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Drug-induced dysphoria is enhanced following prolonged cocaine abstinence and dynamically tracked by nucleus accumbens neurons

Rachel M. Haake, Elizabeth A. West, Xuefei Wang, and Regina M. Carelli

Department of Psychology and Neuroscience, The University of North Carolina, Chapel Hill, NC 27599

Abstract

Negative reinforcement models postulate that addicts use drugs to alleviate negative affective states (e.g., dysphoria) associated with withdrawal. In a preclinical model, rats exhibit negative affect to a normally rewarding tastant when it predicts impending, but delayed cocaine, and nucleus accumbens (NAc) neurons dynamically track this state. Here, we examined the effects of short versus prolonged experimenter-imposed cocaine abstinence on negative affect, cocaine seeking and self-administration. Rats were given 14 saccharin-cocaine sessions; NAc activity and affective responses to the taste (i.e., taste reactivity) were measured during sessions 1 and 14. Next, following 1 or 30 days of abstinence, taste reactivity and cell firing were recorded in a 3-phase test session: 1) intraoral saccharin infusions, 2) extinction, and 3) cocaine self-administration. Results showed that 30 days of abstinence led to a significant enhancement of aversive responses to the cocaine-paired tastant, accompanied by a dramatic decline in NAc phasic activity during tastant infusion. While extinction behavior did not differ across groups, NAc phasic firing re-emerged during drug-seeking. Further, when drug was again readily available, greater aversion to the drug-paired tastant before abstinence was associated with increased self-administration following prolonged (30 day) abstinence in rats classified as high (not low) aversive. Collectively, these findings show that drug-induced dysphoria is enhanced following prolonged cocaine abstinence, and that NAc neural signaling is dynamic, dampening when negative affect is at its highest (Phase 1), but transitioning back ‘online’ during subsequent drug seeking and taking (Phases 2 & 3).

Keywords

Abstinence; cocaine; electrophysiology; negative affect; addiction; self-administration

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Corresponding author: Regina M. Carelli, PhD, CB#3270 Davie Hall, University of North Carolina, Chapel Hill, Chapel Hill, NC 27599, Telephone: 919-962-8775, Fax: 919-962-2537, rcarelli@unc.edu.

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Introduction

Substance use disorders are characterized by cycles of uncontrollable drug-seeking, abstinence from drug use, and relapse (Kalivas and Volkow, 2005; Koob and Volkow, 2010). Negative reinforcement models postulate that addicts relapse, in part, due to the negative affective states (e.g., anhedonia, dysphoria, irritability) that emerge during drug withdrawal (Baker et al., 2004; Koob and Le Moal, 2005; Koob and Volkow, 2010). In abstinent cocaine users, these negative emotional states can be evoked by drug-associated stimuli (Robbins et al., 2000; Sinha et al., 2000) and predict both relapse (Paliwal et al., 2008) and the intensity of subjective 'high' upon cocaine administration (Uslaner et al., 1999; Newton et al., 2003). Understanding the neurobiological mechanisms that underlie negative affect, particularly after extended drug abstinence, is important in preventing relapse and redirecting attention toward more adaptive reward procurement.

Our laboratory utilizes a preclinical model in which a cocaine-associated taste cue that predicts impending but delayed cocaine availability elicits a negative affective state associated with heightened drug-seeking (Wheeler et al., 2008, 2011; Carelli and West, 2014; Green et al., 2015; Colechio et al., 2017). Here, rats receive multiple discrete intraoral infusions (3.5 s/infusion) of a palatable saccharin solution over ~30–45 minutes followed immediately by a 2 h cocaine self-administration session. Affective responses to the sweet are examined using taste reactivity (Grill and Norgren, 1978), wherein animals exhibit stereotyped orofacial responses to intraorally infused tastants that reflect the palatability of the stimulus and the affective state it elicits (Berridge, 2000). Following repeated taste-drug pairings the sweet produces a negative affective state, evidenced by a dramatic shift from appetitive to aversive taste reactivity (Wheeler et al., 2008, 2011; Carelli and West, 2014; Green et al., 2015; Colechio et al., 2017; Hurley et al., 2017), and this shift is tracked by the NAc (Wheeler et al., 2008, 2011; Carelli and West, 2014; Hurley et al., 2017). Specifically, NAc neurons shift their cell firing from predominately inhibitory to excitatory responses during intraoral tastant infusions (Wheeler et al., 2008), typically observed during infusions of the bitter, aversive tastant, quinine (Roitman et al., 2005; Wheeler et al., 2008; Wheeler and Carelli, 2009). Additionally, reductions in rapid dopamine signaling occur during intraoral infusion of the cocaine-paired tastant indicative of a pronounced negative affective state (Wheeler et al., 2011). In support, intracranial self-stimulation thresholds are elevated following the cocaine-paired tastant infusion session, but not following intraoral infusion of a non-predictive water tastant (Wheeler et al., 2011). Importantly, these alterations are specific to the cocaine-paired taste, as when a differently flavored but equally palatable sweet is paired with saline, the sweet remains palatable (Wheeler et al., 2008; Colechio et al., 2017).

