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Drug-Motivated Behavior in Rats with Lesions of the Thalamic Orosensory Area

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Abstract

Rats suppress intake of a palatable taste cue when paired with a rewarding or an aversive stimulus in appetitive or aversive conditioning, respectively. A similar phenomenon occurs with drugs of abuse, but the nature of this conditioning has been subject for debate. While relatively little is known about the underlying neural circuitry, we recently reported bilateral lesions of the thalamic trigeminal orosensory area isolate drug-induced suppression of intake of a taste cue. The lesion blocks avoidance of the taste cue when paired with experimenter delivered drugs of abuse, yet has no effect on avoidance of the same cue when paired with an aversive agent or when it predicts access to a highly palatable sucrose solution. We hypothesize the lesion may blunt the rewarding properties of the drug. To test this, we used a runway apparatus, as running speed has been shown to increase with increasing reward value. Our hypothesis was supported by failure of the lesioned rats to increase running speed for morphine. Interestingly, lesioned rats did avoid intake of the drug-paired cue when presented in the runway apparatus and displayed naloxone-precipitated withdrawal. Using a partial crossover design, the lesion prevented avoidance of a cocaine-paired cue when presented in the home cage. We conclude that the lesion disrupts avoidance of a taste cue in anticipation of the rewarding properties of a drug but, at least in the presence of contextual cues, allows for avoidance of a taste cue as it elicits the onset of an aversive conditioned state of withdrawal.

Keywords

Trigeminal Orosensory Area; Thalamus; Ibotenic Acid Lesion; Motivated Behavior; Morphine; Cocaine

Introduction

Rats suppress intake of an otherwise palatable gustatory cue (referred to as the conditioned stimulus or CS) when paired with an aversive or appetitive stimulus (referred to as the unconditioned stimulus or US). In aversive conditioning, a conditioned taste aversion (CTA) develops when rats come to avoid a taste cue that has been paired with an illness-inducing

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agent such as lithium chloride (LiCl) (Nachman, 1963) or x-radiation (Garcia, Kimmeldorf & Koelling, 1955; Smith, 1971), for example. This phenomenon is at play when humans avoid foods that have caused or have been associated with illness. In appetitive conditioning, intake of a similar taste cue (e.g., saccharin) is suppressed when it predicts access to a preferred sucrose solution (Flaherty & Checke, 1982). In this case, reduced intake of the taste cue is thought to reflect an associative process whereby the perceived value of the saccharin CS pales in anticipation of the availability of the impending and preferred sucrose US (Flaherty & Checke, 1982; Flaherty & Grigson, 1988; Flaherty & Rowan, 1985). How then do we explain a third phenomenon in which avoidance of a palatable taste cue develops following pairings with a drug of abuse?

Specifically, rats avoid intake of a taste cue following pairings with all drugs of abuse tested (LeMagnen, 1969; for reviews, see Gamzu, Vincent & Boff, 1985; Goudie, 1987; Hunt & Amit, 1987), across a range of doses (LeBlanc & Cappell, 1975; Parker, 1991), and routes of administration (Bechara & van der Kooy, 1985; Cappell & LeBlanc, 1971; Mucha & Herz, 1986; Shoaib & Stolerman, 1995; Wise, Yokel & DeWit, 1976). Early on, this effect was attributed to presumed aversive properties of the drugs and, thus, to CTA learning (Lester, Nachman & LeMagnen, 1970). This interpretation, however, does not take into consideration the well-known rewarding properties of drugs of abuse: (1) Drugs of abuse are readily self-administered by humans and other animals (for review, see van Ree, 1979); (2) Drugs of abuse support the development of conditioned place preferences (Bardo, Miller & Neisewander, 1984; Blander, Hunt, Blair & Amit, 1984; Katz & Gormezano, 1979; Reicher & Holman, 1977); and (3) Avoidance of a morphine-paired food cue is accompanied by a simultaneous increase, rather than a decrease, in instrumental responding for morphine in the runway (White, Sklar & Amit, 1977). These findings are opposite those obtained with LiCl, supporting Linda Parker's data (Parker, 1995) dissociating avoidance of a drug-paired cue from avoidance of a LiCl-paired cue.

Indeed, when considering a number of manipulations, avoidance of a drug-paired cue more closely matches avoidance of a sucrose-paired saccharin cue (i.e., anticipatory contrast) than a LiCl-induced CTA. Compared to a LiCl-induced CTA, intake of a drug- or sucrose-paired taste cue is more similarly affected by the deprivation state of the rat (Gomez & Grigson, 1999) and a history of drug exposure (Grigson, Wheeler, Wheeler & Ballard, 2001). Central lesions also serve to dissociate the suppressive effects of a drug of abuse from those induced by the illness-inducing agent, LiCl. Specifically, lesions of the gustatory thalamus (Grigson, Lyuboslavsky & Tanase, 2000; Reilly, Bornavolova & Trifunovic, 2004; Schroy et al., 2005) and focal lesions encompassing the agranular insular cortex (Geddes, Han, Baldwin, Norgren & Grigson, 2008; Mackey, Keller & Van der Kooy, 1986; Zito, Bechara, Greenwood & Van der Kooy, 1988) more greatly disrupt avoidance of a gustatory cue when paired with morphine, cocaine, or sucrose than when paired with LiCl-induced malaise (see Schier et al 2015 for a careful analysis of the gustatory cortex). Avoidance of a drug-paired cue, then, appears to more closely approximate avoidance induced by anticipation of access to a highly preferred sucrose US.

