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The role of dose and restriction state on morphine-, cocaine-, and LiCl-induced suppression of saccharin intake: A comprehensive analysis

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Abstract

Rats avoid intake of a taste cue when paired with a drug of abuse or with the illness-inducing agent, lithium chloride (LiCl). Although progress has been made, it is difficult to compare the suppressive effects of abused agents and LiCl on intake of a gustatory conditioned stimulus (CS) because of the cross-laboratory use of different CSs, different unconditioned stimuli (USs), different doses of the drugs, different conditioning regimens, and different restriction states. Here we have attempted to unify these variables by comparing the suppressive effects of a range of doses of morphine, cocaine, and LiCl on intake of a saccharin CS using a common regimen in non-restricted, food restricted, or water restricted male Sprague-Dawley rats. The results showed that, while the putatively aversive agent, LiCl, was effective in suppressing intake of the taste cue across nearly all doses, regardless of restriction state, the suppressive effects of both morphine and cocaine were greatly reduced when evaluated in either food or water restricted rats. Greater sensitivity to drug was revealed, at very low doses, when testing occurred in the absence of need (i.e., when the rats were non-restricted). Together, these results provide the first uniform and comprehensive analysis of the suppressive effects of morphine, cocaine, and LiCl as a function of dose and restriction state. In the present case, the suppressive effects of morphine and cocaine are found to differ from those of LiCl and, in some respects, from one another as well.

Keywords

reward comparison; conditioned taste aversion; contrast; drugs of abuse; natural rewards

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1. INTRODUCTION

In aversive conditioning, rats decrease intake of a saccharin conditioned stimulus (CS) when paired with unconditioned stimuli (US) that cause gastrointestinal distress such as LiCl or X-radiation (Garcia, Kimeldorf et al. 1955; Smith, Morris et al. 1964; Levy, Carroll et al. 1974; Palmerino, Rusiniak et al. 1980). The magnitude of such conditioned taste aversions (CTA) increases with the intensity of the aversive US (Nachman and Ashe 1973; Spector, Smith et al. 1986; Grigson 1997). In a somewhat similar appetitive conditioning paradigm, rats decrease intake of a saccharin CS when it is paired with consumption of a highly preferred concentration of sucrose. This phenomenon is termed anticipatory contrast and is thought to occur because rats avoid intake of the lesser valued saccharin CS in anticipation of the availability of the preferred high concentration of sucrose (Flaherty and Checke 1982; Flaherty and Grigson 1988). Rats also decrease intake of a saccharin CS when paired with the administration of a psychoactive drug such as morphine, cocaine, or amphetamine (Le Magnen 1969; Cappell and LeBlanc 1971; Cappell, LeBlanc et al. 1973; Ferrari, O'Connor et al. 1991). This finding has been traditionally interpreted as a CTA induced by the aversive properties of drugs of abuse and, as such, as evidence that similar mechanisms mediate the suppressive effects of both drugs of abuse and LiCl (Nachman, Lester et al. 1970; Goudie, Dickins et al. 1978; Goudie 1979; Hunt and Amit 1987).

There is much evidence, however, that points to a distinct mechanism for taste avoidance induced by drugs of abuse compared with that induced by LiCl (Parker 1988; Parker 1991; Parker 1995; Grigson 1997; Grigson, Lyuboslavsky et al. 1999; Grigson and Freet 2000; Grigson, Wheeler et al. 2001; Grigson and Twining 2002; Schroy, Wheeler et al. 2005; Wheeler, Twining et al., 2008). For example, the suppressive effects of a rewarding sucrose US and drugs of abuse, but not LiCl, are similarly affected by manipulations of the intensity and palatability of the CS (Flaherty, Turovsky et al. 1994; Bevins, Delzer et al. 1996; Grigson 1997; Risinger and Boyce 2002), selectively bred strains (Kosten, Miserendino et al. 1994; Wheeler 2002; Wheeler 2002; Davis, Roma et al. 2007), bilateral lesions of the gustatory thalamus (Flynn, Grill et al. 1991; Reilly and Pritchard 1996; Scalera, Grigson et al. 1997; Grigson, Lyuboslavsky et al. 2000; Schroy, Wheeler et al. 2005), as well as exaggeration of the suppressive effects by chronic morphine treatment (Grigson, Wheeler et al. 2001).

