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Abstinence-dependent transfer of lithium chloride-induced sucrose aversion to a sucrose-paired cue in rats

John H. Harkness, Sierra Webb, and Jeffrey W. Grimm

Department of Psychology and Program in Behavioral Neuroscience, Western Washington University, 516 High Street, Bellingham, WA 98225-9172, USA

Jeffrey W. Grimm: jeff.grimm@wwu.edu

Abstract

Rationale—Responding for a drug- or sucrose-paired cue increases over forced abstinence (incubation of craving). If the incentive value of a cue depends on the incentive value of the primary reward, devaluing the primary reward should reduce cue reactivity.

Objectives—We investigated whether conditioned taste aversion (CTA) to sucrose would transfer to a sucrose-paired cue after 1 or 30 days of forced abstinence and whether CTA after 1 day of forced abstinence would affect incubation of craving.

Materials and methods—Rats self-administered 10% sucrose paired with a tone + light cue for 10 days. After 1 (Exp.1) or 30 (Exp.2) days of forced abstinence, rats received two home-cage pairings of sucrose with either LiCl (65 mg/kg, IP) to produce CTA or saline as a control. Two days later, rats responded for the cue alone. The following day, sucrose consumption was assessed in the same operant conditioning chamber. Exp.1 rats were tested again 1 month later to determine if CTA would affect incubation of craving.

Results—Exp.1: CTA after 1 day of forced abstinence did not attenuate cue reactivity when tested immediately after CTA, nor did the treatment affect incubation of craving or incubation of sucrose consumption. Exp.2: CTA after 1 month of forced abstinence resulted in a significant reduction in cue reactivity.

Conclusion—The incentive values of sucrose and the conditioned representation of sucrose increase over an extended period of forced abstinence. This incubation appears to facilitate the transfer of an aversion to the primary reward to the conditioned cue.

Keywords

Addiction; Craving; Devaluation; Eating disorders; Incubation; Relapse

Introduction

Addiction and obesity treatments often fail due to relapse when an individual attempts to abstain from a drug or remain on a diet (Volkow and Wise 2005). Relapse into food and drug seeking can occur when a user is exposed to environmental cues that remind the user of their addiction. For example, smells, sights, sounds, and familiar people may induce cravings leading to relapse to drugs (Childress et al. 1999) or food (Hill 2007).

In rats, craving is operationally defined as responding on a lever either for, or in the absence of, a cue previously associated with reward (Grimm et al. 2000; Markou et al. 1993). Cue-induced drug or sucrose seeking increases over a period of forced abstinence in rats, described as an incubation of craving (Grimm et al. 2005; Grimm et al. 2001). Incubation of craving has been observed in human cocaine, heroin, and cigarette-smoking addicts (Bergquist et al. 2006; Kosten et al. 2005; Nava et al. 2006; Piasecki et al. 1998, 2000), and it is possible that humans experience incubated cravings in food addiction. Therefore, identification of manipulations that disrupt the incubation of craving in the animal model may have broad clinical implications for treatment of craving and relapse in protracted abstinence.

So far, several neurotransmitter/receptor (Conrad et al. 2008; Grimm et al. 2007; Lu et al. 2007; Uejima et al. 2007), growth factor (Grimm et al. 2003; Lu et al. 2009), and second messenger (Li et al. 2008; Lu et al. 2005) candidate targets have been identified for possible treatment of the incubation of craving, but only one behavioral approach has been effective, environmental enrichment (Grimm et al. 2008). Our interpretation of those findings was that enrichment positively affected the ability of rats to learn to refrain from lever pressing in extinction conditions. While incubation of craving likely also reflects a heightened motivational response toward reward-paired cues (Grimm et al. 2006), further investigation of such learning-based interventions is warranted.

