week before baseline paw withdrawal testing. Food and water were available *ad libitum* throughout the study. Self-administration sessions were conducted in an operant conditioning chamber, which was placed in a light- and sound-attenuating cubicle (28 × 26 × 20 cm; Med Associates Inc., St Albans, VT). Rats were allowed to self-administer either cocaine (0.5 mg/kg/infusion, n = 18) or heroin (0.06 mg/kg/infusion, n = 44) in 13 daily, 1-h training sessions over two weeks on a fixed-ratio 1 (FR1) schedule. Following stable acquisition of self-administration, animals were divided into ShA (1 h self-administration for both cocaine and heroin) and LgA (6 h self-administration for cocaine and 12 h self-administration for heroin) groups for subsequent sessions. Twelve hours of access to heroin was used based on previous studies showing that this time is optimal for producing escalation of heroin intake (Vendruscolo et al., 2011).

2.4. Measurement of Mechanosensitivity (Paw Withdrawal Thresholds)

Mechanosensitivity testing was conducted before drug exposure (baseline, BL) and during withdrawal, just prior to subsequent self-administration sessions. Evaluation of paw withdrawal thresholds (PWTs) was performed according to methods described by Chaplan et al. (1994). Briefly, rats were acclimated for 15 min in elevated cages with a wire mesh floor. A series of von Frey filaments were applied perpendicularly to the plantar surface of the hind paw for 3 seconds. A sharp withdrawal of the hind paw indicated a positive response. The stimulus was incrementally increased until a positive response was obtained, then decreased until a negative result was observed in order to determine a pattern of responses to apply to the statistical method of Dixon (1980). The 50% paw withdrawal threshold was determined by the formula $X_f + k\delta$, where X_f = last von Frey filament employed, k = Dixon value corresponding to response pattern, and δ = mean difference between stimuli. Baseline mechanical nociceptive thresholds were similar to those reported for the ages of rats employed in this study (Ririe and Eisenach, 2006).

2.5. Pharmacological Testing of Altered Mechanosensitivity

The CRF₁R antagonist N,N-bis(2-methoxyethyl)-3-(4-methoxy-2-methylphenyl)-2,5-dimethyl-pyrazolo[1,5-a]pyrimidin-7-amine (MPZP) was prepared for systemic administration by first solubilizing it in 1 M HCl (10% final volume). It then was diluted using 25% w/v hydroxypropyl β -cyclodextrin (HBC, Cargill, Cedar Rapids, IA) (80% final volume) and backtitrated with descending concentrations of NaOH (2, 1, 0.1 M) (10% final volume) resulting in a final suspension of 10 mg/ml MPZP in 20% HBC (pH 4.5). Lower concentrations were then prepared by serial dilution with vehicle (20% HBC, pH 4.5). Animals were administered the appropriate dose via a 2 ml/kg injection (0–20 mg MPZP/2 ml vehicle/kg body weight, subcutaneously) in a Latin-square, dose-counterbalanced design 60 minutes before testing. For the 0 mg/kg dose of MPZP, animals were given 2 ml 20% HBC vehicle/kg body weight. Doses and pretreatment times were based on previous studies of CRF₁R antagonist effects on reducing excessive ethanol drinking (Richardson et al., 2008) and antinociception (Ji et al., 2007).

2.6. Statistics

Escalation of cocaine or heroin self-administration was compared across sessions using repeated-measures one-way ANOVA followed by Dunnett's multiple comparison tests. Differences in PWTs between LgA and ShA animals or between ethanol-dependent and non-dependent animals were compared using repeated-measures two-way ANOVA (group × test day or group × MPZP dose) followed by a Bonferroni post hoc test. Differences in

PWTs in LgA or ethanol-dependent animals across tests were analyzed using repeated-measures one-way ANOVA followed by Student-Newman-Keuls multiple comparison tests. Direct comparisons between individual preferred levels of heroin intake during the last session and subsequent individual PWTs were analyzed by Pearson correlation. All statistical analyses were performed with Prism 4.0c.

