Published in final edited form as:

Behav Brain Res. 2013 March 1; 240: 87–90. doi:10.1016/j.bbr.2012.10.057.

A Drug-Paired Taste Cue Elicits Withdrawal and Predicts Cocaine Self-Administration

Jennifer E. Nyland^a and Patricia S. Grigson^a

^aThe Pennsylvania State University College of Medicine, Hershey, Pennsylvania

Abstract

Addiction is a chronic disease where periods of abstinence are riddled with instances of craving, withdrawal, and eventual relapse to escalated drug use. Cues previously associated with drug use can have a deleterious effect on this cycle by precipitating withdrawal symptoms. Here we focus specifically on the relationship between avoidance of a drug-paired taste cue and the ability of the drug-paired cue to elicit withdrawal and, ultimately, drug seeking and taking. We used a rat model of drug addiction and naloxone-induced loss of body weight to test whether a taste cue elicits withdrawal in anticipation of drug availability. Experiment 1 investigated the ability of a taste cue to elicit signs of withdrawal when it predicted experimenter-administered morphine (15 mg/kg, i.p.). In Experiment 2, a saccharin taste cue was paired with the opportunity to actively self-administer cocaine (0.167 mg/infusion, i.v.). The results show that presentation of a morphine- or cocaine-paired taste cue is sufficient to elicit naloxone-induced withdrawal symptoms, and greater withdrawal predicts greater cocaine self-administration in rats.

Keywords

Cocaine; Morphine; Withdrawal; Naloxone; Taste Cue; Reward Comparison

Substance abuse, dependence, and addiction are associated with an apparent devaluation of, and inattention to, natural rewards. Indeed, according to the Diagnostic and Statistical Manual of Mental Health Disorders [1], substance abuse and dependence involve a failure to fulfill major obligations at work, school, or home, the giving up of important social, occupational, or recreational activities, and continued drug use in spite of recurrent physical, legal, social, or psychological problems. Subsequently, addiction and the concurrent devaluation of natural rewards can lead to removal of children from the home [2], loss of home and/or job, negative health consequences [3], and an increase in risky behaviors that affect all members of society [4]. This suggests an urgency for the study of drug-induced devaluation of natural rewards.

Drug-induced devaluation of natural rewards can be investigated using a model where rats avoid intake of a normally preferred gustatory stimulus when it predicts the availability of a drug of abuse. In this model, avoidance of a taste cue that has been paired with

© 2012 Elsevier B.V. All rights reserved.

Correspondence concerning this article should be addressed to Jennifer E. Nyland, Department of Neural and Behavioral Sciences, The Pennsylvania State University College of Medicine, 500 University Drive, MC-H181, Hershey, PA, 17033. Phone: (717) 531-6063, Fax: (717) 531-6916, jen166@psu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

experimenter-administered drugs of abuse [5-14] has traditionally been attributed to unspecified aversive properties of the drug itself, much in the way an aversive agent produces a classic conditioned taste aversion [5, 6, 8-14]. However, over a decade ago, we proposed that the rewarding, not aversive, properties of drugs of abuse were the driving force behind conditioned avoidance of a drug-paired taste cue [15]. We now know that greater avoidance of a taste cue is associated with more, rather than less, cocaine self-administration [16, 17], and that avoidance of the drug-paired taste cue is dependent upon dopaminergic signaling, as the systemic administration of the D1 receptor antagonist, SCH39166, attenuates avoidance of the drug-paired taste cue [18]. That said, recent findings suggest the onset of an aversive process when the taste cue indicates that the rat must wait for drug [19]. Here we argue that avoidance of the drug-paired taste cue may in part also result from the development of an aversive conditioned state of withdrawal that develops when the taste cue comes to predict the availability of drug. Specifically, when the cue signals a delay, the rat experiences a negative affective state accompanied by reduced intake, elevated circulating corticosterone [20], blunted accumbens dopamine [19, 21], and frank aversive taste reactivity behavior following the intraoral infusion of the drug-paired cue [22]. Each of these indices has been linked to conditioned withdrawal [23]. That being said, the present study seeks direct evidence.

