Inflated reward value in early opiate withdrawal

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ABSTRACT

Through incentive learning, the emotional experience of a reward in a relevant need state (e.g. hunger for food) sets the incentive value that guides the performance of actions that earn that reward when the need state is encountered again. Opiate withdrawal has been proposed as a need state in which, through experience, opiate value can be increased, resulting in escalated opiate self-administration. Endogenous opioid transmission plays anatomically dissociable roles in the positive emotional experience of reward consumption and incentive learning. We, therefore, sought to determine if chronic opiate exposure and withdrawal produces a disruption in the fundamental incentive learning process such that reward seeking, even for non-opiate rewards, can become maladaptive, inconsistent with the emotional experience of reward consumption and irrespective of need. Rats trained to earn sucrose or water on a reward-seeking chain were treated with morphine (10–30 mg/kg, s.c.) daily for 11 days prior to testing in withdrawal. Opiate-withdrawn rats showed elevated reward-seeking actions, but only after they experienced the reward in withdrawal, an effect that was strongest in early (1–3 days), as opposed to late (14–16 days), withdrawal. This was sufficient to overcome a negative reward value change induced by sucrose experience in satiety and, in certain circumstances, was inconsistent with the emotional experience of reward consumption. Lastly, we found that early opiate withdrawal-induced inflation of reward value was blocked by inactivation of basolateral amygdala mu opioid receptors. These data suggest that in early opiate withdrawal, the incentive learning process is disrupted, resulting in maladaptive reward seeking.

Keywords  Chronic morphine, incentive learning, instrumental conditioning, opiate withdrawal, reward.

INTRODUCTION

Prescription opiates are highly efficacious for pain management, but carry a high abuse liability (Savage 2003; Fields 2011) and their use may lead to a higher propensity for non-narcotic substance abuse (Michna et al. 2004). Chronic opiate exposure is also associated with a preference for sugary foods and consequent weight gain (Nolan & Scagnelli 2007; Mysels & Sullivan 2010). Opiate abuse itself is characterized by compulsive drug seeking despite a decline in the experienced ‘high’ and often in the face of severe negative consequences. Here, we evaluate a potential common psychological process that may be disrupted by chronic opiate exposure to result in these aberrant behaviors.

Opiate withdrawal is characterized by severe physical aversive symptoms and anhedonia (Hand et al. 1988; Koob, Maldonado & Stinus 1992; Stinus, Caille & Koob 2000) and has been proposed to contribute to the compulsive nature of opiate seeking via a negative reinforcement process (Koob et al. 1989; Koob 1996; Koob & Le Moal 2005). Disruption of positive incentive processes may, however, also play a role. Experiencing a reward (either earned or non-contingently) in a relevant motivational/need state sets—the process referred to as instrumental incentive learning—the incentive value that is recalled when making a decision to engage in an instrumental action to earn that specific reward when in that same state in the future (Balleine 1992; Dickinson & Balleine 1994). It is well established that opiate self-administration in rodents can be increased during opiate withdrawal (Wikler & Pescor 1967; Goldberg, Woods & Schuster 1969; Kenny et al. 2006). Interestingly, experience with opiate in this withdrawal state can enhance opiate-seeking behavior, an effect that occurs independent of an opportunity to learn
that the drug-seeking action will lead to alleviation of withdrawal (i.e. negative reinforcement) (Hutcheson et al. 2001). This suggests that the opiate is more valued in the withdrawn state, thereby acting as a greater incentive for drug seeking. Because a reward’s incentive value is normally tightly linked to the recent emotional experience derived from its consumption (Cabanac 1992; Damasio 1996; Balleine 2001), it is possible that the elevated opiate seeking observed during opiate withdrawal is a secondary consequence of a boost in the emotional experience produced by opiate consumption. However, the observation that opiate addicts desire the drug more than would be expected from self-report of the pleasure derived from its consumption (Lamb et al. 1991) suggests another potential interpretation. That is, that chronic opiate administration and withdrawal disrupts the fundamental incentive learning process, whereby the reward experience is *translated* into the incentive value used to guide future behavior, such that the latter is inflated out of proportion to the former. This account predicts that incentive learning for natural rewards, such as food and water, would also be impacted by opiate treatment and withdrawal.

In support of this account, recent work in rodents has shown that the emotional experience of sucrose consumption, and the updating of the incentive value of that reward (information encoded in the reward representation used to guide reward-seeking actions), were found to rely on anatomically dissociable opioid-dependent neural processes (Wassum et al. 2009). While the former involved endogenous opioid transmission in the nucleus accumbens shell and ventral pallidum, the latter required mu opioid receptor activation in the basolateral amygdala (BLA) (Wassum et al. 2009, 2011a). Chronic opiate administration dysregulates endogenous opioid signaling throughout the brain, including the BLA (Brady et al. 1989). It is possible, therefore, that plasticity in endogenous opioid transmission induced by chronic opiate administration and withdrawal increases the gain on the BLA mu opioid receptor-dependent incentive learning process through which the emotional experience of reward is translated into incentive value, such that rewards generally, not just opiates, experienced in the opiate-withdrawn state become over-valued, out of line with the emotional experience induced by their consumption. In support of this idea, food-motivated behavior is reportedly enhanced following chronic opiate exposure under some circumstances (Babbini, Gaiardi & Bartoletti 1976; Ford & Balster 1976; Ranaldi et al. 2009; Cooper, Shi & Woods 2010).