Indeed, more recent data have made it clear that LiCl and drugs of abuse do not merely differ in their drug properties, but in the nature of the conditioning process that is engaged in anticipation of their delivery. For example, when using intake tests that necessitate voluntarily consumption of the saccharin CS, the suppressive effects of LiCl, but not drugs of abuse, elicit aversive orofacial responses (Grill and Norgren 1978; Grill and Norgren 1978; Parker and Carvell 1986; Parker 1991; Parker 1995). When the CS is intraorally infused, however, we now know that both LiCl and cocaine can support the onset of conditioned aversive taste reactivity (i.e., gapes) to the CS (Grigson, Twining et al. 2008; Wheeler, Twining et al., 2008; Wheeler, Aragona et al. 2011). The underlying mechanism in these cases is, nevertheless, distinct. For LiCl, the taste (CS) merely predicts nausea (US); for cocaine, the taste cue elicits a conditioned compensatory response (CCR) to the physiological and neurochemical impact of cocaine delivery. The CCR occurs during the

waiting period, prior to cocaine consumption, and is, by definition, opposite to the drug's rewarding effects and likely contributes to tolerance (Baptista, Siegel et al. 1998; Kim, Siegel et al. 1999; Ramos, Siegel et al. 2002). Along these lines, avoidance of the drug-paired taste cue also is associated with elevated corticosterone levels (Gomez, Leo et al. 2000) and blunted or even reduced levels of dopamine in the nucleus accumbens (Grigson and Hajnal 2007; Wheeler, Aragona et al. 2011). Finally, as evidence that the CCR involves a negative affective and physiological state, greater avoidance of the taste cue and/or aversive taste reactivity is associated with a shorter latency to the first infusion of cocaine, a greater number of infusions of cocaine self-administered in the first 10 minutes of access (i.e., greater "load-up" behavior), a greater willingness to work for cocaine on a progressive ratio schedule, and more rapid acquisition of cocaine self-administration across trials (Grigson and Twining 2002; Grigson, Twining et al. 2008; Wheeler, Twining, et al., 2008; Twining, Bolan et al. 2009).

Another variable that may dissociate the suppressive effects of LiCl from drugs of abuse is restriction state. For example, when using a sucrose CS, food restriction exerts a greater disruption on the suppressive effects of a sucrose US and a drug of abuse than the suppressive effect of LiCl (Flaherty, Grigson et al. 1991; Gomez and Grigson 1999). Similarly, water restriction also prevents the avoidance of a sucrose CS when paired with morphine, but not LiCl (Grigson, Lyuboslavsky et al. 1999). Finally, when using a saccharin CS, food restriction disrupts the suppressive effects of amphetamine and chlordiazepoxide, while leaving those of a standard dose of LiCl intact (Bell, Thiele et al. 1998). Food- and water restriction, then, can attenuate avoidance of a taste cue and these effects might be greater when a drug of abuse, rather than the aversive agent, LiCl, serves as the US.

Since drugs of abuse and LiCl can vary in intensity, it is critical that the reduction in CS intake is compared across a range of doses. Dose effects, however, also can vary greatly across laboratories using slightly different testing regimens. For example, some have reported no differences in the magnitude of morphine-induced suppression of saccharin intake across a range of doses (9, 10, 20, 17, 50, and 80 mg/kg) of the drug (LeBlanc and Cappell 1975; Riley, Jacobs et al. 1978). Others have reported greater suppressive effects with the lower doses (Farber, Gorman et al. 1976), while others have reported greater suppressive effects with the higher doses of the drug (Siegel, Parker et al. 1995). Given conflicting reports of this nature, and the marked effect that restriction state can have on the willingness to avoid intake of an otherwise palatable gustatory cue (Grigson, Twining et al. 2000; Grigson, Cornelius et al. 2001), a full, systematic investigation is warranted. To this end, the current series of experiments was designed to evaluate suppression of intake of a saccharin CS following pairings with morphine, cocaine, and LiCl across a range of doses in non-restricted, food restricted, and water restricted rats.

2. METHODS

2.1 Subjects

The subjects were 695 male Sprague-Dawley rats (Charles Rivers Laboratories) weighing between 250 and 350 grams (mean: ~290 grams) at the beginning of the experiment. All rats were housed individually in stainless steel hanging cages in a temperature-controlled (21°)

animal care facility with a 12:12 hour light:dark cycle (lights on at 7:00 a.m.). All experimental manipulations were conducted approximately 3 h into the light phase of the cycle. The rats were maintained with free access to dry Harlan Teklad rodent diet (W) 8604 and water, except where noted otherwise.

2.2 Apparatus

All experimental manipulations were conducted in the home cages using inverted Nalgene graduated cylinders and stainless steel spouts affixed to the front of the cage. Total intake was recorded to the nearest ml. All LiCl and morphine injections were administered interperitoneally (ip), while the cocaine injections were administered subcutaneously (sc). A stock solution of 1.5 mg cocaine-HCl/ml saline was used with all rats to prevent skin necrosis typical of higher concentrations (injection volumes varied to obtain the desired dosage). Stock solutions for ip morphine and LiCl injections varied which allowed similar injection volumes across doses; however, injection volumes were still dependent upon body weight and dosage (morphine: 0.25 - 0.70 ml; LiCl: 3.3 - 4.7 ml). Any injection in which the volume was deemed too high for a single injection was split and administered at two separate injection sites (this was rarely a concern and only at the highest drug doses). Sodium saccharin and LiCl were obtained from the Sigma Chemical Company, St. Louis, MO and NaCl was obtained from Fisher Chemical, Pittsburgh, PA. Morphine sulfate and cocaine hydrochloride were generously provided by the National Institute on Drug Abuse and dissolved in sterile saline on the morning of each injection.