In opioid pretreated rats, the opioid antagonist naloxone can precipitate measurable indices of withdrawal and a decrease in body weight is one of the most robust [24]. Accordingly, we used this measure to test if a drug-paired taste cue elicits conditioned withdrawal in anticipation of drug availability. In the first experiment, 15 male Sprague-Dawley rats were used to test whether a morphine-paired taste cue will elicit this index of withdrawal in a within-subjects design. A 0.15% saccharin solution adulterated with either orange or grape flavored Kool-Aid (0.187%) served as the taste cue. The experiment was conducted in the home cages, and the taste cue, referred to as the conditioned stimulus (CS), was presented in inverted graduated Nalgene cylinders with silicone stoppers and stainless steel spouts affixed to the front of each home cage with springs. Intake was measured to the nearest 0.5 ml. Morphine sulfate, provided by the National Institute on Drug Abuse (NIDA; Bethesda), was prepared in sterile saline immediately before use. Before conditioning began, rats were maintained on a water deprivation schedule that allowed for 5 min access to distilled water (dH₂O) in the morning and 1 h each afternoon. This training procedure lasted until morning water intake stabilized (9 days). During acquisition, one flavor (orange or grape) was presented each day in a 0.15% saccharin solution, alternating across days. Rats were randomly assigned to either the orange or grape CS+ group. On each conditioning trial, all rats were given 5 min access to the CS (either orange or grape flavored saccharin), followed 5 min later by an intraperitoneal injection of either 15 mg/kg morphine (if given access to their designated CS+ solution) or an equal volume of saline (if given access to their designated CS- solution). All subjects had one flavor paired with morphine alternated with the other paired with saline for a total of 14 conditioning days (7 grape, 7 orange). To maintain proper hydration, dH₂O continued to be provided for 1 h every afternoon. Two test days followed. On each test day, subjects were weighed and then given 5 min access to the CS+ or the CS- (counterbalanced across 2 days of testing), followed 5 min later by an injection of naloxone (1 mg/kg, s.c.). A second body weight was recorded 2 h after the naloxone injection. To control for the volume of CS intake (i.e., for the possibility that reduced body weight was due to low CS+ intake), half of the rats tested with the CS- (saline-paired flavor) received a volume equal to that consumed by the CS+ (morphine-paired flavor) group at test. This group is hereafter referred to as the CS- Yoked group, while those with unlimited access to the CS- at test are referred to as the CS- Ad Lib group.

Intake of the CS during conditioning trials was analyzed using repeated measures analysis of variance (ANOVA) with condition (CS+ or CS-) and trial (1-7) as within-subject factors.

Post hoc Newman-Keuls tests were conducted where appropriate. Data here and elsewhere were considered statistically significant when $p < .05$. The results showed that intake of the morphine-paired taste cue (CS+) decreased compared with intake of the saline-paired taste cue (CS-) over trials (Fig. 1a). This conclusion was confirmed by a significant main effect of condition, $F(1, 14) = 16.33$, $p < .01$, which established that all rats consumed less of the CS+ than CS- overall. The condition $\times$ trial interaction also attained statistical significance, $F(6, 84) = 8.29$, $p < .001$, and post hoc tests revealed that this difference was significant for conditioning trials 3–7, $ps < .05$.

Intake of the CS during the two test trials was analyzed using paired t-tests. Results demonstrated reduced intake of the CS+ compared with ad libitum intake of the CS- at test, $t(7) = -2.34$, $p < .05$ (Fig. 1b). Furthermore, intake of the CS+ did not differ from yoked intake of the CS-, $t(6) = 2.05$, $p = .09$. The change in body weight following naloxone administration, which served as an index of withdrawal, also was analyzed using paired t-tests. The results showed that, regardless of volume consumed, naloxone administration did not induce a loss of body weight when presented following access to the saline-paired CS- (Fig. 1c). The same naloxone challenge, however, elicited a precipitous and significant loss in body weight when administered following presentation of the morphine-paired CS+. This conclusion was supported by a significant decrease in body weight following presentation of the CS+ compared with the CS- in the Ad Lib group, $t(7) = -3.77$, $p < .01$, and the Yoked group, $t(6) = -5.00$, $p < .01$. Furthermore, an unpaired t-test revealed no significant difference in the change in body weight between the CS- Ad Lib and CS- Yoked groups, $t(13) = -.07$, $p = .946$. Finally, because this was a within-subjects study, withdrawal was shown to be a conditioned response to the morphine-paired taste cue, independent of drug history.