We, therefore, assessed the impact of chronic opiate exposure and withdrawal on food- and water-seeking actions and palatability in a rodent incentive learning procedure. Using such an approach, we sought to determine the following: (1) Can the opiate-withdrawn state support a shift in the incentive value of non-opiate rewards, such as sucrose or water? (2) If there is such a generalized effect of opiate withdrawal on reward seeking, is it consistent or discordant with the emotional experience observed during reward consumption? (3) If opiate withdrawal inflates reward value, does this effect have the potential to overshadow a negative reward experience, thereby producing ‘compulsive’ behavior? (4) Does this withdrawal-induced reward inflation depend on BLA opioid transmission?

**MATERIALS AND METHODS**

**General approach**

The goal of these experiments was to evaluate if and how withdrawal from chronic opiate exposure alters the incentive learning process to disrupt subsequent value-driven reward-seeking actions. We assessed the effects of opiate withdrawal on value-driven reward seeking, palatability and goal approach for a sucrose (experiments 1, 2 and 4) or water (experiment 3) reward.

Each experiment followed the same general structure. Rats were trained on a 2-lever sequence of actions to earn a sucrose solution (experiments 1, 2 and 4) or water (experiment 3) reward wherein lever pressing on a ‘seeking’ lever to the left of the magazine introduces a second ‘delivery’ lever to the right of the magazine, pressing of which results in reward delivery to the magazine. The initial lever-press action in the sequence has been demonstrated to be selectively sensitive to changes in the learned incentive value of the earned reward and relatively immune to the general activational effects of motivational state (e.g. hunger or thirst) and of reward-paired cues (Balleine et al. 1995; Corbit & Balleine 2003; Balleine, Paredes-Olay & Dickinson 2005; Wassum et al. 2009, 2011a). Following training to stable response rates (see Supporting Information Appendix S1), rats were split into two groups: one given once-daily administration of morphine for 11 days (10–30 mg/kg, s.c.) on an administration protocol known to induce opiate dependence (Harvey-Lewis, Perdrizet & Franklin 2012) and one given saline vehicle control injections. Following drug treatment, rats were tested in early (1–3 days) or late (14–16 days; experiment 1) or only in early (experiments 2–4) withdrawal for their reward-seeking performance on the chain of actions, first under non-rewarded conditions, to assess any general effect of chronic opiate exposure and the withdrawal state on reward seeking. Rats were then given an incentive learning opportunity wherein they were non-contingently re-exposed to the food or water reward in the opiate-withdrawn state. The effects of this incentive learning opportunity were then...
tested in a second, post-re-exposure test of reward seeking conducted in the absence of reward. Importantly, to prevent negative reinforcement from contributing to the results, rats were never given an opportunity to learn that their reward-seeking lever-press actions would lead to any potential alleviation of the negative withdrawal symptoms that might be conferred by sucrose or water experience in this state.

During the non-contingent sucrose/water re-exposure, we evaluated the palatability responses elicited during consumption using a contact lickometer (see below for full description), a measure previously reported to provide a similar assessment of palatability to the commonly used taste reactivity measures that are often termed reward ‘liking’ (Davis & Smith 1988, 1992; Berridge 1991; Davis & Perez 1993). This allowed us to assess whether chronic opiate exposure and withdrawal induced reward seeking that was discordant with the emotional impact of the reward. During this session, we also evaluated the effects of opiate withdrawal on goal approach behavior signaled by the contextual or sucrose pump cues, measured as magazine entries with a photobeam detector. This procedure, therefore, allowed for within-subject measures of the effects of opiate withdrawal on reward palatability, goal approach behavior and value-driven reward-seeking actions.

**Subjects**

Male, Long Evans rats (280–300 g at the outset of the study; experiment 1: early withdrawal group n = 15, 8 vehicle-treated, 7 morphine-treated; late withdrawal group n = 15, 8 vehicle-treated, 7 morphine-treated; experiment 2: n = 24, 12 vehicle-treated, 12 morphine-treated; experiment 3: n = 15, 8 vehicle-treated, 7 morphine-treated; experiment 4: n = 36, 11 in group vehicle-treated, intra-BLA vehicle, 8 vehicle-treated, intra-BLA CTOP, 8 morphine-treated, intra-BLA vehicle, 9 morphine-treated, intra-BLA CTOP: Charles River Laboratories, Wilmington, MA, USA) were group housed and handled daily prior to training for 5–7 days. Rats were maintained on a food- or water-restriction schedule whereby they were either deprived of food for 4 hours (experiments 1 and 4) or 22 hours (experiment 2), or water-deprived for 18 hours (experiment 3) prior to each day’s training or testing session (see each experiment description below). Food or water was returned 2–4 hours after each daily training session. Unless it was restricted as stated, rats were provided free access to food or filtered tap water in the home cage. All procedures were conducted in accordance with the National Research Council’s Guide for the Care and Use of Laboratory Animals and were approved by the UCLA Institutional Animal Care and Use Committee. Training and testing took place during the dark phase of a 12:12 hour reverse dark: light cycle in 16 Med Associates (St. Albans, VT, USA) operant chambers described in the Supporting Information Appendix S1.

**Chronic morphine treatment**

Following training as described previously (Wassum et al. 2009, 2011a,b) and in the Supporting Information Appendix S1, rats underwent chronic drug treatment. The food/water deprivation schedule of training was maintained during drug treatment. Drug injections were conducted in a room different from that of all behavioral training and testing to avoid any effects of a morphine-paired context on test performance. Morphine (generously provided by NIDA) was dissolved in sterile saline vehicle and injected at a volume of 1 ml/kg. Half of the rats in each group were administered morphine once daily on an escalating dose regime: 2 days of 10 mg/kg, 2 days of 20 mg/kg, 1 day of 25 mg/kg, 6 days of 30 mg/kg, s.c. The remaining rats were administered sterile saline vehicle (1 ml/kg, s.c.). This dosing regime has been previously shown to result in physical dependence marked by tolerance to morphine analgesia and enhancement of naloxone precipitated withdrawal, as well as altered reward seeking in a delay-discounting task (Harvey-Lewis et al. 2012). Moreover, we show evidence of withdrawal in these rats marked by a significant loss in body weight (Martin et al. 1963) compared to the last day of drug exposure and to vehicle-treated controls (see Supporting Information Fig. S1). A passive drug administration procedure was chosen so that solely the pharmacological effects of the drug treatment and withdrawal from it could be assessed without being confounded with previous drug-seeking experience. Drug groups were counterbalanced based on seeking lever-press rate on the last day of training. All behavioral training (pre-drug) and testing (opiate withdrawal) was conducted drug free.