2.7. Experimental Design

Figure 1 shows a schematic timeline for the experiments conducted in this study. For intravenous drug self-administration studies, paw withdrawal threshold (PWT) tests (indicated by arrows) were conducted before (baseline, BL) and after the initiation of drug exposure. Tests 1 & 2 were conducted during the training phase (1h, ShA conditions for all animals) following sessions six and thirteen, respectively. Animals were divided into ShA and LgA groups at this point (balanced according to drug self-administration levels and extant PWTs), and subsequent mechanosensitivity tests (3 & 4 after post-transition sessions seven and fifteen, respectively) were conducted during this LgA escalation phase. In a separate experiment, rats ($n=17$) were allowed to self-administer heroin under the same schedule, and the effect of CRF₁R antagonism on PWTs was subsequently determined. For ethanol studies, rats were first trained to self-administer alcohol as described in the methods section. Mechanosensitivity tests were conducted at baseline (before ethanol self-administration) and then before and after chronic intermittent ethanol vapor exposure (ethanol-dependent group) or air exposure (non-dependent group). Following ten weeks of vapor exposure, the effect of CRF₁R antagonism on PWTs was subsequently tested in ethanol-dependent and non-dependent animals during weeks eleven and twelve of vapor exposure.

3. Results

3.1. Paw Withdrawal Thresholds in Heroin Self-Administering Animals

Animals trained to self-administer heroin (0.06 mg/kg/infusion) under ShA (1 h) conditions displayed a limited intake (Figure 2A) yet developed a modest reduction in PWTs that was not significantly different from baseline levels (Figure 2B), although it appeared to persist for the duration of the experiment. Animals that transferred to extended access (LgA, 12 h) heroin self-administration displayed an escalation of intake (one-way repeated-measures ANOVA significant effect of session in LgA group, $F_{14, 224} = 10.66$, Figure 2A) that became significant by session eight ($p < 0.01$ – 0.05 , Dunnett's multiple comparison tests). Concomitantly, PWTs in LgA animals were also significantly lower compared to animals remaining on the ShA (1 h) schedule (two-way repeated-measures ANOVA significant main effect of group, $F_{1, 48} = 4.535$, Figure 2B). Heroin LgA animals developed a significant mechanical hypersensitivity by PWT test 3 (i.e., after seven self-administration sessions), and PWTs continued to decline after eight more sessions of LgA heroin self-administration at PWT test 4 (one-way repeated-measures ANOVA significant effect of test day in LgA group, $F_{14, 44} = 4.026$; significantly lower PWTs at PWT 3 vs. BL, $q = 2.903$, $p < 0.05$; significantly lower PWTs at PWT 4 vs. BL, $q = 3.851$, $p < 0.05$; Figure 2B). Overall, these changes in mechanical hypersensitivity appeared to mirror the escalation of heroin intake in LgA rats. In fact, after 15 sessions on the LgA schedule, individual PWTs in heroin-dependent rats at PWT 4 were significantly and negatively correlated with preferred levels of heroin self-administration during the last session (Figure 2C, $r = -0.609$, $p = 0.016$). Given the role of CRF receptor signaling in the escalation of heroin intake (Greenwell et al., 2009) and heroin withdrawal-associated behaviors (Heinrichs et al., 1995; Stinus, et al., 2005) including thermal hyperalgesia in precipitated morphine withdrawal (McNally and Akil, 2002), we next tested the effects of systemic CRF₁R antagonism on altered mechanosensitivity during early (10–12 h) spontaneous withdrawal in LgA animals. In a

separate group of rats ($n=17$), previously lowered PWTs in LgA rats (two-way repeated-measures ANOVA significant main effect of group, $F_{1, 30} = 11.14$) were significantly elevated near levels observed in ShA rats after systemic administration of MPZP (Figure 2D). One-way ANOVA conducted on LgA rat thresholds revealed a significant improvement in mechanical hypersensitivity after administration of the 20 mg/kg MPZP dose that constituted 61.9% of the difference in thresholds between LgA and ShA groups following vehicle treatment (one-way repeated-measures ANOVA significant effect of dose, $F_{2, 26} = 4.186$; significantly higher PWTs after administration of 20 mg/kg MPZP vs. 0 mg/kg, $q = 3.932$, $p < 0.05$).