The purpose of the present study was to evaluate if it would be possible to reduce environmental cue-induced sucrose craving, and potentially incubation of such craving, by pairing sucrose with illness in sucrose self-administration experienced rats. The approach was to mimic the clinical approach of aversion therapy used in the treatment of alcoholism. For example, disulfiram taken before alcohol consumption will lead to illness following alcohol ingestion, reducing future alcohol intake (Banys 1988; Tampier et al. 2008). Stimuli paired with alcohol will come to remind the individual of the now negative consequences of alcohol ingestion (Banys 1988; Tampier et al. 2008). Originally described for pairings between LiCl and an odor paired with food (Bermudez-Rattoni et al. 1986), such aversion conditioning in rats typically involves pairing the illness-inducing effects of LiCl with a flavored sweet solution (Limebeer and Parker 2006; Palmerino et al. 1980; Parker 1998). Following pairings, rats will reduce consumption of that flavor when introduced in solution with water, demonstrating that the illness associated with LiCl can lead to a conditioned taste aversion (CTA) (Parker 1998).

As stated above, we were interested in whether creating an aversion to sucrose would generalize to an environmental cue that was previously associated with sucrose. Holland et al. found that CTA to a sweet solution can transfer to an explicitly paired tone (Holland et al. 2008). Our operant-based self-administration procedure differs from Holland's Pavlovian-based procedure in several ways, but most distinctively in that self-administration requires response-contingent presentations of the cue. Our cue functions as a response-contingent cue, a conditioned reinforcer in our cue-reactivity testing conditions (Grimm et al. 2000).

Rats in the present study self-administered 10% sucrose for 10 days. A tone + light cue accompanied each sucrose delivery. For some rats, an aversion to sucrose was then created by pairing LiCl with sucrose. Exp.1 evaluated the effect of a CTA produced on days 1–2 post-sucrose self-administration on cue reactivity immediately thereafter (day 4) and again 1 month later (day 34). This allowed examination of whether CTA would transfer to a sucrose-paired cue early in forced abstinence and then whether the treatment would have effects on cue reactivity when tested 1 month later. That is, would CTA alter the development of the incubation of craving? Exp.2 evaluated the effect of a CTA created 1 month into forced abstinence on subsequent cue reactivity (day 34). This experiment allowed determination of whether the treatment would be effective in subjects where incubation of craving has already been established.

Materials and methods

Subjects

Eighty-nine male Long-Evans rats (3 months old; 366.2 ± 6.9 g at start of study; Simonsen-derived) bred in the Western Washington University vivarium were housed individually on a 12-h reverse day/night cycle (lights off at 7AM) with Purina Mills Inc. Mazuri Rodent Pellets and water available ad libitum. Rats were weighed each Monday, Wednesday, and Friday for the duration of the experiment. Immediately prior to the training phase, the animals were deprived of water for 17 h to encourage sucrose self-administration on the first day of training. Rats were then returned to ad libitum water access except for as noted below under “CTA acquisition phase”. All procedures followed the guidelines outlined in the “Principles of laboratory animal care” (NIH publication no. 85-23) and were approved by the Western Washington University Institutional Animal Care and Use Committee.

Apparatus

Operant training and testing took place in operant conditioning chambers ($30 \times 20 \times 24$ cm; Med Associates) containing two levers (one stationary and one retractable), a tone generator, a white stimulus light above the retractable lever, and a red house light on the opposite wall. An infusion pump delivered sucrose into a reward receptacle to the right of the active lever. Operant conditioning chambers were enclosed in sound-attenuating cabinets with ventilation fans.

Materials

Molecular Biology grade LiCl (Research Organics) was dissolved in saline to 65 mg/ml to be injected (IP) at 1 ml/kg. This dose of LiCl was described previously to be sufficient to produce a CTA (Limebeer and Parker 2006), yet results in negligible levels of serum LiCl 24 h post-injection (Olesen et al. 1976).

Design

A flow chart for the study is provided in Fig. 1. As indicated in the figure, rats in Exp.1 were tested for cue reactivity and sucrose consumption twice in forced abstinence (cue reactivity days 4 and 34; sucrose consumption days 5 and 35). Rats in Exp.2 were tested only once for cue reactivity and sucrose consumption (cue reactivity day 34; sucrose consumption day 35).