Drug- and sucrose-induced suppression of CS intake, however, are not one and the same. For example, recent data show that lesions centered on the thalamic trigeminal orosensory area

(TOA) disrupt avoidance of a saccharin cue when paired with a drug of abuse, but not when paired with a highly preferred sucrose solution in the anticipatory contrast paradigm or with the aversive agent, LiCl, in the CTA paradigm (Liang, Freet, Grigson & Norgren, 2012; Nyland, Alexander, Liang & Grigson, 2012). Therefore, while avoidance of the drug-paired cue resembles that normally seen in appetitive conditioning (i.e., when anticipating access to a palatable sucrose reward), we have evidence that drug-induced suppression of CS intake may, in fact, be its own phenomenon and that the drug-paired cue may come to elicit the onset of a conditioned state involving craving and withdrawal (Nyland & Grigson, 2013). In accordance, avoidance of the drug-paired taste cue also is associated with an increase in circulating corticosterone (Gomez, Leo & Grigson, 2000), a blunting of accumbens dopamine to the saccharin cue (Grigson & Hajnal, 2007), onset of aversive taste reactivity behaviors (i.e., gapes) following intraoral infusion of the drug-paired cue (Wheeler et al., 2008; Colechio & Grigson, 2014; Colechio, Imperio & Grigson, 2014), and a precipitous short-term loss of body weight (Nyland & Grigson, 2013).

This dissociation by the TOA lesion (i.e., disruption of drug-, but not LiCl- or sucrose-induced suppression of CS intake) provides insight into the function of the area. Specifically, we must conclude that TOAx rats can detect the gustatory cue and can associate a gustatory CS with a US. They also can reduce intake of a sucrose- or LiCl-paired taste cue. To what, then, do we attribute the lesion-induced failure to suppress intake of a morphine- or cocaine-paired taste cue? One possibility is that the TOAx rats are unable to experience the rewarding properties of the drug. If so, the failure to avoid a drug-paired cue in our previous report (Nyland et al., 2012) would have been due to a reduction in contrast between the natural reward and the perceived value of the drug of abuse. If, on the other hand, the motivation to obtain drug is unaffected by the lesion, then the lesion may have a direct effect on drug-induced devaluation of the otherwise palatable cue. Experiment 1 used the runway to test this hypothesis. Rats increase running speed in the runway to reach the Goal Box paired with experimenter-administered morphine (Zernig et al., 2002) and amphetamine (Ettenberg, 1990), as well as self-administered heroin (McFarland & Ettenberg, 1995). This also is true for natural rewards such as sex (Lopez, Olster & Ettenberg, 1999), water (Ettenberg & Horvitz, 1990), and food (Chausmer & Ettenberg, 1997; Ettenberg & Camp, 1986) and greater running speed is associated with greater reward magnitude (Flaherty, Coppotelli, Hsu & Otto, 1998). Consequently, in this study, the taste cue was presented in the Start Box and saline or morphine was experienced in the Goal Box. If the lesion interferes with detection of the rewarding properties of the drug, then TOAx rats will not run faster for morphine than for saline. If, on the other hand, the lesioned rats can detect the rewarding properties of the drug, then TOAx rats in the morphine condition will run faster to the Goal Box than will the saline controls. Of course, as has been found previously (Nyland et al., 2012), avoidance of the morphine-paired saccharin cue also should be disrupted in rats with bilateral TOA lesions.

Finally, body weight loss following naloxone-precipitated withdrawal has been shown to be a reliable test for dependence and for estimating addiction liability (Nurimoto, 1972; Nozaki, 1976). We have shown in intact rats, that avoidance of the drug-paired cue is accompanied by a precipitous loss of body weight when access to the drug-paired cue is followed, at test, by an injection of a 1.0 mg/kg dose of naloxone (an opiate antagonist),

rather than morphine (Nyland & Grigson, 2013). Given that body weight loss is a component of withdrawal (Becker, Gerak, Li, Koek & France, 2010; McDonald, Parker & Siegel, 1997), this finding was taken as evidence that the drug-paired taste cue is not only devalued, but that it elicits the onset of a conditioned aversive state – an opponent process, if you will, that opposes the impact of the impending drug (Solomon & Corbit, 1974). The present study, then, also challenged rats with naloxone at the end of testing to determine whether the TOA lesion will block not only avoidance of the drug-paired taste cue, but also the conditioned loss of body weight as well.