3.2. Paw Withdrawal Thresholds in Cocaine Self-Administering Animals

LgA cocaine (6 h) self-administering animals displayed a significant intake escalation (one-way repeated-measures ANOVA significant effect of session in LgA group, $F_{14, 134} = 6.892$, Figure 3A) that was apparent by session seven ($p < 0.01$, Dunnett's multiple comparison tests). However, in contrast to LgA heroin self-administering animals, LgA cocaine animals failed to develop altered mechanosensitivity over fifteen sessions of LgA conditions (Figure 3B).

3.2. Paw Withdrawal Thresholds in Ethanol-Dependent and Non-Dependent Animals

We next sought to determine the relationship between the induction and maintenance of ethanol dependence and mechanical hypersensitivity (Figure 4). Animals were initially trained to self-administer alcohol, and subsequently, half of the animals were made dependent via chronic, intermittent ethanol vapor exposure (Gilpin et al., 2008). The time course of weekly BALs in ethanol-dependent animals is presented in Figure 4A. While limited ethanol self-administration (average ethanol intake ranging from 0.54–0.66 g/kg on the last 3 of 12 training sessions) failed to alter PWTs (BL vs. 0-week vapor tests), animals subsequently made dependent on ethanol displayed reduced PWTs by eight weeks of vapor exposure (two-way repeated measures ANOVA significant group $\times$ time interaction, $F_{2, 68} = 3.266$; significant difference at 8-week PWT test by Bonferroni post hoc test, $t = 2.722$; Figure 4B), similar to a previous study utilizing an ethanol liquid diet to induce dependence (Dina et al., 2000). Given the role of stress-related signaling in the development of ethanol withdrawal-related behaviors (Valdez et al., 2002; Valdez et al., 2003; Huang et al., 2010), including altered mechanosensitivity (Dina et al., 2008), we tested the effects of CRF₁R antagonism on compromised thresholds in ethanol-dependent animals. During weeks 11–12 of ethanol vapor exposure, the lowered PWTs in ethanol-dependent rats (two-way repeated-measures ANOVA significant main effect of group, $F_{1, 66} = 21.70$) were significantly elevated by systemic administration of the CRF₁R antagonist MPZP (Figure 4C). One-way ANOVA conducted on ethanol-dependent rat thresholds revealed a significant improvement in mechanical hypersensitivity after administration of the 20 mg/kg MPZP dose that constituted 54.9% of the difference in thresholds between ethanol-dependent and non-dependent groups following vehicle treatment (one-way repeated-measures ANOVA significant effect of dose, $F_{2, 35} = 3.733$; significantly higher PWTs after administration of 20 mg/kg MPZP vs. 0 mg/kg, $q = 3.823$, $p < 0.05$). The efficacy of MPZP in alleviating heroin and alcohol withdrawal-induced mechanosensitivity approximated the efficacy of another CRF₁R antagonist (NBI 27914) in reducing hyperalgesia in an arthritis pain model (Ji et al., 2007).

4. Discussion

4.1. Enhanced Mechanosensitivity as a Negative Motivational Symptom of Dependence

The elucidation of opiate- and alcohol-induced neuroadaptations within brain reward and stress systems has provided valuable insights into potential mechanisms underlying drug

dependence in vulnerable individuals. In these circuits, reward neurotransmitter systems including certain opioid peptides are compromised, while brain stress systems such as CRF signaling are recruited (Koob and Le Moal, 2008). Strong evidence suggests that the neural substrates associated with addiction may also overlap with substrates of emotional aspects of nociceptive processing in areas such as the central amygdala (Neugebauer et al., 2004), cingulate cortex (Vogt, 2005), and nucleus accumbens (Gear and Levine, 1995; Barrot et al., 2002), where ascending pain pathways connect for processing the emotional components of pain perception. Specifically, pain-responsive neurons are abundant in the lateral part of the central amygdala (also known as the “nociceptive amygdala”; Bernard and Besson, 1990). As a potentially powerful negative motivational symptom, pain represents a subjective experience that can have a substantial influence on drug reinforcement, possibly facilitating the transition to drug addiction (Miller and Gold, 2007). Although mechanisms of nociception, stress, and anxiety are difficult to dissociate, pain represents a unique negative affective valence and is often intimately associated with a host of psychiatric disorders (Elman et al., 2011). Moreover, agents that alleviate pain-like states can be rewarding by acting via negative reinforcement mechanisms in animal models (King et al., 2009).