**Experiment 1**

The training and testing procedures for experiment 1 are outlined in Fig. 1. Rats were maintained 4 hours food deprived throughout training and testing to avoid ceiling effects on lever pressing and sucrose incentive value. Following training and chronic morphine treatment, rats were tested either 1–3 days (early withdrawal group) or 14–16 days (late withdrawal group) after the last drug injection. For both groups, testing was identical and began with a test (test 1a) of responding on the instrumental chain under non-rewarded conditions for 5 minutes. This non-rewarded test was conducted just as in training, with rats responding on the seeking lever on random ratio (RR) 4 schedule to receive the second delivery lever, which was retracted once pressed, but no reward was delivered allowing evaluation of the immediate (i.e. pre-exposure) impact event.
of opiate withdrawal on reward seeking. The next day, rats were given non-contingent re-exposure to the sucrose training outcome (30 exposures/35 minutes) in the operant box with the levers retracted. Lickometer measures were collected during this phase of the experiment to assess sucrose palatability. These non-contingent sucrose deliveries provide an incentive learning opportunity wherein the value of the sucrose reward may be updated in the new opiate-withdrawn state. The day following re-exposure, rats were tested again (test 1b) for their responding on the chain under non-rewarded conditions for 5 minutes in order to assess the effects of the previous day’s incentive learning opportunity on reward-seeking actions.

Experiment 2

The training and testing procedures for experiment 2 are outlined in Fig. 2 and were similar to experiment 1, with the exception that rats were maintained 22 hours food deprived throughout the training and were tested in early withdrawal 1 hour food deprived to assess the effects of a downshift in need state on reward seeking. In this experiment, the sucrose re-exposure provided rats the opportunity to experience, for the first time, the sucrose while sated; a negative incentive learning opportunity wherein the rats learn that when sated the sucrose is less valuable (Balleine 1992; Dickinson & Balleine 1994; Wassum et al. 2011a). The effect of this negative incentive value change on sucrose seeking was
Figure 3 Experiment 3: In early opiate withdrawal the value and subsequent seeking actions for a water reward are inflated. Table: Experiment 3 Design. A. Effect of early opiate withdrawal on water seeking (normalized to pre-drug baseline reward-seeking response rate) prior to (Test 1a- open bars) and after (Test 1b- shaded bars) an opportunity to experience the water training outcome in the opiate withdrawn state. B. Effect of early opiate withdrawal on water palatability, assessed as lick frequency, during the non-contingent water re-exposure. C. Effect of early opiate withdrawal on goal approach (normalized to pre-drug baseline entry rate) during the non-contingent water re-exposure. D. Effect of early opiate withdrawal on instrumental reward seeking (normalized to pre-drug baseline entry rate) during the non-contingent water re-exposure.

Experiment 3

The training and testing procedures for experiment 3, in which we tested the effects of opiate withdrawal on the palatability, goal approach and reward seeking for a water reward, are outlined in Fig. 3. Rats were maintained 18 hours water deprived with full access to food throughout the training and testing, and testing was conducted entirely in early withdrawal. Training and testing were otherwise identical to experiment 1. In addition to weight, food and water consumption were monitored in this experiment (see Supporting Information Fig. S2).

evaluated by comparing reward-seeking response rate prior to (test 1a) and after (test 1b) the re-exposure. Rats were given both a reward-seeking (test 2a) and re-exposure test 22 hours food deprived to serve as a control. The order of testing was not counterbalanced to allow for the critical comparison between the sated test in this experiment and the effects seen in early withdrawal in experiment 1.

Experiment 4

The training and testing procedures for experiment 4 were largely similar to experiment 1 and are shown in Fig. 4. Following training, rats underwent surgery for implantation of guide cannula targeted above the BLA (see below). Rats were single-housed following surgery. After a 5-day recovery period, rats were retrained for 2 days prior to the start of morphine treatment as described earlier. Testing was conducted in early withdrawal and was identical to experiment 1 with the exception that rats received an infusion of either the selective mu opioid receptor antagonist, CTOP (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH₂; 1 μg/0.5 μl/side—see Supporting Information Appendix S1), or sterile water vehicle, into the BLA immediately prior to non-contingent re-exposure to sucrose. There were four groups, counter-balanced based on seeking lever-press rate on the last day of instrumental training, as follows: chronic morphine-treated intra-BLA vehicle; chronic morphine-treated intra-BLA CTOP; chronic vehicle-treated
intra-BLA vehicle and chronic vehicle-treated intra-BLA CTOP. Sucrose was used as the training outcome, as in experiments 1 and 3, to allow for absolute control over reward exposure, which was critical to assess the effects of intra-BLA CTOP on incentive learning.

**Surgery**

Standard stereotaxic procedures were used for implantation of bilateral guide cannulae (Wassum et al. 2009, 2011a). Rats were anesthetized with isoflurane (4–5 percent induction, 1–2 percent maintenance) and implanted bilaterally with 22 gauge stainless steel guide cannulae (Plastics One, Roanoke, VA, USA) 1 mm above the intended BLA injection site (coordinates from bregma and the skull surface: anterior-posterior, −3.0; medial-lateral, ±5.1; ventral, 8.0 mm).