We hypothesize that the cocaine-paired tastant serves as a predictor of cocaine's impending, but delayed, availability and precipitates the expression of a conditioned aversive state in learned anticipation of the future opportunity to self-administer cocaine. As such, a critical feature of this model is that the rat must 'wait' an extended period for drug availability, and this waiting period is aversive. In support, aversive taste reactivity and reduced dopamine signaling is not observed when cocaine co-occurs with or immediately follows tastant infusion (Parker, 1995; Wheeler, 2011). Further, the negative affective state is 'corrected' by

cocaine, as the degree of aversion to the drug-paired tastant predicts subsequent motivation to self-administer drug (Wheeler et al., 2008; Colechio et al., 2014; Green et al., 2015). Additionally, a drug-paired taste elicits physiological withdrawal and predicts cocaine self-administration in rats (Nyland and Grigson, 2013). As such, drug self-administration here may reflect an effort to alleviate negative mood, particularly in a situation where the taste cue signals delayed cocaine availability. In humans, once in this state, interest in other rewards (e.g., food, family, friends) pale in comparison to their desire for drug and these negative feelings play important roles in relapse (Baker et al., 2004; Koob and Le Moal, 2005; Koob and Volkow, 2010).

The length of time away from drug use (i.e., abstinence) plays a key role in the propensity to relapse in addicts (Baker et al., 2004; Koob and Le Moal, 2005; Koob and Volkow, 2010). In animals, the ability of cocaine-associated stimuli to elicit heightened drug-seeking increases as a function of abstinence duration (Gawin and Kleber, 1986; Grimm et al., 2001; Lu et al., 2004), a finding that has been attributed to numerous neuroadaptations in reward-related brain regions, including the NAc (Lu et al., 2004; Bossert et al., 2005; Hollander and Carelli, 2005, 2007; Pickens et al., 2011; Cameron et al., 2016; Wolf, 2016). Further, anhedonia such as that experienced during drug withdrawal is associated with weak ventral striatal activity in humans (Der-Avakian and Markou, 2012). As such, the objective of the present study was to examine if the devaluation of a sweet associated with impending, but delayed cocaine availability would become heightened following extended abstinence from cocaine self-administration. We also examined if cocaine seeking and self-administration differed across rats categorized as showing low versus high aversive behaviors (Colechio et al., 2014; Colechio and Grigson 2014; Colechio et al., 2017). Finally, we determined if this abstinence-related negative affective state would be reflected in alterations in behavior and NAc neuronal firing dynamics during subsequent drug seeking (extinction) and resumption of drug taking behavior.

Materials and Methods

Subjects

Twenty-two, adult male Sprague-Dawley rats (Envigo/Harlan, Indianapolis, IN, USA) aged 60–90 days (~300–325 g) upon arrival were used. Animals were housed individually on a 12h/12 h light-dark cycle and maintained at no less than 85% of pre-experimental body weight by water restriction (~30 ml/day) for the duration of behavioral testing except during the post-operative recovery period. Rat chow (Purina, St. Louis, MO, USA) was available *ad libitum*. All procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and approved by the University of North Carolina Chapel Hill Institutional Animal Care and Use Committee (IACUC).

Surgery

Rats were anesthetized with ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg) and prepared for chronic indwelling catheter, intraoral cannula, and microwire electrode array implantation in the same surgery. Rats were implanted with a custom-made (Access Technologies, Skokie, IL, USA) intrajugular catheter as well as an

intraoral cannula, using established procedures (Wheeler et al., 2008, 2011; Green et al., 2015). Microwire electrode arrays (8 microwires/array; 50 μ m diameter; NB Labs, Denison TX, USA) were bilaterally implanted into the NAc core (+1.7 AP, $\pm$ 1.4 ML, -7.0 DV) or shell (+1.7 AP, $\pm$ 0.8 ML, -7.0 DV) using established methods (Carelli et al., 2000; West and Carelli, 2016). Animals were allowed at least 7 post-operative recovery days.