Procedure

Operant training phase—Rats spent 2 h/day for 10 consecutive days in operant conditioning chambers where they were allowed to press the retractable (active) lever for a 0.6-ml delivery of 10% sucrose solution into the receptacle to the right of the lever. This response also activated a compound stimulus consisting of a tone (2 kHz, 15 dB over ambient noise) and the white light. The compound stimulus lasted for 5 s and was followed by a 40-s time out, during which presses on the active lever were recorded but had no programmed consequence. A response on the inactive (stationary) lever did not have a programmed consequence, but presses were recorded. Four photobeams crisscrossed the chamber. The total number of beam breaks was recorded during cue-reactivity testing. At the end of each training session, rats were returned to home cages.

Forced-abstinence phase—The forced-abstinence phase began as day 1 (“d1”) following the 10th day of the training phase.

CTA acquisition phase—On days 9 and 10 of the training phase, rats in Exp.1 received an IP injection of saline (1 ml/kg) in their home cages to acclimate the animals to receiving

injections. Rats in Exp.2 received acclimation injections of saline on d29 and d30 of forced abstinence. On the 10th day of the training phase (Exp.1), or d30 (Exp.2), water bottles were removed from home cages for 48 h, and access to water was limited to 1 h/day for those 48 h. During these hours, water was available as 10% sucrose. That is, 200 ml of 10% sucrose solution was measured into water bottles and inserted into the home cage for 1 h every 23 h. Consumption was measured as the post-consumption period volume subtracted from 200 ml. Immediately following the removal of the sucrose bottles, control groups received an IP injection of saline; CTA groups received LiCl (65 mg/kg). Following injections on the second injection day, water bottles were returned to the home cages, and consumption remained ad libitum for the duration of the study. Sucrose consumption was then measured on d3 (Exp.1) or d33 (Exp.2) in home cages with the same 1-h procedure for sucrose bottle access, except that this session was not followed with a saline or LiCl injection.

Testing phase—On d4 and d34 (Exp.1) or d34 only (Exp.2), rats were tested in the operant conditioning chambers for sucrose cue reactivity. This session was identical to the 2-h training procedure, except that sucrose was not delivered following a lever response. Rats were then returned to home cages. The following day (d5 or d35 in Exp.1; d35 only in Exp.2), rats were returned to the operant boxes with the active lever retracted and the house light turned off. They were given access to a 200-ml bottle of 10% sucrose solution for 1 h. At the end of the 1-h period, consumption was measured. This measure was used to verify that CTA-trained animals found sucrose aversive in an environment other than where CTA pairing took place (as indicated above, LiCl was only administered in the context of home cages followed by immediate housing in home cages).

Statistical analyses

Operant training phase—Active lever responding during operant training was analyzed using two-way repeated measures (RM) ANOVA of the last 4 days of training using between-group factors of Day (1 or 30) and Treatment (saline or LiCl). This analysis was used to verify that all groups across experiments received equal training.

CTA acquisition phase—Sucrose consumption during CTA acquisition was analyzed for Exp.1 and Exp.2 using RM ANOVAs of the three CTA consumption trials (days 1–3 or days 31–33) using the between-groups factor of Treatment (saline or LiCl). This examination was carried out to verify that LiCl-treated rats acquired a CTA for sucrose.