**Histological analysis**

Histology was conducted as described previously (Wassum et al. 2009). See Supporting Information Fig. S5.

**Palatability analysis**

The sucrose solution or water was delivered into a custom-built electrically isolated magazine through a stainless steel tube. A lickometer circuit (Med Associates), connecting the grid floor of the box and the stainless steel tubes, with the circuit closed by the rats’ tongue, allowed recording of individual lick events. Lickometer measures were amplified and fed through an interface to a PC programmed to record the time of each lick to the nearest 1 millisecond. Based on previous reports (Davis & Smith 1992; Kaplan, Roitman & Grill 1995; Baird et al. 2006; Thornton-Jones et al. 2007; Wassum et al. 2009, 2011b), we used licking frequency as a measure of sucrose or water palatability. This measure of licking microstructure during consumption provides a similar analysis of palatability changes as those assessing taste reactivity following oral infusions (Davis & Perez 1993).

**Data analysis**

In order to control for pre-test response variability and allow comparison across tests conducted in different deprivation states (see Wassum et al. 2009, 2011a,b), lever pressing and magazine entry data are presented as a percentage of pre-drug baseline response rate, with the baseline as the rate of performance (seeking lever press or head entry rate) during the last training session before drug treatment and test. For all hypothesis tests, the α level for significance was set to $P < 0.05$. Data were analyzed with $t$-tests and ANOVAs as described below using SPSS (IBM Corp, Armonk, NY, USA), GraphPad Prism (La Jolla, CA, USA) and Excel. Bonferroni post hoc analyses correcting for multiple comparisons were used to clarify main effects and interactions.

**RESULTS**

**Experiment 1: Early opiate withdrawal disrupts incentive learning to elevate value-driven reward seeking**

Experiment 1 was designed to test the hypothesis that chronic opiate administration and withdrawal disrupts the fundamental incentive learning process, whereby the reward experience is translated into the incentive value used to guide future behavior. As illustrated in Fig. 1, rats were trained 4 hours food deprived on a seeking-delivery chain of lever-pressing actions to earn sucrose. All rats acquired the action chain and, at the end of training, prior to drug treatment and testing, performed the instrumental reward-seeking response at similar rates. For the group tested in early withdrawal, the seeking response rate was 9.94 seeking presses/minute for the future vehicle-treated group (SEM = 0.93) and 9.64 seeking presses/minute (SEM = 1.19; $t_{11} = 0.21$, $P = 0.84$) for the future morphine-treated group. For those tested in late withdrawal, the seeking response rate was 9.53 seeking presses/minute for the future vehicle-treated group (SEM = 0.49) and 9.17 seeking presses/minute (SEM = 0.99; $t_{11} = 0.34$, $P = 0.74$) for the future morphine-treated group. Testing was conducted drug free (i.e. in withdrawal; Fig. 1) and commenced 24 hours after the last drug injection for the early withdrawal group and 14 days after the last injection for the late withdrawal group. Rats were tested for the effects of opiate withdrawal on reward seeking both prior to and after an incentive learning opportunity (non-contingent re-exposure to sucrose in the opiate withdrawn state).

As observed in Fig. 1a, there was no significant difference in reward-seeking response rate between the vehicle- and morphine-treated rats during the pre-exposure reward-seeking test in early withdrawal, but there is a readily apparent difference in reward seeking during the second post-exposure test due to an increase in reward seeking in the morphine-treated rats after sucrose exposure in early withdrawal, an effect that was absent in vehicle-treated rats. These observations were confirmed by ANOVA, which indicated a significant main effect of Exposure ($F_{1,11} = 6.32$, $P = 0.03$), no significant main effect of Drug treatment ($F_{1,11} = 1.11$, $P = 0.31$), but a significant Drug × Exposure interaction ($F_{1,11} = 5.47$, $P = 0.04$). Post hoc analyses, controlling for multiple comparisons, clarify this interaction to reveal that there was no significant difference in seeking rate between the vehicle- and morphine-treated rats during the first...
pre-exposure test of reward seeking (test 1a, \( P > 0.05 \)), but that there was a significant difference in reward-seeking rate during the second post-exposure reward-seeking test (test 1b, \( P < 0.05 \)). Indeed, rats in early opiate withdrawal showed a significant increase in their seeking response rate after exposure to the sucrose training outcome in the withdrawn state (\( P < 0.05 \)), while control, vehicle-treated rats showed no significant change (\( P > 0.05 \)). These data suggest that early opiate withdrawal has no general effect on reward seeking prior to an opportunity for incentive learning, but after such an opportunity, reward-seeking actions are increased, relative to controls. Analysis of these data taking body weight into consideration is provided in the Supporting Information Appendix S2 and Fig. S2.

Incentive value is normally related to, although can be dissociated from, the emotional experience of reward consumption (Wassum et al. 2009), which we assayed here with a lick frequency measure (Berridge & Kringelbach 2008; Wassum et al. 2009) during sucrose re-exposure. Rats in opiate withdrawal displayed significantly higher sucrose lick rates than those treated with vehicle \((t_{11} = 2.53, P = 0.03;\) Fig. 1b), suggesting that the elevation in reward seeking was concomitant with an increase in sucrose palatability. No significant effect of Drug treatment on goal approach was detected \((t_{11} = 1.57, P = 0.14;\) Fig. 1c), suggesting that, as expected, the effects of early withdrawal following chronic opiate exposure were limited to instrumental actions.