Our results describe significant increases in mechanical hypersensitivity in animal models of opiate and alcohol dependence, suggesting a possible emergence of hyperalgesia that may relate to excessive drug intake in dependent animals. Opiates and alcohol activate similar neuroanatomical and molecular systems (Koob and Bloom, 1988; Herz, 1997; Koob et al., 2003; Siggins et al., 2003; Modesto-Lowe and Fritz, 2005; Gianoulakis, 2009), and also share a close similarity with regard to withdrawal symptomatology (West and Gossop, 1994). In addition, mice that display an alcohol deprivation effect (elevated drinking levels as a consequence of withdrawal) also display greater thermal hyperalgesia during concomitant morphine withdrawal (Salimov et al., 1993). However, there remains a gap in our knowledge regarding how specific negative emotional states that precede or develop during chronic drug use influence compulsive drug-seeking behaviors. A link between hyperalgesia and an enhanced motivation to obtain opiates has been demonstrated in rats (Martin et al., 2007). In animals with reduced paw withdrawal thresholds following spinal nerve ligation, only heroin doses that effectively produced alleviation of hyperalgesia maintained opiate self-administration, whereas lower doses were only effective in maintaining opiate self-administration in control (sham-operated) rats, suggesting that the driving force for the motivation to self-administer drugs in individuals with a sensitized nociceptive system may be in part to seek relief of chronic pain. Nerve-injured rats would seem to require higher doses of heroin to register reward, and are also more sensitive to mu-opioid receptor blockade (Martin et al., 2011).

In our study, paw withdrawal thresholds during heroin withdrawal were significantly and negatively correlated with individual, preferred levels of heroin intake after two weeks of extended access self-administration (Figure 2C). This relationship may represent a direct dose-response between heroin intake and resultant mechanical hypersensitivity, but could also indicate that animals more sensitive to tactile stimulation self-administered more heroin to overcome this condition. In contrast to heroin-dependent animals, we did not detect paw withdrawal threshold changes in either cocaine LgA or ShA rats (Figure 3). Cocaine is an analgesic (Altier and Stewart, 1999; Pamplona et al., 2007) that activates both opponent motivational processes (Ettenberg, 2004) and endogenous opioid systems (Kreek, 1996; Roth-Deri et al., 2003; Boutrel, 2008), although endogenous opioid signaling may play a more direct role in incentive sensitization mechanisms related to cocaine-seeking behaviors (Self, 2004; Simmons and Self, 2009). A recent study found that induction of a chronic pain-like state induced by spinal nerve ligation was able to block morphine-induced, but not cocaine-induced, potentiation of rewarding electrical brain stimulation (Ewan and Martin,

2011), suggesting a lack of association between exaggerated nociception and cocaine reward in line with results from the present study.

In accordance with prior reports (e.g., Dina et al., 2000), protracted exposure to dependence-inducing ethanol concentrations resulted in a lowering of nociceptive thresholds (Figure 4B). Dina and colleagues have further characterized the relationship between ethanol exposure patterns and the establishment of neuropathy-like conditions (Dina et al., 2006; Dina et al., 2007). The results of these studies indicate that enhanced mechanosensitivity appears more rapidly by increasing the withdrawal periods between alcohol exposures in what the authors considered a model of binge-like drinking. They also discovered that the hypersensitivity persists or is even exacerbated during withdrawal, consistent with human data (e.g., Yokoyama et al., 1991). Finally, Dina et al. (2008) implicated glucocorticoid receptor signaling in the development of alcohol withdrawal-induced reduction of thresholds. The present study extends these findings by implicating another critical component of stress-related signaling, the CRF₁ receptor, in the maintenance of enhanced mechanosensitivity in ethanol-dependent animals. Indeed, it is well documented that glucocorticoids can sensitize extra-hypothalamic CRF systems (Imaki et al., 1991; Makino et al., 1994; Shepard et al., 2000), although future studies are required to delineate central vs. peripheral actions of CRF signaling on drug-induced mechanical hypersensitivity.