2.3 Procedure: Water restricted experiments

Rats were water restricted and trained to come to the front of the cages to drink for 4 days. During this time the rats received 5 min access to purified H₂O in the morning and 1h in the afternoon to maintain hydration. Baseline water intake was evaluated over 8 days. Groups were matched on the basis of their 5-min a.m. water intake on the final two days of baseline into the following groups: LiCl: saline (n=16), 0.002 M LiCl (n=8), 0.004 M LiCl (n=8) 0.009 M LiCl (n=8), 0.018 M LiCl (n=8) 0.037 M LiCl (n=8) 0.075 M LiCl (n=8), and 0.15 M LiCl (n=8); Morphine: saline (n=18), 0.5 mg/kg (n=8), 1.5 mg/kg (n=8), 3 mg/kg (n=8), 5 mg/kg (n=10), 15 mg/kg (n=10), 30 mg/kg (n=10), and 60 mg/kg (n=8); Cocaine: saline (n=33), 1.25 mg/kg (n=8), 2.5 mg/kg (n=8), 5.0 mg/kg (n=15), 7.5 mg/kg (n=15), 10 mg/kg (n=15), 15 mg/kg (n=10), 20 mg/kg (n=10), and 40 mg/kg (n=9). Drug dose groups were run over several replications and each replication had a control (saline) group. As the saline animals received the same experience, they were pooled resulting in a larger control group compared with the drug dose groups. In addition, drug dose group size generally started at n=8, although when possible, additional rats were included depending on the replication which also contributed to different group sizes. Regarding the dose range, the 0.15 M dose of LiCl is a very high, but also standard dose of LiCl that is used in CTA experiments (Spector, Norgren et al. 1992; Scalera, Grigson et al. 1997). The suppressive effect of the 0.009 M dose of LiCl on 5-min CS intake was found to be roughly equivalent to that induced by a 15 mg/kg dose of morphine and a 10 mg/kg dose of cocaine when assessed in water restricted rats (Grigson, 1997). During successive taste-drug pairings (which served as both the conditioning and testing stages), all rats were given 5 min access to a 0.15% saccharin solution, and after a 5 min wait, were injected with their assigned drug and dose or

saline. Each dose of LiCl had an appropriate amount of NaCl added to bring the total salt molarity to 0.15 M, which is isotonic with physiological saline. There was one pairing every other day for a total of 8 pairings. All subjects received 1h access to purified H₂O every afternoon to rehydrate. On the mornings between injections, they also received 5 min access to purified H₂O.

2.4 Procedure: Food restricted experiments

Rats were food restricted to 82% of their body weights prior to testing. Rats were given their daily ration 45 minutes after testing each day. All rats were water restricted as described previously in the water restriction experiments. Free access to water was then restored and baseline intake (5-min morning H₂O) was evaluated over 8 additional days. The subjects were matched on the basis of their 5-min H₂O intake on the final two days of this baseline period and assigned to the following groups: LiCl: saline (n=11), 0.002 M LiCl (n=7), 0.004 M LiCl (n=7), 0.009 M LiCl (n=4), 0.018 M LiCl (n=5), 0.037 M LiCl (n=4), 0.075 M LiCl (n=5), and 0.15 M LiCl (n=5); Morphine: saline (n=22), 0.5 mg/kg (n=12), 1.5 mg/kg (n=12), 3 mg/kg (n=11), 5 mg/kg (n=10), 15 mg/kg (n=10), 30 mg/kg (n=10), and 60 mg/kg (n=10); Cocaine: saline (n=22), 5.0 mg/kg (n=15), 7.5 mg/kg (n=14), 10 mg/kg (n=15), 15 mg/kg (n=8), 20 mg/kg (n=10), and 40 mg/kg (n=8). Taste-drug pairings and preparation/presentation of solutions were identical to that previously described in the water restriction experiments.

2.5 Procedure: Non-restricted experiments

Rats were water restricted as described in the water restriction experiments and then evaluated for baseline intake in the food and water ad lib condition. The rats were matched on the basis of their 5-min H₂O intake during the final two days of the baseline period and were placed into the following groups: LiCl: saline (n=13), 0.002 M (n=7), 0.004 M (n=7), 0.009 M (n=6), 0.018 M (n=6), 0.037 M (n=6), 0.075 M (n=6), and 0.15 M (n=6); Morphine: saline (n=20), 0.5 mg/kg (n=12), 1.5 mg/kg (n=11), 3 mg/kg (n=11), 5 mg/kg (n=8), 15 mg/kg (n=8), 30 mg/kg (n=8), and 60 mg/kg (n=8); Cocaine: saline (n=8), 5.0 mg/kg (n=8), 7.5 mg/kg (n=8), 10 mg/kg (n=8), and 20 mg/kg (n=8). Taste-drug pairings and preparation/presentation of solutions were identical to that previously described in the water restriction experiments. In this non-restricted state, all rats received 24 h access to H₂O from a lilit at the back of the cage. In addition, a cylinder containing purified H₂O was presented at the front of each rat's cage for 5 min each morning between conditioning trials.