Experimental Design

Experiments were conducted in standard operant chambers (see Supplemental Information). Figure 1A shows a schematic diagram of behavioral training. Rats were initially trained in daily 30 min sessions to lever press for water (3–5 days) then underwent surgery and recovery (1 week) followed by re-establishment of lever pressing (1–2 days) and habituation to intraoral (water) infusions (1 day). On subsequent training days 1–14, rats were given 45 discrete intraoral infusions of 0.15% saccharin (0.13 ml delivered over 4 s/infusion, $\sim$ 1 infusion/min for 45 trials). An i.v. catheter line was then attached and a lever was extended into the chamber with the cue light above it illuminated. Rats were then allowed to self-administer cocaine on a fixed-ratio 1 schedule of reinforcement for 2h. Here, lever depression resulted in intravenous cocaine delivery (0.33 mg/infusion, $\sim$ 1 mg/kg/infusion, 6s) paired with termination of the cue light and simultaneous onset of a tone (67 dB, 1 kHz)/house light conditioned stimulus complex (20 s).

Following 14 training sessions, rats were divided into two groups: 1 day ($n=10$) or 30 days ($n=12$) of abstinence and remained in their home cages. Rats were then tested in a single session consisting of three phases: (1) intraoral tastant infusions, (2) extinction, and (3) resumption of cocaine self-administration (Figure 1B). In Phase 1, rats received intraoral infusions of the same 0.15% saccharin solution as delivered during training. Next, the lever previously associated with cocaine delivery during self-administration was extended into the chamber, the cue light illuminated, and Phase 2 was initiated. Here, each lever press resulted in termination of the cue light and presentation of the audiovisual CS, but no drug delivery. After 2 h, Phase 3 was initiated by administration of 1–3 priming infusions of cocaine (0.33 mg/inf; 6 s) paired with the audiovisual CS (20 s). Each subsequent lever press resulted in a cocaine infusion (0.33 mg/inf; 6 s) and presentation of the audiovisual CS (20 s). The test session ended 2 h after Phase 3 initiation.

Taste Reactivity

A video camera was positioned to face a mirror below the chamber to record orofacial responses to intraoral infusions on days 1 and 14 of training and on test day. Responses were categorized as appetitive or aversive, described previously (Grill and Norgren, 1978; Berridge, 2000; Wheeler et al., 2008, 2011; Green et al., 2015; Hurley et al., 2017). Briefly, lateral tongue protrusions were classified as appetitive and gaping as aversive. Taste reactivity data were analyzed as the number of appetitive (licks) or aversive (gapes) performed per 45 trials during each recorded session. In some cases, intraoral infusions of saccharin elicited other aversive behavioral responses including dripping of the fluid from the mouth (a behavior consistent with responses to concentrated quinine; Grill and Norgren, 1978, and tastants paired with illness; Dwyer et al., 2017), as well as face wipes, forelimb flails, head shakes, and mouth-to-floor wiping (Grill and Norgren, 1978; Berridge, 1996;

Berridge, 2000). Animals that exhibited a high frequency of these other aversive behaviors were analyzed separately (see Supplemental Information).

Electrophysiology

Electrophysiology procedures are well-established and have been described in detail previously (Carelli et al., 2000; and Supplemental Information). Electrophysiological recordings were completed on days 1 and 14 of training and during the test session.

Data Analysis

Behavior—To determine whether behavior prior to abstinence differed between animals destined for the 1 and 30 day groups, the number of cocaine infusions earned during training and changes in appetitive and aversive taste reactivity from training day 1 to 14 were compared using two-way repeated measures ANOVAs. Upon confirmation of similar behavior, taste reactivity counts on day 1 vs 14 of training were collapsed across groups and analyzed with paired *t*-tests. The effects of abstinence on taste reactivity following abstinence (test, Phase 1) was examined via a two-way repeated measures ANOVA. Additionally, to further examine the effects of abstinence on taste reactivity a difference score of aversive responses was calculated; the number of gapes on day 14 was subtracted from those observed on test day and compared with Student's *t*-tests. One-sample *t*-tests compared each group's difference score to a theoretical value of zero (reflecting no change between day 14 and test). Student's *t*-tests were used to compare lever pressing during extinction (Phase 2) and the resumption of cocaine self-administration (Phase 3) across abstinence groups (2 rats from 30 day group excluded from Phase 3 analyses due to catheter patency loss). To determine if the degree of aversion to the cocaine-paired tastant prior to abstinence was associated with behavior during Phases 2 and 3 after abstinence, each animal was categorized as having a low or high degree of aversion by median split of aversive gapes on day 14. Animals that exceeded the median number of gapes were classified as high aversive, and animals that exhibited less than the median number of gapes were considered low aversive. Two-way ANOVAs were used to compare the number of lever presses (Phase 2) and cocaine infusions (Phase 3) for low versus high aversive rats across abstinence groups. Pearson's correlations were used to examine the relationship between aversive taste reactivity on day 14 and cocaine seeking/self-administration in each group.