Experiment 1

Methods

Subjects—The subjects were 42 naive male, Sprague-Dawley rats delivered from Charles River at approximately 50 days of age. Following one week of quarantine, the rats were housed individually in stainless steel hanging cages in a temperature-controlled (21 °C) animal care facility with a 12-h light-dark cycle (lights on at 7 a.m.). Testing was conducted during the light phase of the cycle. The rats were maintained on dry Harlan Teklad rodent diet (Madison, WI) and dH₂O ad libitum, except where noted otherwise.

Surgery—Following acclimation to the colony room, the subjects (at least 300 g) underwent either bilateral ibotenic acid lesions targeting the thalamic trigeminal orosensory area (n=24 TOAx) or vehicle infusions (n=18 Sham). Twenty min prior to anesthesia, all rats were injected with atropine sulfate (0.1 mg, ip) and Bicillin (200,000 U/0.3ml, im). They were anesthetized with sodium pentobarbital (50 mg/kg, ip) and supplemented as necessary throughout surgery. Body temperature was maintained at 37+/-1 °C. The rat's head was mounted in a stereotaxic instrument using non-traumatic earbars. The skin over the skull was cleaned with Betadine and opened with a mid-line incision. Using a 4 mm diameter trephine, two holes were drilled in the skull centered 3.0 mm posterior to β and 1.2 mm lateral of the midline on either side. The dura mater remained intact and moist throughout surgery with physiological saline. The skull was leveled between β and λ and a search electrode (glass-insulated tungsten microelectrode; Z = 0.5 – 1.2 M Ω at 1 kHz) was lowered into the left or right gustatory thalamus. The taste area was located by recording neural activity while stimulating the anterior tongue with 0.3 M NaCl (approximate coordinates: -3.8 mm posterior to β , +/- 1.2 mm lateral to the midsagittal suture, and 6.0 mm below dura). Once having located the gustatory thalamus, the search electrode was removed and the lesion micropipette lowered. The lesion was made using an ibotenic acid filled glass micropipette (diameter 20 – 24 μ m at the tip) that was glued directly onto the needle of a 1.0 μ l Hamilton microsyringe. Sham surgery rats were treated similarly, except that sodium phosphate buffer, rather than ibotenic acid was infused. The presence of taste neurons was confirmed by recording background taste responses with the glass micropipette. The micropipette was then raised and lowered again 500 microns lateral to the recorded area and 0.2 μ l (20 μ g/ μ l) of ibotenic acid was infused over 10 min. The pipette remained *in situ* for another 10 min. This same procedure was repeated on the contralateral side. After removal of the pipette, the holes in the skull were filled with Gelfoam and the wound closed with wound clips. The

animals recovered over 2–3 days and body weight returned to presurgical levels within a week.

Apparatus—All experimental manipulations were conducted in the runway (Med Associates Inc., VT). The floor, walls, and ceiling of the runway (7.85 ft × 7 in × 5.5 in) were made of Plexiglas. The Start Box and Goal Box (19.5 in × 9.25 in × 5.5 in) of identical dimensions were located at opposing ends of the runway. The grid floors of the Start Box consisted of thirty 4.8 mm stainless steel rods spaced 2 cm apart. The outer ceiling and walls of the entire runway were covered with 30% tint. The Goal Box was changed as to appear different from the Start Box. A tile was fitted over the grid floors in the Goal Box to give it a different texture than the rest of the runway. The Goal Box had 1-inch thick white and black diagonal stripes covering the walls and ceiling. Computer operated sliding doors provided access from the Start Box to the runway and from the runway to the Goal Box. The Start Box and Goal Box were each equipped with one automated, retractable sipper dispenser, such that a bottle could be advanced through a 1.3 cm diameter hole in the end wall, approximately 10 cm above the grid floor. A closed circuit lick-o-meter was used to monitor licking behavior. The latency to leave the Start Box and enter the Goal Box was detected by interruption of six pair of infrared photocell emitters. Output of these photocells and lick-o-meter was collected on-line by a computer. Programs were written in the Medstate notation language and the behavioral data were stored as Notepad files.

Solutions—Morphine sulfate, provided by the National Institute on Drug Abuse (Bethesda, MD) and naloxone hydrochloride (Sigma Chemical, St. Louis, MO) were prepared in sterile saline immediately before testing. Sodium saccharin (Sigma Chemical, St. Louis, MO) was dissolved in dH₂O and presented at room temperature.

Procedure—The protocol was approved by the Pennsylvania State University College of Medicine's Institutional Animal Care and Use Committee and was conducted in compliance with federal guidelines, the Animal Welfare Act, Animal Welfare Regulations, and the principles of the Guide for the Care and Use of Laboratory Animals. All rats were placed on a water deprivation schedule that allowed for 5 min access to dH₂O in the morning and 1 h each afternoon. Intake was closely monitored during both morning and afternoon sessions. Following stabilization of morning dH₂O intake (approximately 7 days), the Sham and TOAx rats were matched for 5 min dH₂O intake and divided into two drug treatment groups: saline and morphine.