These data suggest that in acute opiate withdrawal, the value of a sucrose reward can be inflated upon experience in the withdrawn state driving elevated reward-seeking actions. In an additional experiment (see Supporting Information Appendix S1 & S2 and Fig. S3), we evaluated the persistence of this effect into late withdrawal. Here, we find that the value encoded during sucrose experience in early withdrawal continues to influence reward seeking 14 days into withdrawal—a timepoint at which the palatability of the sucrose is no longer elevated above controls (Supporting Information Fig. S3). To fully evaluate this ‘late withdrawal’ stage, a second group of rats in experiment 1 was tested beginning 14 days after the last drug injection (Fig. 1d–f). As shown in Fig. 1d, there was no apparent difference in reward-seeking rate between vehicle- and morphine-treated groups in either the pre- or post-exposure test conducted in late withdrawal. This was confirmed by ANOVA, which showed no main effect of Exposure \((F_{1,11} = 0.28, P = 0.61)\) or Drug \((F_{1,11} = 2.27, P = 0.16)\), and no significant interaction between these factors \((F_{1,11} = 1.86, P = 0.20)\). These data seem to suggest that the late opiate withdrawal state is not effective in altering instrumental incentive learning. Interestingly, however, time course analysis shows a significant a three-way interaction between Exposure, Time in test and Drug treatment \((F_{4,12} = 2.47, P = 0.05)\) (see Supporting Information Appendix S2 for full analysis), providing a hint that the late withdrawal state may be effective in altering instrumental incentive learning. Based on this analysis, we next re-examined the data including only the first two minutes of the reward-seeking tests (Fig. 1d, inset), a period in which we have previously shown incentive learning effects to be largest (Wassum et al. 2011b).

Analysis of these data still shows no overall main effect of Drug \((F_{1,11} = 0.88, P = 0.37)\), but does reveal a significant effect of Exposure \((F_{1,11} = 4.90, P = 0.05)\) and, importantly, an Exposure × Drug interaction \((F_{1,11} = 4.38, P = 0.05)\); the vehicle-treated group showed a significant decrease in reward seeking after sucrose re-exposure (i.e. in test 1b; \( P < 0.05 \)), while the morphine group showed no such change (\( P > 0.05 \)). This is interesting when one considers that 14 days in withdrawal both morphine- and vehicle-treated rats show greater sucrose reward seeking during the pre-exposure test than rats tested 1 day off drug (main effect of Drug-to-test time: \( F_{1,26} = 5.37, P = 0.03 \)). This may represent an incubation of craving effect (Grimm, Fyall & Osincup 2005) such that when, during the sucrose re-exposure session, this ‘incubated’ sucrose value is not realized, vehicle-treated rats adjust their reward seeking down accordingly. Indeed, experiencing sucrose after a period of forced abstinence following training can attenuate the abstinence-induced elevation of uncued, but not conditioned reinforced, sucrose-seeking behavior (Grimm et al. 2005). In this interpretation, opiate withdrawal may create a state that prevents this value down-shift.

When tested in late withdrawal, there was no effect of drug treatment on sucrose lick frequency \((t_{11} = 0.81, P = 0.43;\) Fig. 1e), suggesting that the effects of opiate withdrawal to alter sucrose palatability are short lasting. These results suggest that opiate withdrawal has the ability to alter the emotional experience of a sucrose reward, but only in the short-term, and also has the ability to alter the incentive learning process so as to inflate the instrumental incentive value of sucrose—an effect that is clearly strongest in early withdrawal, but remains present after 2 weeks of withdrawal. The temporal dissociability of these effects suggests that the ability of opiate withdrawal to alter incentive learning may not depend on its reward palatability effect.

**Experiment 2: Opiate withdrawn rats fail to reduce sucrose reward seeking following satiety-induced reduction in experienced sucrose palatability**

We next evaluated if opiate withdrawal would inflate the value of a reward in the face of a negative shift in the reward experience. Experiment 2 was designed to test...
the hypothesis that opiate withdrawal-induced inflation of incentive value would be sufficient to counteract the normal reduction in such value induced by experience of the sucrose reward in a sated state (Fig. 2), perhaps reflecting ‘compulsive’ behavior. Rats were trained 22 hours food deprived to earn sucrose reward. At the conclusion of training, prospective vehicle and morphine groups displayed similar reward-seeking rates (vehicle-treated: 14.30 presses/minute SEM = 2.02, morphine-treated: 11.29 presses/minute SEM = 1.22; t_{12} = 1.27, \( P = 0.22 \)). Following chronic morphine or vehicle treatment, all rats were tested in early withdrawal. The first series of tests was conducted in a novel sated state (1 hour food deprived) to assess the effects of opiate withdrawal on negative incentive learning, i.e. reduction in reward seeking after experiencing the sucrose reward under sated conditions for the first time (Balleine 1992; Wassum et al. 2011a). Following initial testing, rats were tested again for their reward seeking and sucrose palatability in the control 22 hours food-deprived state.

Observation of the data in Fig. 2a suggests equivalent response rates in the vehicle- and morphine-treated groups during the pre-exposure test, but higher responding in morphine relative to vehicle groups in the post-exposure test due to the opposite direction of the change in morphine relative to vehicle groups in the post-exposure test. This was confirmed by statistical analysis: there was a significant main effect of Exposure (This was confirmed by statistical analysis: there was a significant main effect of Exposure (\( F_{1,22} = 4.69, \ P = 0.04 \)). Post hoc analysis confirmed the significance of the difference between the drug treatment groups in the second test conducted after negative incentive learning (\( P < 0.05 \)). A negative incentive learning effect is marked by a decrease in reward seeking for a food reward when sated relative to hungry, but only after the reward has been experienced sated (Dickinson & Balleine 1994; Balleine et al. 1995). We, therefore, used planned comparisons to show that the vehicle control group’s reward-seeking response rate was not reduced relative to the 22 hours deprived control training state (see Supporting Information Fig. S4) when tested 1 hour food deprived (test 1a, \( t_{12} = 0.45, \ P = 0.66 \)) until the rats had the opportunity to experience the sucrose reward in the 1 hour deprived state (i.e. on the post-exposure reward-seeking test 1b; \( t_{12} = 3.62, \ P = 0.004 \)). This was not the case for the opiate-withdrawn rats; after the negative incentive learning opportunity, opiate-withdrawn rats reward-seeking rate was not different from their 22 hours food-deprived control state (\( t_{12} = 0.73, \ P = 0.45 \)).