Electrophysiology—Classification of phasic activity during intraoral tastant infusions, lever pressing under extinction, and cocaine self-administration, and analysis of changes in neural firing patterns relative to task events are described in the Supplemental Information. Analyses of magnitude, latency, and duration of neural responses are in the Supplemental Information.

The shift in the population response of NAc neurons to tastant infusion from training day 1 to 14 was determined by calculating the number of cells excited versus inhibited during tastant infusions on each day (collapsed across groups; see Supplemental Information) and compared with Fisher's exact test. To determine the effects of abstinence on neural activity during tastant infusions in Phase 1, the percentages of phasic cells were determined for each animal on training day 14 and test day and examined with a two-way repeated measures

ANOVA. Further, Student's *t*-test was used to compare difference scores in mean percent phasic cells (test day minus day 14) during tastant infusions for 1 vs 30 day groups and one-sample *t*-tests compared each group's difference score to a theoretical value of zero (reflecting no change between day 14 and test day). Student's *t*-tests were also used to compare mean percent phasic cells during extinction (Phase 2) and self-administration (Phase 3) for 1 versus 30 day groups. Animals with <2 cells recorded during any session (1 per group), less than 5 lever presses under extinction (1, 1 day) or that lost catheter patency (2, 30 day) were excluded from electrophysiology analyses.

Statistical analyses were performed using GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA). Post hoc analyses (Sidak's Multiple Comparisons) were used when appropriate.

Histology

Histological reconstruction of electrode positions was accomplished using established procedures (Hollander & Carelli, 2005; Wheeler et al., 2008; West and Carelli, 2016; also see Supplemental Information and Supplemental Figure S1).

Results

Behavior before abstinence

Rats destined for the 1 versus 30 day groups did not differ in cocaine self-administration or taste reactivity behaviors prior to abstinence (see Supplemental Information, and Supplemental Figures S2 & S3).

Taste cues that predict impending but delayed cocaine are devalued and elicit a negative affective state tracked by NAc neurons (before abstinence)

Following 14 taste-drug sessions, responses to intraoral saccharin infusions shifted from primarily appetitive to mostly aversive behaviors (Figure 2A), consistent with prior reports (Wheeler et al., 2008, 2011; Green et al., 2015; Hurley et al., 2017). On day 1 of taste-drug pairings, rats exhibited classic appetitive taste reactivity during intraoral saccharin infusion with minimal aversive responses. However, following repeated taste-drug pairings aversive taste reactivity emerged (Figure 2A). Paired *t*-tests revealed a significant decrease in appetitive taste reactivity ($t_{21}=3.88$; $p<0.001$), as well as a significant increase in aversive gapes ($t_{21}=8.06$; $p<0.0001$) following 14 taste-drug pairing sessions.

The emergence of this negative affective state was reflected in NAc cell firing dynamics. NAc neurons were classified as exhibiting phasic inhibitions (INH), excitations (EXC), or as nonphasic during tastant infusion as previously described (Roitman et al., 2005, 2010; Wheeler et al., 2008; also see Supplemental Information), and illustrated in Figure 2B. Critically, as shown in Figure 2C, the population neural response shifted from primarily INH during intraoral infusions of the sweet on day 1, to mostly EXC firing following repeated taste-drug pairings on day 14 (Fisher's exact test, $p<0.01$). These findings demonstrate that an initially palatable saccharin solution that predicts impending, but delayed cocaine is

devalued and elicits an aversive state that is tracked by NAc neurons, consistent with prior reports (Wheeler et al., 2008, 2011; Green et al., 2015; Hurley et al., 2017).

Enhancement of negative affect and dampening of phasic NAc neural signaling following one month cocaine abstinence