The rats were placed in the runway one at a time. They were first habituated to the runway apparatus for 20 min, once daily for 2 days. During habituation, all rats were removed from their home cage, weighed and placed into the Start Box where they received 5 min access to dH₂O through a retractable sipper spout located on the wall opposite the exit door. After the 5 min period was over, the spout retracted and the door to the Start Box opened. Rats were then allowed to explore the runway freely (except for the Goal Box, which remained closed during habituation) for 5 min. Once the session was over, the rat was removed and placed back in the home cage. One h after being removed from the runway, the rat was given 1 h access to dH₂O in the home cage. Habituation was necessary to prevent confounding influence from the novelty of the environment.

Following habituation to the runway apparatus, conditioning commenced. During conditioning trials, the rat was removed from the home cage, weighed and placed into the Start Box where it received 5 min access to 0.15% saccharin via a retractable sipper spout opposite the exit door. Intake of the saccharin taste cue was recorded as the number of licks on the retractable spout. After the 5 min access period was over, the doors to the Start Box and Goal Box opened, allowing the rat access to the Start Box, runway, and Goal Box. The rat was then given a maximum of 15 min to reach the far wall of the Goal Box. Once detected by the photocell at the back of the Goal Box, the Goal Box door closed and the rat immediately received an experimenter-administered injection of morphine (15mg/kg, ip; Sham: n=9; TOAx: n=14) or saline (Sham: n=9; TOAx: n=10), depending on the random group assignment. The time required to reach the Goal Box was used to determine the running speed. The rat was then placed back into the Goal Box for 5 min. Following the 5 min post-injection rest period, the rat was immediately removed from the runway and placed back in the home cage. One hour later, the rat was given 1 h access to dH₂O in the home cage to rehydrate. The same procedure occurred every other day for a total of six conditioning trials followed by a seventh test trial. On days between conditioning trials, rats received 5 min access to dH₂O in the morning and 1 h in the afternoon in the home cage.

Naloxone-precipitated withdrawal, as indicated by a loss in bodyweight, is a well-documented assay for the addictive liability of a substance (Nurimoto, 1973; Nozaki, 1976). We have previously shown that such a reduction in body weight is reliably induced by a drug-associated cue 2 h after administration (Nyland & Grigson, 2013). On the final test day (trial 7), the procedure was run as described above with the exception that all rats received subcutaneous injections of naloxone (1 mg/kg) rather than saline or morphine following entry into the Goal Box. Body weight was recorded again 2 h after the rat was injected with naloxone. Thereafter, all rats were given free access to dH₂O in the home cage.

Histology—At the end of all behavioral tests, the rats were given an overdose of Pentobarbital Sodium (100 mg/kg, ip) and, once deeply anesthetized, perfused transcardially for 5 min with cold physiological saline plus heparin (1.5 U/ml), immediately followed by cold 4% paraformaldehyde for 20 min. The brains were removed and stored in paraformaldehyde for 2 h, then in a mixture of 20% sucrose + 0.1 M phosphate buffer overnight. They were frozen and sliced coronally in 50 µm sections. One series of alternate sections was stained for cell bodies with cresyl echt violet and another series of sections underwent immunohistochemical detection for Neuronal Nuclei protein (NeuN). The adequacy of the lesions was judged by comparing the acellular areas in the lesioned subjects to that of the sham-lesioned controls. The boundaries of the gustatory and oral tactile nuclei have been defined from neuroanatomical and electrophysiological data (Kosar, Grill & Norgren, 1986a, 1986b; Norgren, 1976; for reviews, see Norgren, 1984; 1995). Data from subjects found to have misplaced or incomplete lesions were excluded from further analysis (see Figure 1).

Statistics—Data were analyzed using a mixed factorial analysis of variance (ANOVA) with lesion (Sham or TOAx) and drug (morphine or saline) as the between-subjects factors

and trial (1–6) as the within-subjects factor. Post hoc Newman-Keuls tests were conducted where appropriate. Data were considered statistically significant when $p < .05$.

Results

Histology

Data from six TOAx rats were excluded from statistical analysis due to incomplete lesions (see Figure 1). The resulting group numbers were as follows: Sham Saline $n=9$, Sham Morphine $n=9$, TOAx Saline $n=9$, TOAx Morphine $n=9$. Complete lesions damaged the middle third of the VPM as well as the majority of the taste area in the parvocellular area of the VPM (VPMpc). Additionally, the lesion encompassed small parts of adjacent nuclei such as the parafascicular nucleus (Pf), posterior nucleus (Po), and subparafascicular nucleus (SPF).