3. RESULTS

3.1 Experiment 1: LiCl

3.1.1 Experiment 1A. Water restricted—An 8 × 8 mixed factorial ANOVA varying drug (saline, 0.002, 0.004, 0.009, 0.018, 0.037, 0.075, 0.15) and trials (1-8) was conducted on the data. Newman-Keuls post hoc tests of a significant Drug × Trials interaction, $F(49, 448)=11.49$ $p<0.0001$, indicated that suppression of the saccharin CS occurred in the later trials at lower doses and in the earliest trials at higher doses (see Figure 1).

Specifically, at the three lowest doses, significant suppression began at the sixth, fifth, and third pairing, respectively, and persisted to trial 8, $p < .05$. At the remaining four higher doses, significant suppression began after a single saccharin-LiCl pairing and persisted to trial 8, $p < .05$.

3.1.2 Experiment 1B. Food restricted—Relative to the water restricted state, the suppressive effect of LiCl on saccharin intake is not as potent at the lowest dose tested when examined in the food restricted state (see Figure 2).

This was indicated by the results of an 8×8 mixed factorial ANOVA varying drug (saline, 0.002, 0.004, 0.009, 0.018, 0.037, 0.075, 0.15) and trials (1-8), and confirmed by Newman-Keuls post hoc tests of the significant Drug $\times$ Trials interaction, $F(49,280) = 2.09$, $p < .0001$. Specifically, the 0.009, 0.037, 0.075, and 0.15 M doses of LiCl suppressed intake of the saccharin solution after a single taste-drug pairing, $p < .05$, while the 0.018 M dose significantly suppressed intake by trial 3, $p < .05$. The only real deviation from this robust pattern of suppression occurred at the two lowest doses. The 0.004 M dose did not suppress intake until trial 4, $p < .05$, and the lowest dose (0.002 M), which was effective when tested in the water restricted state, was ineffective at suppressing intake of the saccharin solution in the food restricted condition, $p > .05$.

3.1.3 Experiment 1C. Non-restricted—The results of an 8×8 mixed factorial ANOVA varying drug (saline, 0.002, 0.004, 0.009, 0.018, 0.037, 0.075, 0.15) and trials (1-8) showed that the suppressive effects of LiCl on saccharin intake in the non-restricted condition are similar to those observed in the water restricted condition (see Figure 3).

This finding was confirmed by post hoc tests of a significant Drug $\times$ Trials interaction, $F(49,343) = 3.06$, $p < .0001$. Specifically, the 0.009, 0.018, 0.037, 0.075, and 0.15 M doses of LiCl suppressed intake of the saccharin solution after a single taste-drug pairing, $p < .05$. The 0.004 M dose suppressed intake by the third pairing, and the lowest dose (0.002 M) suppressed intake by the fourth pairing, $p < .05$.

Taken together, these data clearly show that LiCl-induced suppression of saccharin intake (i.e., CTA), which traditionally has been run in the water restricted state, is an extremely robust and orderly dose-dependent phenomenon regardless of restriction state. Indeed, even at the lowest dose tested (0.002 M LiCl), a significant reduction in saccharin intake was observed by the sixth taste-drug pairing in the standard water restricted condition. The non-restricted condition actually enhanced the suppressive effects of LiCl on saccharin intake, despite reducing overall intake. In contrast, the food restricted condition, which also reduced overall intake relative to the water restricted condition, diminished the suppressive effects of LiCl on saccharin intake, but only at the lowest dose tested. Therefore, the 0.002 M LiCl dose, while effective in the water restricted and non-restricted conditions, was ineffective at reducing saccharin intake when examined in the food restricted state. Manipulating the restriction state, then, had no effect on the efficacy of LiCl to support CTA at each of the other 6 doses tested.

3.2 Experiment 2: Cocaine

3.2.1 Experiment 2A. Water restricted—When examined in the water restricted state, there appeared to be an orderly dose response function where lower doses of cocaine were less effective than higher doses to induce avoidance of the saccharin cue. To verify this, a 9×8 mixed factorial ANOVA varying drug (saline, 1.25, 2.5, 5, 7.5, 10, 15, 20, 40 mg/kg cocaine) and trials (1-8) was conducted. Indeed, post hoc tests of a significant Drug $\times$ Trials interaction, $F(56,798) = 7.73$, $p < .0001$, revealed an orderly dose response function (see Figure 4).