Following abstinence, rats were placed back in the chamber and underwent a single test session completed in 3 phases. During Phase 1, rats were given 45 discrete intraoral infusions of saccharin, identical to procedures utilized during training. We examined whether aversive taste reactivity that emerged after 14 days of taste-drug pairings was exacerbated by prolonged abstinence. Figure 3A shows the number of gapes on day 14 and during Phase 1 of the test session for 1 and 30 day groups. A two-way repeated measures ANOVA revealed no main effect of group ($F_{1,16}=0.57$, $p>0.10$), but a main effect of session ($F_{1,16}=24.34$, $p<0.001$) and a significant group by session interaction ($F_{1,16}=10.38$, $p<0.01$). Post hoc analyses revealed a significant increase in aversive gapes from day 14 to test day for the 30 day group only ($t_{16}=5.77$, $p<0.0001$). Figure 3B shows the difference scores in aversive taste reactivity between day 14 of training prior to abstinence and test day after abstinence for each group. The increase in aversive taste reactivity was significantly greater in the 30 day compared to 1 day abstinence group ($t_{16}=3.22$; $p<0.01$). Critically, this increase in aversive gapes was only observed following prolonged abstinence as evidenced by difference scores significantly greater than zero in the 30 day ($t_8=4.50$; $p<0.01$), but not 1 day group ($t_8=2.03$; $p>0.05$). These findings indicate that the negative affective state that emerges following repeated taste-drug pairings is significantly heightened following prolonged drug abstinence.

Importantly, this behavioral enhancement of negative affect in Phase 1 was reflected in a dampening of NAc patterned activity during intraoral infusions. Here, we calculated the mean percentage of neurons that exhibited phasic firing patterns (i.e., EXC and/or INH cells vs. nonphasic) during intraoral infusions on training day 14 (before abstinence) and test day after abstinence across groups. Figure 4A shows that there was a significant and pronounced *decrease* in the mean percentage of cells that exhibited phasic activity after 30 days of abstinence compared to the 1 day group. A two-way repeated measures ANOVA revealed no main effect of group ($F_{1,18}=0.69$, $p>0.10$), but a main effect of session ($F_{1,18}=15.54$, $p<0.01$) and a significant group by session interaction ($F_{1,18}=7.30$, $p<0.05$). Post-hoc analyses revealed a significant decrease in mean percent phasic from day 14 to test day for the 30 day group only ($t_{18}=4.95$, $p<0.001$). Figure 4B shows the significant difference in mean percent phasic difference scores between day 14 prior to abstinence and test day after abstinence across groups ($t_{18}=2.70$, $p<0.05$). This change in mean percent phasic from day 14 of training to test day was significantly different than zero for the 30 day ($t_{10}=5.39$, $p<0.001$), but not the 1 day ($t_8=0.77$, $p>0.10$) group. Importantly, this reduction in phasic activity following prolonged abstinence did not result from a significant decrease in the *total* number of cells recorded across sessions for each group ($\chi^2=1.93$, $p>0.10$). Interestingly, the few cells that exhibited phasic activity in the 30 day group (6 of 57 total cells) were all classified as excitations. In contrast, cells showing phasic activity following 1 day of abstinence (18 of 65 total cells) included both excitations ($n=13$ cells) and inhibitions ($n=5$ cells). These

findings support the view that prolonged abstinence exacerbated negative affective responses to the cocaine-paired tastant and dramatically reduced NAc firing to the devalued sweet.

Cocaine seeking and taking behavior and its tracking by NAc neurons following tastant infusions are not altered by one month cocaine abstinence

Following intraoral infusions on test day, the lever was extended into the chamber and each press resulted in CS presentation only ('extinction', Phase 2). Lever press responding during extinction did not significantly differ between abstinence groups ($t_{20}=1.01$, $p>0.10$). To examine whether phasic neuronal activity patterns during cocaine seeking were altered following 30 days of abstinence, the mean percent of cells showing phasic excitations or inhibitions relative to lever presses during extinction was calculated, as described previously (West et al., 2014). Of the 117 cells recorded for the 1 and 30 day groups in Phase 2, 46 neurons (39%) exhibited phasic changes (increases or decreases) in firing rate relative to the press. While the percentage of phasically active cells dramatically increased for the 30 day group from Phase 1 (6.44% phasic) to Phase 2 (41.86% phasic; $t_{11}=3.20$, $p<0.01$), neuronal tracking of cocaine seeking was not significantly different between the 1 and 30 day groups ($t_{18}=0.33$, $p>0.10$).

In Phase 3, each lever press again resulted in cocaine infusion paired with the CS. The total number of cocaine infusions earned did not significantly differ between groups ($t_{18}=0.24$, $p>0.10$), consistent with prior reports (Hollander and Carelli, 2005, 2007; Cameron and Carelli, 2012; West et al., 2014). Next, we examined if phasic NAc activity during cocaine self-administration differed between abstinence groups. Of the 106 cells recorded for the 1 and 30 day groups in Phase 3, 32 neurons (30%) exhibited one of four types of patterned discharges relative to the lever press for intravenous cocaine (Supplemental Figure S4), as described previously (Carelli, 2000; Hollander & Carelli, 2005, 2007). While the mean percent phasic cells for the 30 day group remained elevated in Phase 3 ($t_{11}=1.71$, $p>0.10$ for mean % phasic in Phase 2 compared to Phase 3), the mean percent of phasic cells did not significantly differ between 1 and 30 day groups ($t_{17}=0.96$, $p>0.10$). In sum, although drug seeking and related NAc activity were not altered by 1 month cocaine abstinence, phasic NAc activity that was significantly dampened during Phase 1 following prolonged abstinence transitioned back online for the 30 day group during Phases 2 and 3 as animals engaged in drug seeking and taking.