Based on these findings, we hypothesized that if avoidance of the taste cue was due to the development of a conditioned state of withdrawal in anticipation of drug availability, rather than to a specific property of the drug, this index of withdrawal should be evident with any type of abused drug. Therefore, we conducted a separate between-subjects study with naïve rats where a saccharin taste cue was paired with the opportunity to self-administer cocaine. Briefly, 17 male Sprague-Dawley rats underwent jugular catheter implantation surgery with catheters custom made in our laboratory as previously described [16]. Cocaine hydrochloride (NIDA, Bethesda) was prepared in sterile saline immediately before use. Once having recovered from surgery, rats were habituated to the experimental chambers for 3 days as outlined [16]. The experimental chambers were equipped with three retractable spouts; the leftmost spout was used to present the taste cue, and the remaining two spouts were empty and served as the active (rightmost) and inactive (center) operant spouts. During each acquisition trial, the left CS spout advanced, allowing 5 min access to 0.15% saccharin. After the 5 min CS access period concluded, the CS spout retracted and the empty active and inactive spouts advanced. A stimulus light was illuminated above the active spout and the rats were placed on a fixed ratio schedule of reinforcement where completion of 10 licks on the active spout resulted in an intravenous infusion of either saline or cocaine (0.167 mg/infusion) over a 6 sec period, depending on the group assignment. Drug or saline delivery was signaled by offset of the stimulus light and onset of a tone and house light, which remained on for a 20 sec period. Further responding during this time was not reinforced, as both the active and inactive operant spouts were retracted. The access period to the drug or saline lasted for 1 h, after which the spouts retracted and the subjects were returned to their home cages. There was one acquisition trial per day for 7 days. Following the final acquisition trial, one test day occurred in which rats received 5 min access to the saccharin taste cue in the self-administration chambers followed by an injection of naloxone (1 mg/kg, s.c.) as described in the first experiment. Body weight was recorded before and 2 h after naloxone administration.

Intake of the saccharin taste cue during the acquisition trials was analyzed using a mixed factorial ANOVA with drug (cocaine or saline) as the between-subjects factor and trial (1-7) as the within-subjects factor. Post hoc Newman-Keuls tests were conducted where appropriate. Results indicate that intake of the taste cue was significantly reduced when it predicted the opportunity to self-administer cocaine compared with saline (Fig. 2a). This was confirmed by a significant main effect of drug, $F(1, 15) = 8.11$, $p < .05$, and a significant drug x trial interaction, $F(6, 90) = 6.74$, $p < .001$. Post hoc analyses confirmed that this effect was significant for trials 2–7, $ps < .05$. The number of infusions per acquisition trial also was analyzed using a mixed factorial ANOVA varying drug (cocaine or saline) and trial (1-7). The results indicated that rats self-administered more cocaine than saline (Fig. 2b). This is demonstrated by a significant main effect of drug, $F(1, 15) = 6.10$, $p < .05$, and a significant drug x trial interaction, $F(6, 90) = 5.20$, $p < .001$. Post hoc analyses verified that the infusions were significantly higher for the cocaine group compared with the saline group for trials 2–7, $ps < .05$.

The CS intake at test was analyzed using an unpaired t-test with drug (cocaine or saline) as the variable. Results revealed that intake of the taste cue was significantly lower for the cocaine group compared with the saline group on the test day, $t(15) = 4.67$, $p < .001$ (Fig. 2c). The change in body weight following naloxone administration was analyzed similarly. As occurred with experimenter delivered morphine, the cocaine group had a significant loss in body weight 2 h after naloxone administration compared with the saline group, $t(15) = 3.41$, $p < .01$ (Fig. 2d). Additionally, follow up analyses revealed that a greater loss in body weight was significantly correlated with greater avoidance of the saccharin cue at test, $R^2 = .73$, $F(1, 13) = 35.49$, $p < .001$ (Fig. 2e) and with a greater number of infusions of cocaine on the final conditioning trial, $R^2 = .42$, $F(1, 13) = 9.54$, $p < .01$ (Fig. 2f).

Although avoidance of the drug-paired taste cue may initially result from drug-induced devaluation of the saccharin cue, with experience, aversion develops as would be predicted by the opponent process theory of motivation [25]. The opponent process theory proposes that the pleasurable effects of the drug are automatically opposed by central nervous system mechanisms that serve to reduce the impact of the drug. With experience, the pleasurable effects of the drug reduce in intensity due, at least in part, to tolerance. The aversive opponent process, however, gains in strength and is conditioned to drug-paired cues. As such, the opponent process is elicited in anticipation of drug-delivery and can be experienced as withdrawal in rats and man [26].