Specifically, the 15, 20, and 40 mg/kg doses of cocaine suppressed intake of the saccharin solution after a single taste-drug pairing and persisted to trial 8, $ps < .05$. Suppression occurred by trial 3 for the 10 mg/kg dose, and on trials 4 and 6-8 for the 7.5 mg/kg dose. The three lowest doses (1.25 mg/kg, 2.5 mg/kg, and 5 mg/kg) were ineffective at suppressing intake of the saccharin solution in water restricted rats, $ps > .05$.

3.2.2 Experiment 2B. Food restricted—Given the lack of suppressive effect of the two lowest doses of cocaine in the water restricted groups, examination of the suppressive effects of cocaine in the food restricted state (and in the non-restricted state below) began with a 5 mg/kg dose of the drug. As shown in Figure 5, cocaine-induced suppression of intake of the saccharin cue remained dose-dependent when examined in the food restricted state. On the whole, the suppressive effects of cocaine are quite similar under both water- and food-restricted conditions.

To confirm this observation, a 7×8 mixed factorial ANOVA varying drug (saline, 5, 7.5, 10, 15, 20, 40 mg/kg cocaine) and trials (1-8) was conducted. Post hoc tests of a significant Drug $\times$ Trials interaction, $F(42,595) = 7.79$, $p < .0001$, indicated that the 40 mg/kg dose of cocaine suppressed intake of the saccharin solution after a single taste-drug pairing while the 15 mg/kg and 20 mg/kg doses suppressed intake by trial 3, $ps > .05$. The 10 mg/kg dose suppressed intake by trial 5 and the 7.5 mg/kg dose suppressed intake by trial 4, $ps > .05$. Deviating slightly from the water restricted condition at the 5 mg/kg dose, where there was no significant suppression of CS intake, a significant but transient suppression of saccharin intake occurred on trials 5 – 7, $ps > .05$.

3.2.3 Experiment 2C. Non-restricted—Relative to the deprived conditions, the non-restricted condition revealed marked suppressive effects of cocaine on saccharin intake at all doses tested (see Figure 6).

To verify this observation, a 5×8 mixed factorial ANOVA varying drug and trials was conducted. Post hoc tests of a significant Drug (saline, 5, 7.5, 10, 20 mg/kg cocaine) $\times$ Trials (1-8) interaction, $F(28,245) = 10.61$, $p < .0001$, indicated that the suppressive effects of cocaine on saccharin intake are robust in the non-restricted state. Specifically, the 5 mg/kg, 7.5 mg/kg, 10 mg/kg, and 20 mg/kg doses of cocaine suppressed intake of the saccharin solution after a single taste-drug pairing, $ps < .05$.

In sum, cocaine-induced suppression of saccharin intake was reliably observed in the water restricted state in doses ranging between 10-40 mg/kg. While the effect was intermittent

across trials at the 7.5 mg/kg dose, it was non-significant at the lower 1.25 to 5 mg/kg doses. When examined in the food restricted state, suppression of saccharin intake was evident at each of the cocaine doses tested. The effect, however, dissipated by trial 8 at the 5 mg/kg dose and was marginally slower to develop for doses ranging from 10 – 40 mg/kg when compared to rats tested in the water restricted state. Finally, in contrast, cocaine-induced suppression of saccharin intake was found to be robust at all doses when rats were tested in the non-restricted condition. This is most evident at the 5 mg/kg dose which was completely ineffective in the water restricted rats and only transiently effective in the food restricted subjects.

3.3 Experiment 3: Morphine

3.3.1 Experiment 3A. Water restricted—The lower doses of morphine were not effective when examined in water restricted rats (Figure 7).

This observation was verified by an 8×8 mixed factorial ANOVA varying drug (saline, .5, 1.5, 3, 5, 15, 30, 60 mg/kg morphine) and trials (1-8). Newman-Keuls post hoc tests of a significant Drug $\times$ Trials interaction, $F(49,504) = 3.44$, $p < .0001$, indicated that only the 15 mg/kg and 60 mg/kg doses of morphine suppressed intake of the saccharin solution following a single taste-drug pairing. Suppression by the 30 mg/kg dose occurred on the fifth, seventh and eighth pairings. The 0.5 mg/kg, 1.5 mg/kg, 3 mg/kg, and 5 mg/kg doses, on the other hand, were ineffective in suppressing intake of the saccharin cue relative to their saline treated controls, $ps > .05$.

3.3.2 Experiment 3B. Food restricted—When evaluated in the food restricted state, morphine-induced avoidance was attenuated relative to water restricted conditions at almost every dose tested. An 8×8 mixed factorial ANOVA varying drug (saline, 0.5, 1.5, 3, 5, 15, 30, 60 mg/kg morphine) and trials (1-8) was conducted and revealed an unusual dose response profile (see Figure 8).