4.2. Corticotropin-Releasing Factor, Pain, and Drug Dependence

The role of CRF in pain signaling is complex, and CRF typically interacts with opioid peptide signaling at both central and peripheral nervous system components (Mousa et al., 2007). In the periphery, CRF, potentially acting via both CRF₁ and CRF₂ receptors (Mousa et al., 2003), can also directly activate immune cells that have accumulated at injured peripheral nerves to produce antinociception via opioid-dependent mechanisms (Schafer et al., 1997; Labuz et al., 2006; Labuz et al., 2010). Early studies also described an antinociceptive role of CRF in the context of stress-induced analgesia (for review, see Lariviere and Melzack, 2000), although CRF produced hyperalgesic effects in models of visceral hypersensitivity (Tache et al., 2005). More recent studies have attempted to reconcile these differences, with significant progress. For example, Lariviere et al. (2011) revealed differential effects of central CRF on distinct behavioral responses in the formalin test. Additionally, with the development of more selective pharmacological tools for distinguishing specific CRF receptor-subtype function, recent work suggests that the central anti-nociceptive effects of CRF are most likely mediated through CRF₂ receptors (Ji and Neugebauer, 2008). In contrast, CRF₁ receptors mediate the pro-nociceptive effects of this peptide, and this relationship is mediated at least partly via the central amygdala (Ji and Neugebauer, 2007; Fu and Neugebauer, 2008). CRF₁ receptors also mediate pain-related anxiety-like behavior (Ji et al., 2007). At the intracellular level, several recent studies have delineated a critical role for amygdala extracellular signal-regulated kinase (ERK) signaling (Carrasquillo and Gereau, 2007; Fu et al., 2008) in pain-associated synaptic plasticity and behavior. ERK phosphorylation in the amygdala is increased during withdrawal from either escalated heroin self-administration (Edwards et al., 2009) or after ethanol vapor-induced dependence induction (Sanna et al., 2002), while selective CRF₁R antagonism reduces both ethanol (Richardson et al., 2008) and heroin self-administration (Greenwell et al., 2009) in dependent animals. Thus, enhanced CRF₁R-ERK signaling in the amygdala represents one possible molecular node of intersection between pain-related negative affect and drug addiction.

The antinociceptive effects of CRF₁R antagonists have been demonstrated across several pain models, although this class of drugs does not alter various pain-related indices (e.g., audible or ultrasonic vocalizations, paw withdrawal thresholds) in non-injured animals (e.g., Fu and Neugebauer, 2008). Similarly, CRF₂R antagonism does not alter pain-related

behavior in non-dependent animals in the tail flick test (McNally and Akil, 2002) or in any non-dependent model in terms of paw withdrawal thresholds in the present study. Since mechanical hypersensitivity is produced by chronic drug exposure (in the case of opiates and alcohol) and since we hypothesize that this state drives renewed drug intake as a negative reinforcement mechanism, we believe that CRF1R antagonism represents a viable therapeutic strategy targeting this condition.

In the present study, mechanical hypersensitivity was observed during spontaneous heroin withdrawal (i.e., without opioid receptor antagonist administration), a condition hypothesized to be associated with a sensitization of pronociceptive systems following chronic heroin exposure (Celerier et al., 2001). In comparison to opiate-induced nociceptive sensitization via descending facilitation following continuous drug exposure (e.g., Vanderah et al., 2001; Ossipov et al., 2004) we feel that our observations better model an opiate withdrawal-induced state of hypersensitivity (Gutstein, 1996) whereby neurotransmitter signaling mechanisms opponent to mu-opioid receptor stimulation (including CRF signaling) are recruited and subsequently unmasked on an intermittent (daily) basis during withdrawal selectively in LgA animals. Based on our daily observations, in addition to reduced paw withdrawal thresholds, heroin LgA animals readily exhibit a host of spontaneous physical withdrawal symptoms prior to self-administration including excessive grooming, diarrhea, and vocalization upon handling (Vendruscolo et al., 2011). These symptoms emerge in parallel with the manifestation of brain reward deficits (Kenny et al., 2006) and enhanced heroin-seeking behavior (Lenoir and Ahmed, 2007) observed during withdrawal in rats given extended access to heroin self-administration. Attempts to alleviate such physical and/or motivational withdrawal states may underlie compulsive heroin seeking, while tolerance to the antinociceptive and other positive rewarding effects of opiates may in part drive the need to escalate heroin intake across days in LgA rats.