Enhanced negative affect was associated with greater cocaine self-administration in high (not low) aversive rats after one month abstinence

To determine whether the degree of negative affect before abstinence (day 14) was associated with drug seeking or self-administration following abstinence, lever presses under extinction (Phase 2) and cocaine infusions earned (Phase 3) were compared across animals classified as low versus high aversive for 1 versus 30 day abstinence groups, similar to prior work by others (Colechio et al., 2014; Colechio and Grigson, 2014; Colechio et al., 2017). Importantly, rats classified as high aversive exhibited a significantly greater number of gapes compared to their low aversive counterparts for both the 1 day ($t_8=4.54$, $p<0.01$) and 30 day ($t_{10}=4.68$, $p<0.001$) groups. No differences were observed in lever press responding during Phase 2 across low- versus high-aversive animals (Figure 5A). A two-way ANOVA revealed

no main effect of abstinence group ($F_{1,18}=0.98$, $p>0.10$), degree (low versus high) of aversion ($F_{1,18}=0.44$, $p>0.10$), nor a group by degree of aversion interaction ($F_{1,18}=0.44$, $p>0.10$). Likewise, there were no significant correlations between number of gapes and lever presses during Phase 2 for the 1 day ($R^2=0.04$, $p>0.50$) or 30 day ($R^2=0.05$, $p>0.50$) groups. However, the degree of aversion exhibited during tastant infusions on day 14 was associated with the amount of drug consumed in Phase 3 for the 30 day, but not 1 day, group (Figure 5B). Specifically, a two-way ANOVA revealed no main effect of group ($F_{1,16}=0.12$, $p>0.10$), but a main effect of degree of aversion ($F_{1,16}=11.47$, $p<0.01$) and a significant group by degree of aversion interaction ($F_{1,16}=10.82$, $p<0.01$). Post hoc analyses revealed that high aversive animals earned significantly more cocaine infusions compared to their low aversive counterparts for 30 day rats only ($t_{16}=4.72$, $p<0.001$). Importantly, there were no significant differences in amount of drug consumed on day 14 between the low versus high aversive groups. Specifically, a two-way ANOVA revealed no main effect of group ($F_{1,18}=0.23$, $p>0.10$), degree of aversion ($F_{1,18}=2.45$, $p>0.10$), nor an interaction ($F_{1,18}=3.08$, $p>0.05$) on self-administration behavior. There was no correlation between number of gapes and cocaine infusions for the 1 day group ($R^2=0.09$, $p>0.50$), but a trend between number of gapes and cocaine infusions for the 30 day group ($R^2=0.38$, $p=0.057$).

We also examined whether degree of aversion during Phase 1 was associated with cocaine seeking/self-administration. Here, rats were classified into low and high aversive groups as before. For the 30 day group, animals that exhibited a high frequency of other aversive behaviors were considered maximally aversive (see Supplemental Information). While the categorization into low and high groups remained mostly stable from day 14 to test day for both groups, the aversive behavioral profile for some rats shifted following abstinence (see Supplemental Information). Nevertheless, 30 day rats classified as high aversive (Phase 1) earned significantly more cocaine infusions compared to their low aversive counterparts. A two-way ANOVA revealed no main effect of abstinence group ($F_{1,16}=0.08$, $p>0.50$), but a main effect of aversion group ($F_{1,16}=5.41$, $p<0.05$) and an abstinence group by aversion group interaction ($F_{1,16}=5.03$, $p<0.05$). Post hoc analyses revealed that 30 day high aversive rats earned significantly more cocaine infusions than their low aversive counterparts ($t_{16}=3.23$, $p<0.05$). Collectively, these findings suggest that stronger negative affect before and after abstinence was associated with greater resumption of cocaine taking following 30, but not 1 day, abstinence.