Specifically, post hoc tests of a significant Drug $\times$ Trials interaction, $F(49, 623) = 4.03$, $p < .0001$, indicated that the 0.5 mg/kg dose of morphine, the lowest dose tested, suppressed intake of the saccharin solution by trial 5, while the next two higher 1.5 mg/kg and 3 mg/kg doses were without effect. At the 5 mg/kg dose, intake of the saccharin solution was significantly suppressed on trial 2, and then intake began to increase on trials 3-7 at a rate on par with the saline controls until trial 8 where the morphine-injected rats consumed significantly more saccharin than the saline injected controls (i.e., a reinforcement effect). This type of transient suppressive effect also was evident for both the 15 and 30 mg/kg dose, where suppression was established after a single taste-drug pairing and gradually waned to insignificance by the fifth trial. Even at the highest dose tested, 60 mg/kg, there was a tendency for weakened suppressive effects. Although rats tested with this high dose of morphine suppressed intake relative to saline controls on trials 2-7, they did so with a great deal of between-subject variability.

3.3.3 Experiment 3C. Non-restricted—Morphine induced avoidance of the saccharin cue under non-restricted conditions was similar and robust across all doses of the drug (see Figure 9).

This observation was confirmed by the results of an 8×8 mixed factorial ANOVA varying drug (saline, 0.5, 1.5, 3, 5, 15, 30, 60 mg/kg morphine) and trials (1-8). Newman-Keuls post hoc tests of a significant main effect of drug, $F(7, 78) = 5.11$, $p < .0001$, indicated that all doses of morphine significantly reduced intake of saccharin CS relative to intake by the saline treated controls ($ps < .02$). The fact that the Drug $\times$ Trials interaction was not significant, $F(49, 546) = 1.07$, $p = 0.35$, speaks to the symmetry of morphine's suppressive effects at each dose.

The impact of restriction state on morphine-induced suppression of saccharin intake was less straightforward than was the effect of restriction state on either cocaine- or LiCl-induced suppression of CS intake. In the water restricted state, lower doses (0.5 to 5 mg/kg) were ineffective at reducing intake of saccharin. The 15 mg/kg and 60 mg/kg doses appeared to be the most effective, while the intermediate 30 mg/kg dose was less effective. In the food restricted condition, the highest and lowest doses were the most effective (suppression by trial 2 with 60 mg/kg and by trial 4 with 0.5 mg/kg) while the doses in between were only transiently effective or not effective at all. Finally, as mentioned above, the Drug $\times$ Trials interaction was not significant in the non-restricted condition. This lack of significance was due to the fact that all doses effectively suppressed CS intake and to a similar degree when examined in the non-restricted state. Thus, while the impact of restriction on morphine-induced suppression of CS intake is more varied, it is clear that the suppressive effects of morphine are most robust when examined in the non-restricted state.

4. DISCUSSION

The current set of experiments was designed to compare LiCl-, morphine-, and cocaine-induced avoidance of a preferred 0.15% saccharin solution in rats that were water restricted, food restricted, or non-restricted using an extensive dose-response analysis. Importantly, all of the doses employed for each of the three drugs produced significant conditioned avoidance of the saccharin CS when taste-drug pairings were evaluated in a non-restricted state. However, this avoidance was disrupted when many of these very same doses were evaluated in rats that were deprived of either food or water. Of the three drugs tested, LiCl was the most resistant to the disruptive effects of either water or food restriction (see Table 1).

When evaluated under non-restricted conditions, the suppressive effects of LiCl, morphine, and cocaine, at every dose tested, induced significant avoidance of the saccharin CS after just one or two taste-drug pairings. Thus, there were no differences in the suppressive effects across the compounds unless a restriction state was imposed. As such, the disrupted suppressive effect of morphine and cocaine following restriction cannot be attributed to learning deficits or to dose, per se, because these interpretations would have predicted a similar pattern of behavior regardless of restriction state. Clearly, restriction enhanced the drive to consume saccharin. This enhanced drive was sufficient to override the suppressive effects of cocaine and morphine at lower doses, but was less effective in overriding the suppressive effects of LiCl, even at extremely low doses.

Relative to the non-restricted condition, water restriction reduced the suppressive effects of both cocaine and morphine on saccharin intake. Thus, even at doses found highly effective in the non-restricted state (see Table 1), both morphine and cocaine were rendered either completely ineffective or marginally effective when evaluated in the water restricted state. Water restriction also reduced the suppressive effects of LiCl, but in this case, saccharin-LiCl pairings still led to a fairly robust, approximate 50% reduction, in CS intake. Again, of the three drugs tested, LiCl was most resistant to the disruptive effect of water restriction on drug-induced suppression of CS intake.