As shown in Fig. 2b, there was a significant main effect of Deprivation (1 hour versus 22 hours food deprived; \( F_{1,22} = 19.76, \ P = 0.0002 \)) on sucrose lick frequency, with no significant main effect of Drug (\( F_{1,22} = 0.58, \ P = 0.45 \)) and no interaction between these factors (\( F_{1,22} = 0.02, \ P = 0.88 \)); sucrose lick frequency was significantly lower when sated relative to hungry for both vehicle-treated (\( P < 0.05 \)) and opiate-withdrawn rats (\( P < 0.01 \)). These data suggest that while opiate withdrawal may increase sucrose palatability (Fig. 1), this effect is not sufficient to overcome a satiety-induced reduction in palatability (Fig. 2b). Opiate withdrawal is, however, capable of overcoming the reduction in reward value induced by satiety (Fig. 2a), highlighting the dissociability of the effects of opiate withdrawal on the emotional experience of reward consumption and value-driven reward seeking. Indeed, there was a significant positive correlation between sucrose palatability (lick frequency) and subsequent sucrose reward seeking (\( r^2 = 0.39, \ P = 0.03 \)) in vehicle-treated rats, while no such correlation was apparent in opiate-withdrawn rats (\( r^2 = 0.0002, \ P = 0.97 \); Fig. 2c), suggesting that, in opiate withdrawal, reward seeking can be inconsistent with the most recent emotional experience derived from reward consumption.

Lastly, we found only a main effect of Deprivation on goal approach (Fig. 2d; \( F_{1,22} = 26.36, \ P < 0.0001 \)), with no effect of Drug treatment (\( F_{1,22} = 0.06, \ P = 0.80 \)), and no Drug × Exposure interaction (\( F_{1,22} = 0.13, \ P = 0.72 \)); both vehicle-treated and chronic morphine-treated rats approached the magazine less when sated than when hungry (\( P < 0.01 \)).

**Experiment 3: In opiate withdrawal the value and subsequent seeking actions for a water reward are inflated**

Evidence suggests that opiate exposure alters weight gain and metabolism (Gosnell, Levine & Morley 1983; Levine et al. 1985; Levine & Atkinson 1987; Ferenczi et al. 2010), which could potentially account for the withdrawal-induced changes in sucrose value and palatability detected in experiments 1 and 2. Opiate-withdrawn rats may value sucrose more because of a greater metabolic need for food rather than a primary effect of the withdrawal itself. Indeed, our morphine-treated rats weighed significantly less than their vehicle-treated counterparts (see Supporting Information Appendix S2 and Figs 1 & 2) and this was taken as evidence of withdrawal symptoms. Although we can statistically rule out that these effects on weight or metabolism explain the effects of opiate withdrawal on value-guided reward-seeking actions for sucrose (see Supporting Information Appendix S2), experiment 3 was designed to experimentally rule this out using a water reward, which provides no calories. This also provided an opportunity to test the generality of the effect.
All rats were trained on the action chain to earn water while 18 hours water deprived and at the end of training pressed the seeking lever at an average rate of 9.89 (SEM = 1.13) presses/minute (prospective vehicle group) and 9.23 (SEM = 0.99) presses/minute (prospective morphine-treated group: \( t_{13} = 0.43, P = 0.67 \)). Morphine-treated rats weighed less at the end of drug treatment than vehicle-treated controls and during drug treatment did, on some days, consume less food, but, importantly, not less water (Supporting Information Fig. S2 and Supporting Information Appendix S2). Testing was conducted in early withdrawal and was identical to experiment 1.

As can be seen in Fig. 3a, there was no significant difference in water-seeking response rate between the vehicle- and morphine-treated rats during the pre-exposure reward-seeking test, but after water exposure in withdrawal, morphine-treated, but not vehicle-treated, rats escalated their water seeking. Analysis reveals a marginally insignificant main effect of Drug (\( F_{1,11} = 3.56, P = 0.08 \)) on water seeking, a significant main effect of Exposure (\( F_{1,11} = 12.23, P = 0.004 \)), and, importantly, a significant Exposure \( \times \) Drug treatment interaction (\( F_{1,11} = 16.47, P = 0.001 \)). There was no significant difference in water seeking between the vehicle- and morphine-treated rats during the first pre-exposure test of reward seeking (\( P > 0.05 \)), but there was a significant difference in water seeking during the second post-exposure test (\( P < 0.01 \)); opiate-withdrawn rats showed a significant increase in water seeking after water re-exposure in withdrawal (\( P < 0.001 \)), while vehicle-treated rats showed no change (\( P > 0.05 \)), suggesting that, as with a sucrose reward, the opiate withdrawal state inflates the value of a water reward, resulting in elevated water seeking.

Unlike experiment 1, where sucrose was the reward, in this experiment there was no significant difference between opiate-withdrawn and vehicle-treated rats in lick frequency (\( t_{11} = 1.05, P = 0.31; \) Fig. 3b), suggesting that opiate withdrawal-inflated water value is not commensurate with emotional experience of water consumption. As in previous studies, there was no significant effect of opiate withdrawal on goal approach during the re-exposure test (\( t_{13} = 1.99, P = 0.07 \); Fig. 3c).