Testing phase—Exp.1: The effects of treatments for the dependent measures were evaluated using RM ANOVAs. The within-subjects factor was Day (4 vs. 34 or 5 vs. 35 depending on the dependent measure) and the between-groups factor was Treatment (saline or LiCl). Active lever responding during the cue-reactivity test was also examined as a time course where responding over the 2-h sessions was divided into 12 10-min bins. These data were statistically tested using RM ANOVA (Time $\times$ Treatment). Day 4 and day 34 responding was tested separately for this measure. Exp.2: An initial Day $\times$ Treatment ANOVA of active lever responses during the cue-reactivity test was calculated to compare the effect of CTA on cue reactivity between Exp.1 and Exp.2. The effects of treatments for the other dependent measures in Exp.2 were evaluated using *t* tests. As in Exp.1, active lever responding during the cue-reactivity test was also examined as a time course where responding over the 2-h session was divided into 12 10-min bins. These data were statistically tested using RM ANOVA (Time $\times$ Treatment).

For all ANOVAs, post hoc comparisons were made with either LSD tests for between-group comparisons or paired *t* tests for within-group comparisons. As described in the “Discussion” section, two incubation-related comparisons between saline groups in Exps. 1 and 2 were made

with *t* tests. In addition, Pearson's *r* correlations were calculated among three dependent measures in select groups, but separately for Exp.1 and Exp.2.

ANOVAs and Pearson's *r* correlations were calculated using SPSS version 16.0. The *t* tests were calculated using Microsoft Excel 2007. Group data are presented as means $\pm$ standard errors of the means (SEMs) in the text and figures. For statistical comparisons, $p < 0.05$ was the criterion for statistical significance. In general, only the statistics for significant effects and interactions are indicated in the text.

Results

Operant training phase (all subjects)

Of 89 rats that were trained for sucrose self-administration, 11 were removed from the study because they did not meet the minimum response criteria of an average 20 presses/day over the last 4 days of training, or their average lever pressing or CTA training sucrose consumption was greater than two standard deviations away from the overall group mean. Therefore, the final *N* for the study was 78 with final groups comprised of: Exp.1 saline ($n=20$), Exp.1 LiCl ($n=19$); Exp.2 saline ($n=19$), Exp.2 LiCl ($n=20$). There were no significant differences in training between groups. Means $\pm$ SEMs for average active lever responding across training days 7–10 were: Exp.1 saline, 95.7 ± 9.4 ; Exp.1 LiCl, 90.4 ± 9.4 ; Exp.2 saline, 95.8 ± 9.9 ; and Exp.2 LiCl, 93.1 ± 7.6 .

Exp.1

CTA acquisition phase—Sucrose consumption decreased over CTA acquisition days in LiCl-treated, but not saline-treated, rats (Treatment $F(1,37)=56.7$, $p < 0.0001$; Time $F(2,74)=8.6$, $p < 0.0001$; Treatment $\times$ Time $F(2,74)=20.0$, $p < 0.0001$). Rats that experienced pairings of sucrose with LiCl almost completely refrained from drinking sucrose by the third consumption trial (Fig. 2).

Testing phase—For active lever responding totals for the 2-h test session, there was a main effect of forced-abstinence period (Day $F(1,37)=12.8$, $p < 0.01$) but no effect of CTA treatment nor an interaction between Day and Treatment (Fig. 3).

For active lever responding as a time course on Day 4 there was only a significant effect of Time ($F(11,407)=60.0$, $p < 0.0001$). This was also observed for Day 34 active lever responding (Time $F(11,407)=41.9$, $p < 0.0001$) (Fig. 4).

Inactive lever responding was greater in the Day 34 test session as compared to the Day 4 test session (Day $F(1,37)=8.3$, $p < 0.01$). There was no effect of CTA treatment on inactive lever responding. Similarly, locomotor activity was greater in the Day 34 test session as compared to the Day 4 test session (Day $F(1,37)=8.1$, $p < 0.01$), and again there was no effect of CTA treatment. Inactive lever pressing means $\pm$ SEMs were (saline d4, LiCl d4, saline d34, LiCl d34) 7.4 ± 1.4 , 10.1 ± 1.6 , 15.9 ± 4.7 , and 14.2 ± 2.7 . Photobeam break means $\pm$ SEMs were (saline d4, LiCl d4, saline d34, LiCl d34) $1,733.9 \pm 126.3$, $1,971.0 \pm 94.9$, $2,037.6 \pm 108.9$, and $2,107.6 \pm 147.9$.