A role for amygdala CRF receptor signaling in regulating thermal hyperalgesia following precipitated opiate withdrawal in morphine-dependent animals was previously demonstrated by McNally and Akil (2002). In this study, rats were made dependent via morphine pellet and withdrawal was precipitated by naloxone. Under these conditions, dependent rats exhibited a reduced tail flick latency compared to sham-pelleted, naltrexone-treated animals, and this thermal hyperalgesia was alleviated by microinjection of the non-selective CRF receptor antagonist alpha helical CRF₉₋₄₁ into the central amygdala (but not the bed nucleus of the stria terminalis). Thus, CRF would appear to play a role in the expression of both mechanical and thermal hypersensitivity during opiate dependence. Importantly, administration of a CRF receptor antagonist into the CeA also reduces the reward associated with negative reinforcement during morphine withdrawal (Heinrichs et al., 1995), further suggesting possible links among negative affect, hyperalgesia, and CRF signaling after chronic opiate exposure.

One remaining unexplored issue is how extended periods of withdrawal influence paw withdrawal thresholds. Ethanol-dependent rats display heightened anxiety-like behavior up to four weeks into withdrawal (Valdez et al., 2002). The ability of naltrexone to precipitate hyperalgesia in heroin-exposed animals is also maintained for up to 25 days following discontinuation of heroin (Celerier et al., 2001). Ren et al. (2009) found that a hyperalgesic state can persist for up to five months in abstinent opiate addicts, while addicts with more pain sensitivity also displayed greater cue-induced craving at this time point. Further investigation of the effects of persistent pain-like states in the context of long-term self-administration and drug-seeking behaviors is warranted for the benefit of both pain and addiction fields (Barrot, 2011).

5. Conclusions

This study revealed a differential ability of three common drugs of abuse to alter paw withdrawal thresholds, with chronic heroin and alcohol (but not cocaine) producing a significant mechanical hypersensitivity that could represent part of a negative emotional state associated with excessive drug exposure. Pharmacological blockade of CRF₁ receptors partially alleviated this hypersensitivity, which may in part explain the efficacy of CRF₁R antagonists in reducing excessive heroin and ethanol self-administration in dependent animals. Since a magnified CRF₁ receptor signaling appears to be linked to chronic pain-related mechanisms and elevated heroin and alcohol intake, CRF₁R antagonism, by attenuating the negative motivational effects of mechanical hypersensitivity during drug withdrawal, may represent a viable therapeutic strategy for ethanol- and opiate-dependent individuals, particularly those suffering from hyperalgesia.

Highlights

1. Mechanical hypersensitivity is observed after escalation of heroin intake.
2. Mechanical sensitivity is unaltered after escalated cocaine self-administration.
3. Mechanical hypersensitivity is also observed in rats made dependent on ethanol.
4. Heroin- and ethanol-induced hypersensitivity are alleviated by CRF₁R antagonism.

Abbreviations

BAL	blood alcohol level
CRF	corticotropin-releasing factor
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, fourth edition
FR1	fixed ratio 1 schedule of reinforcement
HBC	hydroxypropyl β -cyclodextrin
MPZP	N,N-bis(2-methoxyethyl)-3-(4-methoxy-2-methylphenyl)-2,5-dimethylpyrazolo[1,5-a]pyrimidin-7-amine, CRF ₁ R antagonist
PWT	paw withdrawal threshold

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Figure 1.