**Experiment 4: Opiate withdrawal-induced inflated incentive value is blocked by BLA mu opioid receptor inactivation**

BLA mu opioid receptor activation is necessary for a hunger-induced increase in sucrose reward value, but not for increases in sucrose palatability (Wassum et al. 2009, 2011a). Moreover, BLA mu opioid receptor binding sites are upregulated following chronic opiate exposure (Brady et al. 1989). Therefore, we next tested the hypothesis that BLA mu opioid receptor activation is necessary for opiate withdrawal-induced increases in sucrose instrumental incentive value by replicating experiment 1, with the exception that prior to the non-contingent re-exposure opportunity for incentive learning in early withdrawal, rats were given an infusion of either vehicle, or the selective mu opioid receptor antagonist, CTOP, into the BLA (Fig. 4). Following training, there was no difference in reward seeking between the prospective groups [chronic vehicle/intra-BLA vehicle: 6.96 (SEM = 0.97) seeking presses/minute; chronic morphine/intra-BLA vehicle 6.10 (SEM = 0.81); chronic vehicle/intra-BLA CTOP: 7.54 (SEM = 1.08); chronic morphine/intra-BLA CTOP 7.34 (SEM = 0.64): \( F_{1,13} = 0.48, P = 0.70 \)].

Observation of the data in Fig. 4a suggests that intra-BLA CTOP during re-exposure blocks the post-exposure increase in reward seeking evident in intra-BLA vehicle-treated animals (right side of the panel). This is supported by an initial ANOVA, which demonstrates no overall main effect of Chronic drug (vehicle or morphine treated: \( F_{1,32} = 1.50, P = 0.23 \)), but a marginally insignificant effect of Intra-BLA drug (vehicle or CTOP: \( F_{1,32} = 2.88, P = 0.10 \)), a significant Exposure \( \times \) Intra-BLA drug interaction (\( F_{1,32} = 4.66, P = 0.04 \) and a marginally insignificant Exposure \( \times \) Chronic drug interaction (\( F_{1,32} = 3.23, P = 0.08 \)). All other main effects and interactions were non-significant (\( P > 0.05 \)). Given the interactions, this analysis was clarified with separate analyses on the data divided by chronic drug treatment. In morphine-treated rats, there was no effect of Exposure (\( F_{1,15} = 0.50, P = 0.49 \)), but there was a significant effect of Intra-BLA drug (\( F_{1,16} = 6.97, P = 0.02 \)). While there was no significant interaction (\( F_{1,16} = 1.85, P = 0.19 \), reward-seeking rate was significantly higher during the post-exposure test in the rats that experienced the sucrose in opiate withdrawal under intra-BLA vehicle relative to those rats who had this exposure under BLA mu opioid receptor blockade (\( P < 0.05 \)). Within the chronic vehicle-treated group, there was no main effect of Intra-BLA drug (\( F_{1,17} = 0.18, P = 0.69 \), but rather only an effect of Exposure (\( F_{1,17} = 4.82, P = 0.04 \)) with a moderately insignificant interaction between these factors (\( F_{1,17} = 3.30, P = 0.09 \)). The negative effect of exposure is significant (\( P < 0.05 \)) in the rats that received intra-BLA CTOP on the re-exposure test. In both the vehicle- and the morphine-treated rats, response rates on the first pre-exposure test (Fig. 4a, open bars) were higher than those observed in experiment 1 (see Fig. 1a, open bars), despite the fact that the deprivation, training and testing procedures were identical with the exception of intra-BLA drug infusion. This difference may be due to stress from surgery/implant or other differences between these experiments, including differences in the time of day that the training and testing was conducted. Overall, these
data suggest that early opiate withdrawal creates a state in which the value of a reward is increased and that this effect is blocked by inactivation of BLA mu opioid receptors.

There was neither a main effect of Chronic drug ($F_{1,12} = 1.93, P = 0.17$) nor Intra-BLA drug ($F_{1,12} = 0.03, P = 0.86$) on sucrose lick frequency and no interaction between these factors ($F_{1,12} = 1.98, P = 0.17$; Fig. 4b). Contrary to our findings in experiment 1, but similar to experiments 2 and 3, these data suggest that opiate withdrawal did not induce a palatability increase, perhaps because of the infusion stress. In this experiment, a main effect of Chronic drug on goal approach behavior ($F_{1,12} = 6.21, P = 0.02$) was detected, with neither an effect of Intra-BLA drug ($F_{1,12} = 1.87, P = 0.18$) nor an interaction between these factors ($F_{1,12} = 0.01, P = 0.91$; Fig. 4c).

**DISCUSSION**

This study evaluated the effect of opiate withdrawal on value-driven reward seeking and reward palatability. The data show that in early opiate withdrawal, the experience-dependent incentive value of both sucrose and water reward was increased, resulting in enhanced value-driven reward seeking. Importantly, in certain circumstances, such reward seeking was inconsistent with the emotional experience of reward consumption and occurred in the face of circumstances that would otherwise negatively impact reward value. These data, therefore, suggest that early opiate withdrawal is a distinct motivational state in which the general incentive learning process is altered, resulting in maladaptive reward seeking. The incentive value inflated in early withdrawal continued to influence reward seeking in late withdrawal, but the late withdrawal stage itself was only modestly effective in elevating sucrose incentive value, suggesting that this mechanism might contribute to escalation and increased frequency of opiate use, short-term relapse and other effects specific to early withdrawal on natural reward consumption and non-opiate drug use.

This effect of early opiate withdrawal on incentive learning was dependent on BLA mu opioid receptor activation.