Saline-treated rats consumed more sucrose in the operant conditioning chambers than LiCl-treated rats during the free-access tests both on Days 5 and 35 of forced abstinence (Treatment $F(1,37)=408.2$, $p < 0.0001$). There was also a significant effect of Day ($F(1,37)=4.2$, $p < 0.05$), indicating that consumption on Day 35 was generally greater than on Day 5 for all rats (Fig. 5).

The operant box sucrose consumption findings not only demonstrate the strength of CTA to sucrose but also that sucrose consumption incubated from the early time point to the late time point. We observed some indication of an incubation of sucrose consumption in a previous study (Grimm et al. 2005). However, the present data represent our clearest indication of an incubation of sucrose consumption.

Exp.2

CTA acquisition phase—Sucrose consumption decreased over CTA acquisition days in LiCl-treated, but not saline-treated, rats (Treatment $F(1,37)=103.4$, $p<0.0001$; Time $F(2,74)=36.1$, $p<0.0001$; Treatment $\times$ Time $F(2,74)=37.8$, $p<0.0001$). Rats that experienced pairings of sucrose with LiCl almost completely refrained from drinking sucrose by the third consumption trial (Fig. 6).

Testing phase—When comparing the results of active lever responding between d4 in Exp. 1 and d34 in Exp.2, there was a significant Day $\times$ Treatment interaction of $F(1,74)=8.7$, $p<0.01$. There were also significant main effects of Day and Treatment, Day $F(1,74)=16.8$, $p<0.0001$, Treatment $F(1,74)=11.8$, $p<0.01$. CTA treatment led to a significant decrease in cue reactivity only when it occurred after 1 month of forced abstinence (Fig. 7).

Analyses of active lever responding as a time course revealed a significant effect of Treatment ($F(1,37)=13.3$, $p<0.01$), a significant effect of Time ($F(11,407)=68.4$, $p<0.0001$), and a significant Treatment $\times$ Time interaction ($F(11,407)=4.0$, $p<0.0001$) (Fig. 8).

Inactive lever responding and locomotor activity did not significantly differ according to Treatment. Inactive lever pressing means $\pm$ SEMs were (saline d34, LiCl d34) 7.6 ± 1.9 , 8.8 ± 1.3 . Photobeam break means $\pm$ SEMs were (saline d34, LiCl d34) $2,047.0\pm177.9$, $2,279.7\pm133.3$.

Saline-treated rats consumed more sucrose in the operant conditioning chamber than LiCl-treated rats during the free-access test Day 35 of forced abstinence ($t(37)=12.5$, $p<0.0001$) (Fig. 9).

Discussion

The ability of a taste aversion to sucrose to transfer to a sucrose-paired cue was only apparent following 1 month of forced abstinence from sucrose self-administration. As observed in Exp. 1, CTA acquisition immediately following sucrose self-administration training had no effect on cue reactivity immediately thereafter, nor after a subsequent 1 month forced-abstinence period (Fig. 3, Fig. 4, and Fig. 7). In contrast, as observed in Exp.2, rats that had CTA training after 1 month of forced abstinence responded significantly less for a sucrose-paired cue vs. rats trained with saline (Fig. 7 and Fig. 8). This finding of a time dependence for transfer of the negative hedonic effect of LiCl to the sucrose-paired cue (in effect, a generalization of the devalued unconditioned stimulus (US) to the conditioned stimulus (CS)) has not been described previously. There are several possible reasons for the time dependence of the effect.