Discussion

Negative reinforcement theories posit that addicts seek and take drugs, in part, to alleviate dysphoria associated with withdrawal and prolonged abstinence (Baker et al., 2004; Koob and Le Moal, 2005; Koob and Volkow, 2010). In a preclinical model, we previously showed that rats exhibit pronounced aversive responses to intraoral delivery of a normally rewarding tastant when it predicts impending but delayed cocaine, and this dysphoric state is tracked by NAc neurons (Wheeler et al., 2008; 2011; Green et al., 2015; Hurley et al., 2017). Here, we show that following one month of cocaine abstinence this negative affective state is dramatically exacerbated and accompanied by a dramatic decline in NAc cell firing during the devalued sweet. When drug was again readily available (self-administration, Phase 3), greater aversion to the drug-paired tastant both before (day 14) and after (Phase 1 of test)

was associated with increased self-administration following prolonged (30 day) abstinence. Additionally, the decline in phasic NAc activity observed in Phase 1 for the 30-day abstinent rats was partially reversed when animals could work for drug (Phases 2 and 3). Collectively, these findings suggest that drug-induced dysphoria is enhanced following prolonged cocaine abstinence and associated with heightened drug taking, and that NAc neural signaling is dynamic, dampening when negative affect is at its highest (Phase 1), but transitioning back 'online' as the dysphoric state is corrected during subsequent drug seeking and taking (Phases 2 & 3).

It has been proposed that the devaluation of a drug-paired sweet reflects its direct comparison to the more preferred cocaine (Grigson and Twining, 2002; Nyland and Grigson, 2013; Colechio and Grigson, 2014). The present study supports this view by showing an increase in aversive behavioral responses to the drug tastant following repeated taste-drug pairings prior to abstinence, and its pronounced enhancement following extended abstinence. Further, we also show here that rats could be divided into low versus high aversive, also consistent with work by Grigson and colleagues (Colechio et al., 2014, Colechio and Grigson, 2014; Colechio et al., 2017), with greater aversion linked to increased cocaine self-administration in the 1-month group. These findings support the view that withdrawal/extended abstinence leads to a robust devaluation of nondrug rewards, especially when subjects are in a drug-waiting state.

Here, prolonged cocaine abstinence elicited enhanced aversive taste reactivity, with a dramatic decline in NAc phasic cell firing during delivery of the sweet. This dampening of NAc signaling may indicate that when rats are in a strong dysphoric state, the NAc transitions offline and other neural regions become engaged. This concept is consistent with other reports showing that anhedonia, such as that experienced by human addicts undergoing withdrawal/abstinence, is associated with weak ventral striatal activity (Der-Avakian and Markou, 2012), or prefrontal 'hypofrontality' (Kalivas and Volkow, 2005). Indeed, it has been proposed that 'anti-reward' regions such as the extended amygdala (e.g., BNST; Koob and Volkow, 2010) and/or the rostromedial tegmental nucleus and lateral habenula (Jhou et al., 2009; Barrot et al., 2012; Baker et al., 2016) may be recruited to process these aversive states. Future studies are needed to explore these possibilities.

Numerous studies have shown that the ability of cocaine-associated stimuli to elicit intense drug craving and seeking increases (i.e., 'incubates') as a function of abstinence duration (Gawin, 1991; Grimm et al., 2001; Lu et al., 2004; Pickens et al., 2011; Wolf, 2016). Although we have reported similar findings (Hollander and Carelli, 2005, 2007; West et al., 2014; Cameron et al., 2016), heightened extinction responding in our 30 day abstinent group was not observed here, even in high aversive rats. This finding is likely related to specifics of our task, and the severe dysphoric state elicited by it. Specifically, the present study incorporated taste-drug sessions that imposed a prolonged delayed onset of extinction testing (i.e., after 35–45 minutes in Phase 1). As such, we postulate that this drug-waiting period, along with the negative emotional state elicited by extended abstinence, precipitated the onset of a highly dysphoric, conditioned withdrawal state (e.g., Nyland and Grigson, 2013) that was corrected by administration of cocaine, but not drug seeking under extinction. Regardless, the overall dampened NAc activity observed in Phase 1 (30-day group) was

partially reversed in Phase 2, suggesting that once rats began to work for drug again in extinction, the NAc re-engages.

Interestingly, greater negative affect before and after abstinence was associated with significantly increased cocaine self-administration following prolonged abstinence, particularly in high aversive rats, and a continued increase in phasic cell firing compared to Phase 1. These findings parallel clinical studies showing that robust negative affective states that emerge during abstinence are associated with increased drug craving, attrition from treatment programs, and greater cocaine use reported at follow-up examinations (Brown et al., 1998; Kosten et al., 1987; Paliwal et al., 2008). Additionally, ratings of negative emotional states in abstinent cocaine users predict both relapse and the intensity of subjective high experienced upon administration of cocaine (Newton et al., 2003; Uslaner et al., 1999). Our findings are consistent with these reports and suggest that drug-induced dysphoria following repeated taste-drug sessions and its exacerbation following prolonged abstinence represent an indicator of propensity to resume drug taking following extended abstinence.