CS Intake (Licks/5 min)

Intake of the saccharin taste cue during the six conditioning trials was analyzed using a mixed factorial ANOVA with lesion (Sham or TOAx) and drug (morphine or saline) as between-subjects factors and trial (1–6) as a within-subjects factor. The results indicate that Sham and TOAx rats alike drank less of the saccharin cue when it preceded an injection of morphine compared with saline in the runway (Figure 2). This conclusion was supported by a lack of a significant main effect of lesion ($F < 1$), or any interactions thereof ($Fs < 1$). A significant main effect of drug, $F(1,32) = 17.47$, $p < .001$, and a significant drug $\times$ trial interaction, $F(5,160) = 16.62$, $p < .001$, indicate that avoidance of the morphine-paired cue was observed in both the intact and the lesioned rats when tested in the runway apparatus. Independent follow up analyses of each lesion condition indicated that intake was lower for rats treated with morphine compared with saline treated rats on trials 2–6 for the Sham ($p < .05$), and on trials 3–6 for the TOAx group ($p < .05$). These results suggest that, all other things being equal, when trained in the runway, rats with TOA lesions, like intact controls, avoid intake of a saccharin cue that predicts passively administered morphine.

Running Speed

Running speed was determined by the time required to traverse the runway and enter the Goal Box. Initial analyses compared the absolute values of running speed; however, individual differences made interpretation of the data difficult. To adjust for individual differences in running speed, values across conditioning trials were represented as a percent of running speed on the first conditioning trial. The data were analyzed using a mixed factorial ANOVA varying lesion, drug, and trial as factors. There were significant lesion $\times$ drug $\times$ trial, $F(5,160) = 2.65$, $p < .05$, and lesion $\times$ drug interactions, $F(1,32) = 7.24$, $p < .05$, and significant main effects of lesion, $F(1,32) = 4.50$, $p < .05$, and drug, $F(1,32) = 4.20$, $p < .05$. Post hoc analyses of the lesion $\times$ drug $\times$ trial interaction indicate that rats in the Sham group were faster to reach the Goal Box when associated with morphine than when associated with saline (Figure 3). This was significant for trials 2 through 6 ($p < .05$).

This effect was not present in the lesioned group. Lesioned rats showed no difference in time to reach the goal between saline and morphine treated conditions at any time point. Further,

lesioned rats did not differ from rats in the Sham-saline condition on any trial. These results show that the Sham rats ran faster to reach the Goal Box when associated with passively administered morphine compared with saline, and that this effect was absent in the lesioned rats.

Naloxone Test

The naloxone test was utilized as described in our previous publication (Nyland & Grigson, 2013) which showed that the loss in body weight following exposure to a drug-paired cue was exacerbated by the administration of naloxone. Intake of the taste cue and the resultant change in body weight were analyzed using two-way ANOVAs varying lesion and drug.

CS Intake.—The results indicate that both sham and lesioned rats drank less of the taste cue when it predicted experimenter-administered morphine compared with saline on the final conditioning trial (Figure 4a). This was confirmed by a significant main effect of drug, $F(1,32) = 28.57$, $p < .001$, and by lack of a significant main effect of lesion ($F < 1$) or lesion x drug interaction ($F < 1$).

Change in Body Weight.—Results found both sham and TOAx groups to have a significant loss in body weight, used here as an index of withdrawal, following exposure to the taste cue that was paired with experimenter-administered morphine but not saline (Figure 4b). This conclusion was supported by a significant main effect of drug, $F(1,32) = 23.10$, $p < .001$, and a lack of a significant main effect of lesion ($F < 1$) or lesion x drug interaction ($F < 1$). Overall, the results indicate that, when tested in the runway apparatus, intact and lesioned rats avoid intake of a morphine-paired taste cue and reduce body weight in a pattern that is consistent with the onset of cue-induced withdrawal.

Correlation Analyses

Saccharin intake for all rats on the final trial was positively correlated with the change in body weight following naloxone administration, $R^2 = .64$, $F(1,34) = 60.13$, $p < .001$, (Figure 5). This coincides with previous reports in intact rats (Nyland & Grigson, 2013).

This correlation between intake and the change in body weight also was evident when reanalyzed separately for Sham, $R^2 = .65$, $F(1,16) = 29.12$, $p < .001$, and TOAx rats, $R^2 = .67$, $F(1,16) = 32.97$, $p < .001$. Overall, rats that drank the least of the saccharin taste cue also showed a greater loss in body weight following the naloxone test.

Discussion

Intact but not lesioned rats ran faster to reach the goal when it was associated with morphine. This indicates that the lesion affected instrumental performance and presumably the perceived rewarding properties of the drug. Interestingly, when the taste-drug pairings took place in the runway apparatus, the TOA lesion failed to interfere with avoidance of the morphine-paired cue in the way that it has when tested in the home cage (Nyland et al., 2012). Further, rats in both the TOAx and Sham groups displayed a significant loss in body weight following the naloxone test, which, if reflective of withdrawal, would be expected to precipitate drug seeking (i.e., faster running to the drug-paired goal). Overall, it appears as

though the lesion was without effect on drug-induced devaluation of the saccharin taste cue when presented in the runway apparatus. This unexpected finding may have been due to the presence of abundant contextual cues in the runway apparatus. Contextual cues may have enhanced the salience of the saccharin cue, and thus enabled the saccharin-morphine association. To test this hypothesis, the same rats were reassigned in a partial crossover design to control for previous morphine exposure and underwent taste-drug pairings using a novel Polycose CS and cocaine US in the home cage in Experiment 2.