Locomotor activity was higher after 1 month of forced abstinence when comparing d4 to d34 in Exp.1 and also when comparing saline d4 Exp.1 with saline d34 in Exp.2 ($t(37)=2.4$, $p<0.05$). We have observed this effect previously (Grimm et al. 2006, 2008). Locomotor activity correlated fairly well with active lever responding in saline-treated groups in both Exp.1 and Exp.2 (Pearson's r Exp.1=0.5, $p<0.05$; Pearson's r Exp.2=0.4, $p<0.05$) although the relationship was weaker for rats in the LiCl conditions (Pearson's r Exp.1=0.3, $p=0.1$; Pearson's r Exp.2=0.3, $p=0.08$). Regardless, an abstinence-dependent increase in locomotor activity does not appear to account for the abstinence-dependent decrease in cue reactivity in

the LiCl-treated rats in Exp.2. Activity was similar between saline and LiCl-treated rats, yet active lever responding was much less in the LiCl condition. Furthermore, we do not find evidence to suggest that the CTA effect was mediated by generalized changes in response rate or response manipulandum discrimination. Inactive lever responding was similar (and very low) in all groups (both experiments).

Habit is responding unrelated to the current value of the reinforcer (Balleine et al. 2007). Immediately after 10 days of self-administration training, rats may have been pressing the lever not only because it had been previously reinforced with sucrose but also out of habit. It has been found that late in training, some portion of lever pressing may be due to a persistence of responding in the absence of the reinforcer (Di Ciano and Everitt 2004). Such a habit-based explanation of the lack of aversion transfer in Exp.1 could also relate to a possible floor effect in that experiment. Transfer of an aversion may be possible at the early time point, but the baseline rate of responding in extinction conditions (perhaps related to habit as discussed above) is consistent enough to serve as a floor effect.

Habit and/or a floor effect may explain the lack of aversion transfer in Exp.1. However, these phenomena would not entirely explain why aversion transfer was possible in Exp.2. One possible explanation is that the representation in memory of the CS (tone + light) in relation to the US (sucrose) may converge over forced abstinence such that aversion transfer is facilitated. It has been demonstrated that the US can manipulate hedonic properties of the CS after minimal CS-US pairings, but that transfer of the hedonic value of the US to the CS is diminished after extended exposure (Holland et al. 2008). Specifically, transfer of aversion from the aversion target to a conditioned stimulus is effective following relatively few pairings (e.g., 16) of the US to the CS (Holland et al. 2008; Kerfoot et al. 2007) but not following several times that amount (e.g., 112) (Holland et al. 2008). Extended CS-US “overtraining” would appear to limit the extent to which the value of the CS can be learned from the US (Holland 2005; Holland et al. 2008). Over the 10-day training procedure, our rats were exposed to 759 CS-US pairings on average, well above the 112 pairings shown by Holland et al. to reduced aversion transfer. Although our training procedure differed from Holland et al. in several ways, in particular, our procedure was primarily operant-based while theirs was Pavlovian, and there are parallels between their findings and our early time point results. It could be that subsequent time away from the CS and/or US (e.g., forced abstinence) reduces the effect of the overtraining. The relationship between the CS and US would then become more salient to the subject.

Time dependence in the flexibility of the CS-US relationship could further be affected by a time-dependent change in the hedonic values of the US and/or CS. Sucrose consumption was greater following 1 month of forced abstinence from sucrose self-administration (Grimm et al. 2005; Exp.1 present study). Furthermore, this effect was apparent when comparing consumption on the first day of CTA acquisition in saline-treated rats in Exp.1 (d1) vs. Exp.2 (d31) $t(37)=-3.1, p<0.01$. This incubation of sucrose consumption is supporting evidence for the hypothesis that rats respond more for a conditioned cue after a forced-abstinence period in part because the incentive value of the primary reward has increased. This hypothesis complements our previous assertion that incubation of sucrose (cue) craving reflects an increase in motivation to respond for the conditioned rewarding properties of the cue (Grimm et al. 2006, 2008).