Here we provide evidence that presentation of a morphine- or cocaine-paired taste cue elicits a conditioned state of withdrawal, as indexed by a precipitous loss of body weight following injection of the opiate antagonist, naloxone. Furthermore, greater naloxone-induced loss in body weight (i.e., greater withdrawal) was correlated with greater avoidance of the drug-paired cue and, as verified with cocaine, greater cocaine self-administration behavior. These data suggest that the onset of conditioned withdrawal may precipitate a snowball effect involving devaluation of the natural reward cue (i.e., anhedonia), activation of the HPA axis as indicated by elevated corticosterone [20], and blunted accumbens dopamine [19, 22]. Together, these consequences conspire to elicit seeking and taking in an effort to correct the highly aversive state.

In summary, when considered amongst other chronic diseases such as diabetes, Alzheimer's disease, Parkinson's disease, or cancer, addiction is unique because its development, maintenance, and the associated damage to the brain depend upon learning. As such, it is essential that we develop animal models that can track the development of this dynamic disease and, thereby, begin to understand how conditioned changes in behavior, affect, and neurophysiology promote the seeking and taking of drug. Here, greater conditioned

naloxone-induced withdrawal predicts greater devaluation of the saccharin cue and greater drug taking. Understanding the role of this conditioned aversive state in the devaluation of naturally rewarding stimuli and cue-induced drug-seeking and taking is essential if we are to break the cycle of addiction, as cue-induced withdrawal and the concurrent devaluation of naturally rewarding stimuli are risk factors for acquisition of the behavior and for relapse following even extended periods of abstinence.

Acknowledgments

This research article was supported in part by DA012473, F31-DA029369, and under a grant with the Pennsylvania Department of Health using Tobacco Settlement Funds.

References

- [1]. American Psychiatric Association. Diagnostic and statistical manual of mental health disorders. 4th ed. Author; Washington DC: 1994.
- [2]. Nair P, Black M, Schuler M, Keane V, Snow L, Rigney B. Risk factors for disruption in primary care giving among infants of substance abusing woman. *Child Abuse & Neglect*. 1997; 21:1039–51. [PubMed: 9422825]
- [3]. Santolara-Fernandez F, Gomez-Sirvent J, Gonzalez-Reimers C, Batista-Lopez J, Jorge-Hernandez J, Rodriguez-Moreno F, et al. Nutritional assessment of drug addicts. *Drug and Alcohol Dependence*. 1995; 38:11–18. [PubMed: 7648992]
- [4]. National Highway Traffic Safety Administration. Traffic safety facts 2010: Alcohol impaired driving. National Highway Traffic Safety Administration; Washington DC: Apr. 2012 Report No. DOT HS 811 606
- [5]. Cappell H, LeBlanc AE. Conditioned aversion to saccharin by single administrations of mescaline and d-amphetamine. *Psychopharmacologia*. 1971; 22:352–6. [PubMed: 5133436]
- [6]. Cappell H, LeBlanc AE, Endrenyi L. Aversive conditioning by psychoactive drugs: Effects of morphine, alcohol, and chlordiazepoxide. *Psychopharmacology*. 1973; 29:239–46.
- [7]. Grigson PS, Twining RC, Carelli RM. Heroin-induced suppression of saccharin intake in water-deprived and water-replete rats. *Pharmacology, Biochemistry and Behavior*. 2000; 66:603–8.
- [8]. Miller JS, Kelly KS, Neisewander JL, McCoy DF, Bardo MT. Conditioning of morphine-induced taste aversion and analgesia. *Psychopharmacology*. 1990; 101:472–80. [PubMed: 2167492]
- [9]. Sherman JE, Pickman C, Rice A, Liebeskind JC, Holman EW. Rewarding and aversive effects of morphine: Temporal and pharmacological properties. *Pharmacology, Biochemistry and Behavior*. 1980; 13:501–5.
- [10]. Berger BD. Conditioning of food aversions by injections of psychoactive drugs. *Journal of Comparative and Physiological Psychology*. 1972; 81:21–6. [PubMed: 5074304]
- [11]. Gamzu, E. The multifaceted nature of taste-aversion inducing agents: Is there a single common factor?. In: Barker, L.; Best, M.; Domjan, M., editors. *Learning mechanisms in food selection*. Baylor University Press; Waco, TX: 1977.
- [12]. Hunt T, Amit Z. Conditioned taste aversion induced by self-administered drugs: Paradox revisited. *Neuroscience and Biobehavioral Reviews*. 1987; 1:107–30. [PubMed: 3554039]
- [13]. Lester D, Nachman M, LeMagen J. Aversive conditioning by ethanol in the rat. *Quarterly Journal of the Study of Alcohol*. 1970; 31:578–86.
- [14]. Vogel J, Nathan B. Learned taste aversions induced by hypnotic drugs. *Pharmacology, Biochemistry and Behavior*. 1975; 3:189–94.
- [15]. Grigson PS. Conditioned taste aversions and drugs of abuse: A reinterpretation. *Behavioral Neuroscience*. 1997; 111:129–36. [PubMed: 9109631]
- [16]. Grigson PS, Twining RC. Cocaine-induced suppression of saccharin intake: A model of drug-induced devaluation of natural rewards. *Behavioral Neuroscience*. 2002; 116:321–33. [PubMed: 11996317]