Experiment 2

The failure of the lesion to inhibit suppression of taste cue intake in Experiment 1 was unexpected. To test if these results were unique to the runway paradigm, the same subjects underwent a follow up study pairing a novel taste cue with experimenter-administered cocaine in the home cages. The purpose of Experiment 2 was to determine if these subjects were capable of learning to avoid a taste cue when paired with experimenter-delivered drug in the home cage as previously shown (Nyland et al., 2012). Cocaine was selected as the US in the follow-up study for two reasons: 1) previous exposure to morphine may lead to tolerance if used in the follow-up study; and 2) both morphine and cocaine-paired taste cue avoidance was blocked by TOA lesions in previous home cage experiments (Nyland et al., 2012). If the lesioned rats were to exhibit normal drug-induced suppression of CS intake in the home cage (i.e., no deficit), then we would conclude that successful suppression of CS intake in the runway was due to misplaced lesions.

Methods

Subjects

The subjects that served in Experiment 1 were used in Experiment 2 in a partial cross over design in which half of the rats from each group (saline or morphine) were reassigned to one of two new drug conditions (saline or cocaine). Testing was conducted during the light phase of the cycle. Food and dH₂O were freely available, except where noted below.

Apparatus

The experiment was conducted in the home cages. Fluid 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.

Solutions

Cocaine hydrochloride was provided by the National Institute on Drug Abuse (Bethesda, MD). The 10 mg/kg dose of cocaine was prepared in sterile saline and administered subcutaneously (sc) as a 1.5 mg/ml stock solution, adjusted by body weight to avoid necrosis (Durazzo, Gauvin, Goulden, Briscoe & Holloway, 1994). Polycose (Sigma Chemical, St. Louis, MO) was dissolved in dH₂O and presented at room temperature.

Procedure

The rats were placed on a water deprivation schedule that allowed for 5 min access to dH₂O in the morning and 1 h each afternoon. Following stabilization of morning dH₂O intake (approximately 9 days), all rats were given 5 min access to a novel 0.03 M Polycose CS, and after a 5 min interval, injected sc with either saline or a 10 mg/kg dose of cocaine as described (Nyland et al., 2012). There were five such taste-drug pairings occurring at 48 h intervals. To maintain proper hydration, dH₂O was provided for 5 min each morning on the days between conditioning trials and for 1 h each afternoon.

Results

Histology

As mentioned in Experiment 1, data from six TOAx rats were excluded from statistical analysis due to incomplete lesions. This resulted in the following group numbers: Sham Saline n=9, Sham Cocaine n=9, TOAx Saline n=8, TOAx Cocaine n=10.

CS Intake (ml/5 min)

The results of the mixed factorial ANOVA for Polycose intake over the five taste-drug pairings verified that the Sham subjects, but not the TOAx subjects, suppressed intake of the taste cue that was paired with experimenter-administered cocaine (Figure 6).

This conclusion was supported by a significant lesion x drug interaction, $F(1,32) = 6.78$, $p < .05$, and drug x trial interaction, $F(4,128) = 7.94$, $p < .001$. There was a lack of significant 3-way (lesion x drug x trial) interaction; however, there were significant main effects of lesion, $F(1,32) = 9.96$, $p < .01$, and drug, $F(1,32) = 9.04$, $p < .01$. Therefore, follow-up analyses examined each lesion condition separately. The Sham lesion condition had a significant drug x trial interaction, $F(4,64) = 8.62$, $p < .001$; Newman-Keuls post hoc analyses revealed decreased intake for cocaine versus saline-treated rats on trials 3–5, $p < .01$. There was no significant drug x trial interaction for the TOAx rats, $F(4,64) = 1.32$, $p = .28$, as intake did not differ between cocaine and saline-treated groups across trials, and the main effect of drug also was not statistically significant, $F < 1$. Intake of the taste cue also was analyzed as a function of prior drug exposure in Experiment 1. The results of this analysis found no significant main effect of prior drug exposure, $F < 1$ (data not shown). Based on this result, we can conclude that TOAx rats failed to avoid intake of a cocaine-paired taste cue when presented in the home cage, despite successful avoidance of a morphine-paired cue in the runway in the same experimental subjects. Successful performance in the runway, then, was not due to misplaced lesions.

Discussion

When pairings took place in the home cage, TOAx subjects failed to suppress intake of the drug-paired taste cue. Indeed, even when prior drug exposure in the runway was taken into account, all TOAx rats failed to suppress intake of the cocaine-paired cue – even those TOAx rats that successfully avoided intake of the morphine-paired saccharin cue in the runway. This finding confirms and extends the disruptive effect of the TOA lesion on drug-

induced suppression of CS intake when tested in the home cage (Nyland et al., 2012), and shows that use of the runway had a significant influence on the rats' ability to demonstrate avoidance of a drug-paired taste cue.