If it is the case that conditioned stimuli become more valuable later in forced abstinence, cues presented at late time points may be associated more closely to the value of the primary reward, and thus, the hedonic properties of the cues are more accessible to the transfer of aversion from the primary reward. Incubation appears to be a key motivational factor that contributes to the vigor of both cue reactivity and sucrose consumption, and therefore, the hypothesis of

incubated sucrose craving and incubated sucrose cue saliency following forced abstinence, along with Holland's hypothesis of a dynamic CS-US relationship, may explain our observation of abstinence-dependent transfer of aversion. However, the results of the d34 repeated testing in Exp.1 add an interesting twist to this hypothesis in that CTA acquired immediately following sucrose self-administration training resulted in a persistent aversion to sucrose, yet sucrose cue reactivity was unaffected. It is as if the rats in Exp.1 failed to learn that the CS was tied to the newly devalued US when tested at d4 and that this lack of learning carried over to the d34 test. This would imply that some component of responding for the cue occurs outside of its direct association with sucrose. The fact that responding on d34 in Exp.2 was reduced in the LiCl-treated rats, *but not completely abolished*, would support this conclusion. In addition, active lever responding was not predictive of sucrose consumption in the operant conditioning chamber the next day in saline-treated rats in either Exp.1 (d4) or Exp. 2 (d34) (Pearson's r Exp.1=-0.1, $p=0.3$; Pearson's r Exp.2=0.1, $p=0.3$) nor was locomotor activity (perhaps a measure of conditioned appetitive approach behavior) during the cue-reactivity test predictive of sucrose consumption the next day in the operant conditioning chamber (Pearson's r Exp.1=0.03, $p=0.4$; Pearson's r Exp.2=0.005, $p=0.5$). It would appear that responding for the cue is a multi-dimensional behavior composed of at least four dissociable components: habit, value of the US, value of the CS, and incentive motivation. Regarding the results of the present study, we hypothesize that the memory of the value of the US was susceptible to aversion transfer to the CS only after an extended forced-abstinence period.

The neuropharmacological substrates of the acquisition and expression of CTA and related aversion conditioning (e.g., reinforcer devaluation) in rats have been explored in detail beginning with examination of the importance of the amygdala in the association of a food aversion with an initially neutral odor (Bermudez-Rattoni et al. 1986). The role of the amygdala, and of neurotransmitters including glutamate in amygdalar sub-regions such as the basolateral amygdala in conditioned aversion, has subsequently been substantiated in CTA and reinforcer devaluation paradigms (Johnson et al. 2009; Yasoshima et al. 2000) (but see Reilly and Bornoalova (2005) for an alternative neophobia interpretation). In addition to lower brainstem regions, including the parabrachial nucleus (Sclafani et al. 2001), other mesolimbic structures have also been identified as providing key roles for CTA including the nucleus accumbens (Ramirez-Lugo et al. 2007) and cortical regions including orbitofrontal cortex (Pickens et al. 2003). Various neurotransmitters relevant to CTA have been identified in these structures including dopamine (Fenu and Di Chiara 2003), glutamate (Yasoshima et al. 2000), norepinephrine (Ventura et al. 2007), and acetylcholine (Mark et al. 1995). As these mesolimbic structures and their neurotransmitters are key anatomical substrates of learning and motivation (Stalnaker et al. 2009; Wise 2004), we speculate that the transfer of aversion from sucrose to the sucrose-paired stimulus involves similar mechanisms. For example, it is clear that the magnitude of responding for a cue previously associated with sucrose depends on intact basolateral amygdalae (Burns et al. 1993) and nucleus accumbens dopamine (Burns et al. 1993; Phillips et al. 1994). In addition, recent evidence indicates a critical role for central amygdala glutamate in the incubation of sucrose craving (Uejima et al. 2007). Finally, the orbitofrontal cortex contributes to revaluation of a stimulus (Stalnaker et al. 2009). Future studies using transfer of aversion within the incubation of craving procedure that incorporate experimental manipulation of these structures and neurotransmitters may elucidate the particular neuropharmacology underlying the present results.