Experimental timelines. (A) For heroin and cocaine self-administration studies, repeated paw withdrawal threshold (PWT) tests (indicated by arrows) were conducted before (baseline, BL) and during 1 h ShA training sessions. Subsequently, animals were divided into ShA and LgA groups, and PWT tests were conducted over this escalation phase. In a separate group of heroin self-administering animals, the effect of the CRF₁R antagonist MPZP on mechanosensitivity was determined post-escalation. (B) For the alcohol study, animals were tested before (baseline, BL) and after ethanol self-administration (0 weeks vapor exposure), and following 4 and 8 weeks of vapor exposure. Subsequently, the effect of CRF₁R antagonism on mechanosensitivity was determined over weeks 11–12 of vapor exposure.

Figure 2.

Heroin self-administration and paw withdrawal thresholds in ShA and LgA animals. (A) Animals acquired heroin self-administration ($n = 27$) and upon transition to LgA conditions (12 h access, $n = 15$) significantly increased heroin intake. Asterisks indicate $**p < 0.01$ or $*p < 0.05$ significantly higher intake in LgA animals versus session one. (B) Paw withdrawal threshold (PWT) tests conducted during withdrawal revealed a development of mechanical hypersensitivity selectively in LgA animals following 7 and 15 sessions of extended access conditions (PWT tests 3 & 4, respectively). Pound sign indicates $\#p < 0.05$ significantly lower PWTs in LgA vs. ShA animals (main effect of group). Asterisk indicates $*p < 0.05$ significantly lower PWTs in LgA animals versus baseline (BL) test. (C) Individual paw withdrawal thresholds at PWT 4 were significantly and negatively correlated with individual preferred levels of heroin intake during session 15 in LgA animals ($r = -0.609$, $p = 0.016$). (D) In another group of escalated LgA animals ($n = 9$) that displayed a significant reduction in paw withdrawal thresholds vs. ShA animals ($n = 8$), the CRF₁R antagonist MPZP significantly raised PWTs near levels measured in the ShA group, corresponding to a putative alleviation of heroin withdrawal-induced mechanical hypersensitivity. Pound signs indicate $\#\#p < 0.01$ significantly lower PWTs in LgA vs. ShA animals (main effect of group). Asterisks indicate $*p < 0.05$ significant elevation of PWTs in LgA animals.

Figure 3.

Cocaine self-administration and paw withdrawal thresholds in ShA and LgA animals. (A) Animals acquired cocaine self-administration ($n = 18$) and upon transition to LgA conditions (6 h access, $n = 9$) significantly increased cocaine intake. Asterisks indicate $**p < 0.01$ significantly higher intake in LgA animals versus session one (B) Paw withdrawal threshold (PWT) tests conducted during withdrawal did not significantly change over the course of cocaine self-administration under either ShA or LgA conditions.

Figure 4.

Ethanol vapor exposure and paw withdrawal thresholds in ethanol-dependent and non-dependent animals. (A) Blood alcohol levels (BALs) of ethanol-dependent animals ($n = 18$) over the course of the experiment. (B) Paw withdrawal thresholds were not altered by ethanol self-administration (baseline test vs. 0-week vapor exposure test). Following eight weeks of ethanol vapor exposure, paw withdrawal thresholds in ethanol-dependent animals ($n = 18$) were significantly reduced compared to non-dependent animals ($n = 18$). Asterisk indicates $*p < 0.05$ significantly lower thresholds in ethanol-dependent vs. non-dependent animals at the eight week test. (C) During weeks 11–12 of ethanol vapor exposure, CRF₁R antagonism with MPZP significantly elevated the compromised paw withdrawal thresholds in ethanol-dependent animals ($n = 12$) near levels measured in the non-dependent group ($n = 12$), representing a partial alleviation of ethanol withdrawal-induced mechanical hypersensitivity. Pound signs indicate $###p < 0.001$ significantly lower paw withdrawal thresholds in ethanol-dependent vs. non-dependent animals (main effect of group). Asterisk indicates $*p < 0.05$ significant elevation of thresholds in ethanol-dependent animals.