General Discussion

As discussed, rats avoid intake of a saccharin cue when paired with a drug of abuse. Interestingly, for all its methodological simplicity, published data suggest that this phenomenon is, in fact, fairly complex involving both drug-induced devaluation of an otherwise palatable saccharin cue (Geddes et al., 2008; Gomez & Grigson, 1999; Grigson & Freet, 2000; Nyland et al., 2012; Schroy et al., 2005) and the onset of a conditioned aversive state involving craving and withdrawal (Colechio & Grigson, 2014; Colechio, Imperio & Grigson, 2014; Gomez et al., 2000; Grigson & Hajnal, 2007; Nyland & Grigson, 2013; Wheeler et al., 2008; Wheeler et al., 2011). Moreover, greater avoidance of a drug-paired cue, and greater aversive taste reactivity following its intraoral delivery, are highly correlated with greater drug-seeking and drug-taking behavior and this general relationship is true for both cocaine and for heroin (Colechio & Grigson, 2014; Colechio, Imperio & Grigson, 2014; Grigson & Twining, 2002; Twining et al., 2009; Imperio & Grigson, 2015; Nyland & Grigson, 2013; Wheeler et al., 2008).

Given this strong predictive relationship with drug-seeking and taking, it is crucial that we begin to identify the underlying neural substrates. As discussed, published data show that an intact TOA is essential for avoidance of a saccharin cue when paired with a drug of abuse such as morphine or cocaine, but not for avoidance of the same taste cue when paired with either sucrose or LiCl (Liang, Grigson & Norgren, 2012; Liang, Norgren & Grigson, 2012; Nyland et al., 2012). Successful performance in the anticipatory contrast and the CTA paradigms suggests that the failure to exhibit drug-induced avoidance of the gustatory cue following TOA lesions is not due to a failure to detect the gustatory stimulus, a general inability to associate a CS with the US, or a general inability to suppress intake of an immediately available palatable stimulus. Further, as placement of the lesion is proximal to visceral relays, the successful avoidance of a taste cue paired with LiCl-induced malaise indicates that the lesion's effects are not reliant on visceral input. We hypothesized, then, that the TOAx rats may fail to suppress intake of the drug-paired cue because they do not experience the rewarding properties of the drug. This hypothesis was tested here by examining the phenomenon in a runway. The results indicate that the lesion prevented an increase in running speed for morphine compared with saline. Thus, while sham rats traversed the runway more quickly when morphine was experienced in the Goal Box compared with saline, TOAx rats ran at similar rates in both the saline and the morphine condition. We conclude that the lesion interferes with perception of the rewarding properties of morphine. That said, the TOAx rats have been shown to increase operant responding for sucrose (Liang, Freet, Grigson & Norgren, 2012). Rats with TOA lesions, then, may be more responsive to a natural reward, such as sucrose, than to experimenter administered morphine. If the lesion interferes with the perceived rewarding properties of abused drugs, but leaves intact conditioned aversion associated with cue-induced withdrawal, one can conclude that avoidance of a drug-paired taste cue can arise from the onset of the conditioned aversive state of withdrawal, independent of the comparison of two disparate rewards.

The results of the current study also unveiled the unexpected finding that, when tested in the runway, the TOAx rats were capable of avoiding a morphine-paired saccharin cue. This outcome was not due merely to misplaced lesions, as these same TOAx rats were able to avoid a Polycose cue when paired with cocaine in the home cage. Thus, when tested in the runway, rats with TOA lesions were able to detect the taste cue and to use it to predict experimenter-administered morphine. Moreover, like the Sham controls, the lesioned rats also showed a loss in body weight when naloxone was administered instead of morphine on the final test trial. In our previous study, a saccharin yoked control did not show a loss of body weight (Nyland & Grigson, 2013), suggesting that this reduction in body weight is indicative of the onset of a state of conditioned withdrawal and not due to a mere reduction in CS intake. That said, the present study did not include such a yoked control; therefore, we cannot determine how much decreased CS intake contributed to the loss of body weight in this particular study. That said, while intake of the saccharin cue (about 600 licks) could account for the 3 g gain in body weight over 2 h for the saline injected controls (rats ingest about 5 μ l/lick), the modest intake (i.e., about 50 – 75 licks) of the 0.15% saccharin cue by the morphine-treated rats cannot account for the approximate 3 g loss in body weight over the 2 h period. Further, there was a correlation between CS intake and body weight loss in this study (Figure 5), and factors related to withdrawal including decreased food intake and increased defecation and urination likely contribute to the loss in body weight (Buckett, 1964; Lorenzetti & Sancilio, 1970; Martin, Wilker, Eades & Pescor, 1963).