Summary and conclusions

CTA acquired after 1 month, but not 1 day, of forced abstinence led to attenuated responding for a sucrose-paired cue. It is possible that aversion transfer does not readily affect cue reactivity in early forced abstinence because of a relative inflexibility in the CS-US relationship and/or

a predominance of habit responding. However, after a period of forced abstinence, an increase in the rewarding value of both the US and the CS allows the CS–US relationship to become more malleable to aversion transfer from the US to the CS. Consideration of abstinence-dependent changes in the flexibility of the CS–US relationship reveals important implications for the treatment of addiction behaviors such as relapse. That is, treatments that rely on learning procedures need to take into consideration the dynamic intrinsic and incentive-motivational strengths of the CS and US as well as the CS–US relationship to select the most appropriate treatment to lower the risk of relapse elicited by environmental cues. Results from this study may be of particular importance to disulfiram-based aversion treatment for alcoholism, or for other addictions that might benefit from revaluation techniques including addiction to other drugs of abuse or to food.

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Fig. 1.

Design of the study. Following 10 daily sessions of sucrose self-administration in the operant conditioning chamber with each sucrose delivery accompanied with a tone + light cue, rats were given LiCl or saline paired with sucrose over a 3-day CTA acquisition period in the home cage. Pairings occurred either 1 or 30 days following the 10th day of sucrose self-administration. On subsequent days, sucrose cue reactivity and sucrose consumption were assessed in the operant conditioning chamber. Exp.1 rats were tested for these measures at both the early and late force-abstinence time points. Forced-abstinence days are indicated as d1–d5 or d31–d35

Fig. 2.

Exp.1 CTA acquisition. Rats in early forced abstinence from sucrose self-administration that received daily pairings of sucrose with LiCl decreased subsequent sucrose consumption.

* $p < 0.05$ indicates significant difference from saline-treated group at that time point; # $p < 0.05$ indicates significant difference from d1 time point for that group

Fig. 3.

Exp.1 active lever responding totals. Rats were tested for cue reactivity on both d4 and d34 of forced abstinence. # $p < 0.05$ indicates a main effect of forced-abstinence period

Fig. 4.

Exp.1 active lever responding time courses for d4 and d34. Cue-reactivity responses are grouped in 10-min bins. # $p < 0.05$ indicates a significant difference, from the first bin for the saline and LiCl-treated animals combined

Fig. 5.

Exp.1 sucrose consumption in the operant conditioning chamber. Free-access sucrose consumption was measured for 1 h in the operant conditioning chamber on both d5 and d35 of forced abstinence. # $p < 0.05$ indicates a main effect of forced-abstinence period, and * $p < 0.05$ indicates a main effect of drug treatment

Fig. 6.

Exp.2 CTA acquisition. Rats in extended forced abstinence from sucrose self-administration that received daily pairings of sucrose with LiCl decreased subsequent sucrose consumption.

* $p < 0.05$ indicates significant difference from saline-treated group at that time point; # $p < 0.05$ indicates significant difference from d31 time point for that group

Fig. 7.

Exp.1 vs. Exp.2 active lever responding totals. Active lever responses during the cue-reactivity test on d4 in Exp.1 and d34 in Exp.2 were compared with a Treatment $\times$ Day ANOVA.

Following a significant statistical interaction, $*p < 0.05$ indicates a significant effect of drug treatment on d34 (Exp.2), while $\#p < 0.05$ indicates a significant difference between the d4 and d34 time points for saline-treated rats

Fig. 8.

Exp.2 active lever responding time course. Cue-reactivity responses are grouped in 10-min bins. * $p < 0.05$ indicates a significant difference between saline and LiCl-treated rats at that time point; # $p < 0.05$ indicates a significant difference from the first bin for that treatment group at that time point

Fig. 9. Exp.2 sucrose consumption in the operant conditioning chamber. Free-access sucrose consumption was measured for 1 h in the operant conditioning chamber on d35 of forced abstinence. * $p < 0.0001$ indicates a significant effect of drug treatment