In opiate withdrawal, opiate self-administration is elevated (Wikler & Pescor 1967; Goldberg et al. 1969; Kenny et al. 2006). Previous experience with an opiate in withdrawal (i.e. an incentive learning opportunity) can enhance opiate seeking, relative to rats in withdrawal who have not had this experience and this occurs in the absence of an opportunity for negative reinforcement, i.e. the opportunity to learn that a specific drug-seeking action will lead to alleviation of negative withdrawal symptoms (Hutcheson et al. 2001). The latter study supports an incentive motivational account of opiate addiction, wherein opiate withdrawal serves as a motivational state in which the incentive value of opiates used to inform drug-seeking decisions is elevated (Hutcheson et al. 2001). While this could result from an enhanced emotional impact of the drug in the withdrawn state, it is also possible that repeated opiate administration and withdrawal disrupts the fundamental endogenous opioid-dependent incentive learning process such that the incentive value assigned to the reward when experienced in the withdrawn state may be inflated beyond the level appropriate for its emotional impact. A prediction of such an account is that opiate withdrawal should function as a state in which the incentive value of non-opiate rewards is also increased, producing general maladaptive reward-based decision making. Our data provide evidence in support of this possibility. Opiate withdrawal-induced elevation of reward value was not always consistent with an elevated emotional experience. Indeed, although we found no evidence of elevated palatability responses to water in withdrawal, water seeking was nonetheless increased after water was experienced in withdrawal. Moreover, the inflation of incentive value in the opiate-withdrawal state was also sufficient to counter an opposing negative reward value change and decline in the emotional experience of the reward brought about by experiencing a food reward when sated, a finding that is particularly pertinent to the drug-addicted condition, wherein the abused substance continues to be desired and compulsively sought out irrespective of need and despite adverse consequences.

Importantly, in these experiments, rats were never given the opportunity for the reward-seeking action to be negatively reinforced by any potential alleviation of the withdrawal symptoms that may have been conferred by food or water consumption, effectively ruling out a negative reinforcement account. Opiate withdrawal-inflated reward seeking was found to critically depend on reward evaluation and was found not to be the result of an effect on locomotor activity, generally elevated appetitive behavior or on instrumental learning per se. Moreover, this was not the result of a secondary effect of morphine exposure on weight or hunger because identical effects were detected for a food and water reward even though water intake was not significantly impacted by morphine exposure/withdrawal.

A commonly held view is that drug addiction is associated with strong urges to obtain the drug and reduced responding for natural rewards (Kalivas, Volkow & Seamans 2005). The current data provide evidence of a potential mechanism for the first part of this assumption, but could been seen as contradictory to the second, given the finding of elevated responding for natural rewards in early opiate withdrawal. Natural rewards were not
directly compared to drug rewards in this study; nonetheless, our data are consistent with the findings of elevated food- (Babbini et al. 1976; Ford & Balster 1976; Ranaldi et al. 2009; Cooper et al. 2010) and cocaine-seeking behavior (He & Grasing 2004) in opiate withdrawal. Moreover, our data may be considered as contrasting to other data showing that opiate withdrawn rats display reduced preference for food-associated places (Harris & Aston-Jones 2003, 2007) and attenuated acquisition of a food-reinforced lever press response (Harris & Aston-Jones 2003). The duration (8 days in Harris & Aston-Jones 2003 versus 2–3 days in the current study) and type of withdrawal (continuous morphine treatment in Harris & Aston-Jones 2003 versus intermittent in the current study), in addition to the different instrumental tasks used, likely account for the disparate effects on reward-seeking behaviors. Additionally, it has long been established that cues associated with naloxone-precipitated withdrawal are effective conditioned suppressors of operant behavior (Koob et al. 1992; Baldwin & Koob 1993), suggesting that the negative affective state of withdrawal has a detrimental effect on natural reward-related behavior. Indeed, systemic naloxone treatment, which has also been shown to produce a negative emotional state (Mucha & Iversen 1984; Skoubis et al. 2005), can reduce sucrose seeking in an experience-dependent manner (Wassum et al. 2009). This effect is in the opposite direction to those detected here in which reward seeking was enhanced in opiate withdrawal following exposure to the reward in the withdrawn state, suggesting the negative affective state induced by early withdrawal cannot fully account for the current results. Rather, a chronic opiate-induced disruption in the fundamental incentive learning process more readily explains the escalation of food and water seeking in early opiate withdrawal. Indeed, early opiate withdrawal-induced enhanced incentive value was found to be dependent on BLA mu opioid receptor activation— the very same mechanism required for encoding normal hunger-induced increases in incentive value used to drive sucrose reward seeking (Wassum et al. 2009). That the late opiate withdrawal state was not as effective as the early state in elevating sucrose incentive value perhaps suggests that any adaptations in the BLA mu opioid receptor system subside after 14 days off drug. Taken together, these data suggest that early opiate withdrawal disrupts the fundamental incentive learning process such that reward value becomes inflated, out of proportion with reward experience, resulting in maladaptive reward seeking. In addition to providing a potential mechanism for the escalation of opiate seeking in addicts during early opiate withdrawal, these data may also help explain why recovering opiate addicts and those treated with chronic prescription opiates are both more likely to abuse other drugs and to over-eat (Michna et al. 2004; Nolan & Scagnelli 2007; Mysels & Sullivan 2010).

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Authors Contribution

KMW designed the research, analyzed the data and wrote the paper. VYG and KEL conducted the research and assisted with data analysis. SBO and NTM designed the research and wrote the manuscript. All authors have critically reviewed content and approved final version submitted for publication.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

Figure S1 Effects of morphine withdrawal on body weight
Figure S2 Effects of chronic morphine on weight and consumption of food and water
Figure S3 Opiate withdrawal alters performance of reward-seeking actions
Figure S4 Experiment 2: In opiate withdrawal a negative change in reward value is blocked
Figure S5 Experiment 4: Histological verification of BLA cannula placements
Appendix S1 Methods
Appendix S2 Results