Why then, does the lesion prevent drug-induced avoidance of a palatable taste cue when the experiment takes place in the home cage but not in the runway? Pavlovian conditioning is not as straight forward as the association of a CS and a US; the literature describes numerous factors such as phasic and contextual cues that can become associated with either the CS or the US. For example, contextual cues can become associated with the US, and can either block or enhance conditioning to a CS (Bouton & Bolles, 1979; Durlach, 1983; Grahame, Barnett, Gunther & Miller, 1994; Randich & Ross, 1984; Tomie, 1976). Other theories suggest that the context can become associated with the CS and facilitate appropriate responses to an otherwise ambiguous CS (Bouton & Bolles, 1979).

Results from the runway study suggest that the CS-US association is possible in the presence of contextual cues. While both procedures in this study paired a palatable taste cue with experimenter-administered drug, the runway experiment provided contextual cues that may have aided the development of the taste-drug association. In Experiment 2, the CS is presented in the home cage in a same manner as is dH₂O in the afternoons and on the days between drug pairings. In Experiment 1, the CS is presented in the runway only, while dH₂O is presented in the home cage in the afternoons and on days between drug pairings. Therefore, the runway apparatus becomes an environmental context that is specifically associated with the CS and US exposure. The overlap of cues may act by strengthening the valence of the CS, or may serve as an intermediary that is associated with both the CS and US separately.

According to Rescorla and Wagner (1972), the associative value of a cue gains strength when concurrently presented with other stimuli. For example, context can act as an occasion-setter that may strengthen the association of the taste cue and the US (Bouton &

Bolles, 1979). Alternatively, the salient context and the weak cue can form one combined stimulus, that, when presented together, can form a single association with the unconditioned stimulus (Darby & Pearce, 1995). As such, if the associative value of the taste cue is only effective after enhancement by the contextual stimuli, it may take longer to develop avoidance of the taste cue. This would explain the slightly delayed acquisition of avoidance of the taste cue in lesioned rats in the runway.

The hypothesis that the lesion weakens the associative strength of the taste CS with the drug of abuse also explains the results found in the taste-drug studies. For example, the lesioned rats failed to avoid the taste cue when it was paired with experimenter-administered morphine in the home cage, but they avoided it when presented in the runway apparatus. In this case, the main difference between the two experimental procedures was the presence of contextual cues. While the literature is teeming with theories on the role of context in associative learning, most agree that the presence of contextual cues alters the development of the CS-US association. This raises the question: are rats with lesions of the TOA associating the taste cue with drug, are they associating the context with drug, or are they associating the taste cue with the drug when presented in a distinctive environment? Further studies are necessary to elucidate the specific associative deficit that results from the TOA lesion; however, the deficits of this particular lesion have exposed drug-induced suppression of a taste cue as a third type of conditioning, likely including elements of both reward and aversion.

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Figure 1:
Digital photomicrographs of coronal sections of Sham (A) and TOAx (B) stained with NeuN. Abbreviations: fr, fasciculus retroflexus; mt, mammillary tract; Pf, parafascicular nucleus; Po, posterior nucleus; SPF, subparafascicular nucleus; TOA, trigeminal orosensory area; VPM, ventral posteromedial nucleus; VPMpc, parvocellular subdivision of the VPM (thalamic taste area).

Figure 2:

Mean 5-min intake ($\pm$ SEM) of 0.15% saccharin paired with experimenter-administered saline (open circles) or 15 mg/kg morphine (solid circles) across 6 conditioning trials for Sham (left) or TOAx (right) rats. *indicates a significant difference between the saline and morphine conditions ($p < .05$). Sham: Saline N=9, Morphine N=9; TOAx: Saline N=9, Morphine N=9

Figure 3:

Mean latency to reach the Goal Box represented as a percentage ($\pm$ SEM) of the first conditioning trial when it is associated with experimenter-administered saline (open circles) or 15 mg/kg morphine (solid circles) across 6 conditioning trials for Sham (left) and TOAx (right) rats. Sham: Saline N=9, Morphine N=9; TOAx: Saline N=9, Morphine N=9

Figure 4:

(a) Mean 5-min intake ($\pm$ SEM) of 0.15% saccharin at naloxone test for Sham (left) and TOAx (right) rats. (b) Mean change in body weight (g) ($\pm$ SEM) for Sham (left) and TOAx rats (right) 2-h after the naloxone test. *indicates a significant difference between the saline and morphine conditions ($p < .05$). Sham: Saline N=9, Morphine N=9; TOAx: Saline N=9, Morphine N=9

Figure 6: Mean 5-min intake ($\pm$ SEM) of 0.03 M Polycose paired with experimenter-administered saline (open circles) or 10 mg/kg cocaine (solid circles) across 5 trials for Sham (left) or TOAx (right) rats. *indicates a significant difference between the saline and cocaine conditions ($p < .05$). Sham: Saline N=9, Cocaine N=9; TOAx: Saline N=8, Cocaine N=10