- [17]. Twining RC, Bolan M, Grigson PS. Yoked delivery of cocaine is aversive and protects against the motivation for drug in rats. *Behavioral Neuroscience*. 2009; 123:913–25. [PubMed: 19634952]
- [18]. Fenu S, Rivas E, Di Chiara G. Differential role of dopamine in drug- and lithium-conditioned saccharin avoidance. *Physiology & Behavior*. 2005; 85:37–43. [PubMed: 15924904]
- [19]. Wheeler RA, Aragona BJ, Fuhrmann KA, Jones JL, Day JJ, Cacciapaglia F, Wightman RM, Carelli RM. Cocaine cues drive opposing context-dependent shifts in reward processing and emotional state. *Biological Psychiatry*. 2011; 69:1067–74. [PubMed: 21481843]
- [20]. Gomez F, Leo NA, Grigson PS. Morphine-induced suppression of saccharin intake is correlated with elevated corticosterone levels. *Brain Research*. 2000; 863:52–8. [PubMed: 10773192]
- [21]. Grigson PS, Hajnal A. Once is too much: Conditioned changes in accumbens dopamine following a single saccharin–morphine pairing. *Behavioral Neuroscience*. 2007; 121:1234–42. [PubMed: 18085877]
- [22]. Wheeler RA, Twining RC, Jones JL, Slater JM, Grigson PS, Carelli RM. Behavioral and electrophysiological indices of negative affect predict cocaine self-administration. *Neuron*. 2008; 57:774–85. [PubMed: 18341996]
- [23]. Grigson PS. Reward comparison: The Achilles' heel and hope for addiction. *Drug Discovery Today: Disease Models*. 2008; 5:227–33. [PubMed: 20016772]
- [24]. Nozaki M. Assessment of morphine-type physical dependence liability: A screening method using the rat. *Psychopharmacology*. 1976; 47:225–35. [PubMed: 823558]
- [25]. Solomon RL, Corbit JD. An opponent-process theory of motivation: I. Temporal dynamics of affect. *Psychological Review*. 1974; 81:119–45. [PubMed: 4817611]
- [26]. Koob GF, Le Moal M. Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology*. 2001; 24:97–129. [PubMed: 11120394]

Research Highlights

- Morphine-paired taste cues are sufficient to precipitate indices of withdrawal.
- Cue-induced loss of body weight serves as an index of withdrawal.
- Cue-induced loss of body weight is independent of volume of cue intake.
- Indices of withdrawal are measurable for a cue paired with self-administered cocaine.
- Indices of withdrawal are predictive of cocaine self-administration.

Figure 1.

A morphine-paired taste cue elicits withdrawal independent of volume consumed. **(a)** Mean intake of the morphine-associated taste cue (CS+) and saline-associated taste cue (CS-) across pairings, $n=15$. **(b)** Mean intake of the CS+ and CS- on test, and **(c)** mean change in body weight 2 h after naloxone administration (CS- Ad Lib $n=8$; CS- Yoked $n=7$). All error bars represent standard error of the mean (s.e.m.). * $p < .05$, ** $p < .01$, compared with CS- condition (a), and CS- Yoked condition (b-c).

Figure 2.

Suppression of a drug-paired taste cue predicts cocaine self-administration and withdrawal. (a) Mean intake of the taste cue that predicts access to self-administer cocaine or saline. (b) Mean infusions/60 min of cocaine (0.167 mg/infusion) or saline across 7 trials. (c) Mean intake of the taste cue on test day. (d) Mean change in body weight 2 h after naloxone administration. (e) Correlation of saccharin intake and change in body weight on test day. (f) Correlation of change in body weight on test day and the number of infusions taken on the final conditioning trial. All error bars represent s.e.m. * $p < .05$, ** $p < .01$, *** $p < .001$ compared with the saline condition. Saline $n=8$; Cocaine $n=9$