Food restriction resulted in the most complicated pattern of responding across the dose response function for each drug and was less conclusive than water restriction at differentiating between the compounds. While saccharin is sweet, it contains no calories. Without calories, the saccharin may not be as rewarding to a food restricted rat as it is to a water restricted rat. Nevertheless, the efficacy of each drug compound to support conditioned avoidance of the saccharin CS was reduced by food restriction and LiCl was the most resistant to this reduction. Why food restriction disrupted avoidance of saccharin at the lowest dose of LiCl, but enhanced the efficacy of the lowest dose of morphine is unclear. However, the unusual effects of food restriction on morphine-induced suppression were not consistent across the dose response and trials, as the next two intermediate low doses (1.5 and 3 mg/kg) were completely ineffective, and 5 mg/kg actually produced a reinforcement effect by trial 8. In fact, in the food restricted condition, morphine did not suppress intake of a saccharin CS consistently except at the lowest and highest doses. This pattern of transient suppression for the 5, 15, and 30 mg/kg doses (i.e., the establishment of suppression that gradually dissipated) was unique to the food restricted, morphine-injected rats and, therefore, is difficult to explain. On the one hand, if tolerance alone were to explain the waning suppressive effects of morphine (Siegel, Parker et al. 1995) then it would be expected to occur equally across the restriction conditions and at the lowest dose in the food restricted rats. Moreover, if the transient suppression was an effect of food restriction alone, then a similar pattern of suppression would be expected in the food restricted rats that were injected with cocaine. While it is well known that food restriction enhances the rewarding properties of sweets and drugs of abuse (Carr, Kim et al. 2000; Carroll, Campbell et al. 2001), and reduces the threshold for rewarding electrical brain stimulation in the lateral hypothalamus (Carr 2002), it remains unclear why food restriction appeared to enhance tolerance to the suppressive effect of morphine, but not cocaine, across trials. Some clarity may be gained by the evidence that food restriction alters the expression of endogenous opioid peptides (as well as many other peptides responsible for maintaining energy balance) that are known to mediate the rewarding properties of sweets (Pecina and Berridge 1995; Kelley, Bakshi et al. 2002; Pecina and Berridge 2005). Thus, it is possible that sensitization and tolerance to morphine could progress uniquely when animals are food deprived, particularly in a model where the effect of the drug is measured by intake of a sweet cue.

Perhaps the simplest explanation for the potent avoidance of saccharin intake when the rats are not deprived of either water or food is that the hedonic value of the saccharin solution is reduced. Therefore, the motivation to consume saccharin is also reduced and, as such, is more readily avoided in anticipation of drug effects. Depriving a rat of water or food, then, increases the hedonic valence of saccharin and drives its consumption. The relative

motivational apathy inherent to the non-restricted state would be expected to facilitate the devaluation of saccharin induced by pairings with either drugs of abuse or LiCl. Nevertheless, the heightened motivational states of restriction appear to more effectively override the expression of saccharin avoidance induced by cocaine and morphine than by LiCl. The prominent rightward shift in the dose response curve for both morphine and cocaine, but not LiCl, by water restriction extends support for the idea that morphine and cocaine mediate avoidance of a saccharin CS by a different mechanism than LiCl. Thus, despite our best efforts to include “matched” doses of drug, the illness induced by LiCl may simply differ from the suppressive effects of morphine and cocaine. For example, with LiCl, the US may only need to reach a detectable threshold in order to induce avoidance of an associated taste cue. Once the US is detected, a LiCl-induced CTA will occur in spite of restriction state because the primary function of the CS is to avoid illness by avoiding the illness-associated tastant and, of course, this must hold fast even when in need of water or food. In contrast, because malaise is not at the core of morphine- and cocaine-induced suppression of CS intake, the drug doses need to be not only detectable, but also of sufficient motivational strength to elicit the formation of an opposing conditioned compensatory response or to shift the animal out of saccharin-seeking behavior and toward drug-seeking behavior. This is the case even for very low doses of the drug when examined in a non-restricted state suggesting that the hedonic value of saccharin may compete with drugs of abuse under certain parameters (Lenoir, Serre et al. 2007). When water or food restricted, however, the drug must be even more potent to support a reduction in intake of the sweet saccharin cue. In accordance with this view and outside of the complexities of drug effects, food restriction also prevents sucrose-induced suppression of intake of a weak sucrose cue in the anticipatory contrast paradigm (Flaherty, Grigson et al. 1991; Flaherty 1996).

As alluded to above, recent data suggest that aversive taste reactivity can arise in the “CTA” paradigm for at least two very different reasons. With strictly aversive agents (e.g., LiCl, radiation, intense magnetic fields), the parsimonious interpretation is that gapes are dependent on the known illness inducing properties of the US. When the US has a complex mix of rewarding, addictive, and aversive effects, however, that parsimonious interpretation is not adequate. Currently, the only paradigm known to produce aversive taste reactivity to a saccharin cue that predicts a drug of abuse like cocaine is when the taste cue is intra-orally delivered over a relatively long CS access period prior to drug administration (Wheeler, Twining, et al., 2008; Wheeler, Aragona et al. 2011). As described, the magnitude of the aversive taste reactivity is the single-best predictor of a short latency to self-administer the first cocaine infusion, rapid self-administration rates during the first 10 minutes of the session (i.e., “load up”), total number of infusions, and the willingness to work for the drug on a progressive ratio schedule of reinforcement (Wheeler, Twining et al. 2008; Wheeler, Aragona et al. 2011; Colechio and Grigson 2014; Colechio, Imperio et al. 2014). These findings make the prototypical taste aversion hypothesis untenable, as instrumental behaviors are uniformly punished by LiCl administration – rats will not “self-administer” a drug that induces illness and certainly not with increasing rapidity and in copious amounts. The authors conclude that, in the anticipation of drug, the saccharin cue induces a negative affect state (e.g., cue elicited craving/withdrawal) and, thereby, reduces the palatability of the saccharin cue (Wheeler, Twining, et al., Grigson, Twining et al. 2008; 2008). This