In conclusion, the present findings revealed a prolonged abstinence-induced enhancement of negative affect. NAc neural signaling is highly dynamic during this period, exhibiting dampened neural activity while in this dysphoric state, but transitioning back online as this state is corrected by subsequent drug access. Future investigations will explore the larger neural circuitry underlying this process and incorporate techniques such as optogenetics to examine whether dampened NAc activity is causally linked to it.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Experimental timeline (see main text for details). **A.** Rats were first trained to press a lever for water during 3–5 daily sessions, then were surgically implanted with intrajugular catheters, intraoral cannulae, and bilateral electrode arrays into the NAc core and shell. Following 1 week of recovery, rats were given 1–2 sessions to re-establish lever pressing and were then habituated to tastant infusions. Next, rats received 14 daily taste-drug pairing sessions. Briefly, rats received 45 discrete intraoral infusions of a 0.15% saccharin solution (0.13 ml delivered over 4 s/infusion, ~1 infusion/min for 45 trials). A lever was then extended into the chamber and rats self-administered cocaine (0.33 mg/inf) on a fixed ratio 1 schedule of reinforcement for 2 h. Following 14 taste-drug sessions, rats underwent experimenter-imposed abstinence (no drug) for 1 or 30 days and were subsequently tested during a single test session. Affective responses to the tastant and NAc cell firing were recorded on days 1 and 14 of training and during the test session. **B.** Following abstinence, affective responses and NAc cell firing were recorded during a single test session consisting of three phases: 1) intraoral tastant infusions, 2) extinction (press for cues, no drug), and 3) self-administration. Phase 3 was preceded by 1–3 priming infusions of cocaine (0.33 mg/inf) and presentation of the drug-paired CS.

Figure 2.

Shift in behavioral and neural responses to the sweet that predicts impending, but delayed cocaine. **A.** Repeated taste-drug pairings resulted in a decrease in appetitive taste reactivity (left) and an increase in aversive taste reactivity (right). **B.** PEHs for representative neurons showing either phasic inhibitions (INH, top) or excitations (EXC, bottom) during tastant infusions. **C.** On day 1 (left), a majority of phasic NAc cells exhibit inhibitions to the tastant, but this population response shifts to predominately excitations following 14 taste-drug pairing sessions (right). Error bars represent $\pm$ SEM, here and in subsequent figures.

Figure 3.

Emergence of enhanced negative affective state following 30 days (not 1 day) abstinence. **A.** Number of gapes per 45 trials on day 14 and during Phase 1 of the test session for animals destined for the 1 day versus 30 days abstinence groups. Thirty days, but not 1 day, of abstinence led to a significant increase in aversive taste reactivity. * $p < 0.05$ for test day gapes compared to day 14 gapes in 30 day group. **B.** Change in number of aversive gapes per 45 trials from day 14 to test day in 1 versus 30 day groups. Thirty days of abstinence led to a significantly larger increase in aversive taste reactivity from day 14 to test day compared to 1 day of abstinence. * $p < 0.05$ compared to 1 day group, # $p < 0.05$ compared to theoretical value of zero. Animals with a high frequency of other aversive behaviors on test day excluded (1 day, 1; 30 day, 3).

Figure 4.

Change in NAc phasic activity relative to tastant infusion from day 14 to test day following 1 or 30 days of abstinence. **A.** Mean % of cells showing either phasic excitations or inhibitions relative to tastant infusion on day 14 and test day across 1 and 30 day abstinent groups. There was a significant decrease in phasic activity following 30 days of abstinence. * $p < 0.05$ for day 14 mean percent phasic to test day mean percent phasic for 30 day rats. **B.** Change in phasic activity from day 14 to test day for 1 and 30 day groups. There was a significantly larger and negative change in phasic activity for the 30 day group compared to the 1 day group. * $p < 0.05$ compared to 1 day group; # $p < 0.05$ compared to theoretical value of zero.

Figure 5.

Behavioral responding in low and high aversive rats during extinction and resumption of cocaine self-administration. **A.** One versus 30 day abstinent rats did not significantly differ in mean number of lever presses under extinction. **B.** Number of infusions earned during cocaine self-administration for low and high aversive animals across abstinence conditions. High aversive animals in the 30 day group pressed significantly more for cocaine than their low aversive counter parts. * $p < 0.05$ for 30 day, high aversive animals compared to 30 day, low aversive animals.