interpretation is consistent with the finding that a sweet taste can be conditioned to elicit aversive taste reactivity when it has been associated with naloxone-precipitated withdrawal in morphine dependent rats (McDonald, Parker et al. 1997).

Despite decades of research on conditioned taste avoidance induced by morphine, cocaine, and LiCl, it was not possible before now to evaluate the effect of restriction state on the individual dose response functions of these drugs, let alone to compare them directly to each other. This is mostly because laboratories, understandably, use different testing parameters, making a comparative analysis quite difficult. Here, the experiments were conducted using standardized procedures and parameters that differed only with respect to the dose and drug employed and, thus, allowed for a reasonable comparative analysis across drug compounds, doses, and restriction states. It should be noted however that, while this analysis is extensive, it is by no means exhaustive. Although we were able to test all three restriction states across seven doses of both LiCl and morphine, we were unable to test two of the cocaine doses (i.e., 15 and 40 mg/kg) in the non-restricted state due to logistics and resources. We chose not to conduct these costly studies because avoidance of the cocaine-paired saccharin cue already is maximal with the 10 mg/kg dose of cocaine when tested in the non-restricted state. However, future studies could incorporate additional drugs of abuse (e.g., heroin, alcohol, nicotine, etc.) and doses into the analysis and sex differences would be an interesting variable to investigate. Suppression of saccharin intake by cocaine (Cason and Grigson 2013), morphine (Jenney, Schroy et al. in prep), and LiCl (Foy and Foy 2003) is attenuated in female rats compared with male rats and it would be informative to evaluate the influence of restriction state in female rats and compare it with the current data set.

In conclusion, the results in the current manuscript showed that the suppressive effects of lower doses of morphine and cocaine were attenuated or abolished when the rats were either hungry or thirsty. LiCl, on the other hand, remained a potent suppresser of saccharin intake even at very low doses under both food restricted and water restricted conditions. This pattern of data suggests that different mechanisms mediate suppression of CS intake by LiCl and by drugs of abuse such as morphine and cocaine. While attenuated by food and water restriction, the suppressive effects of morphine also differed qualitatively from those of cocaine. Finally, it should be pointed out that lesion studies have dissociated not only LiCl and drug-induced suppression of intake of a saccharin cue (Grigson, Lyuboslavsky et al. 2000; Geddes, Han et al. 2008), but also the suppressive effects of a drug of abuse from those of a sweet sucrose reward (Nyland, Alexander et al. 2012). Drug-induced suppression of intake of a gustatory cue, then, is a seemingly unitary and complex phenomenon involving the comparison of a natural reward cue with a drug of abuse that is anticipated in the near future and modulated by the current state of need. This is a critical model for further study, as reduced responding for the natural reward predicts individual vulnerability for drug-seeking and drug-taking in rats (Wise, Yokel et al. 1976; Grigson and Twining 2002; Wheeler, Twining et al. 2008; Twining, Bolan et al. 2009; Wheeler, Aragona et al. 2011; Colechio and Grigson 2014; Colechio, Imperio et al. 2014; Imperio and Grigson 2015) and in humans (Wilson, Delgado et al. 2014).

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Highlights

- We analyzed the effect of restriction state on drug-induced suppression.
- Cocaine efficacy was decreased by water and food restriction.
- Morphine efficacy was decreased by water and food restriction.
- LiCl was the most resistant to the disruptive effects of water or food restriction.

LiCl-induced suppression (water-restricted)

Figure 1.

LiCl-induced suppression of saccharin intake in water restricted rats. Mean ($\pm$ SEM) intake (ml/5 min) of 0.15% saccharin following pairings with either saline (open squares) or 7 doses of LiCl expressed as moles/L (closed circles).

LiCl-induced suppression (food-restricted)

Figure 2. LiCl-induced suppression of saccharin intake in food restricted rats. Mean ($\pm$ SEM) intake (ml/5 min) of 0.15% saccharin following pairings with either saline (open squares) or 7 doses of LiCl expressed as moles/L (closed circles).

LiCl-induced suppression (non-restricted)

Figure 3.

LiCl-induced suppression of saccharin intake in non-restricted rats. Mean ($\pm$ SEM) intake (ml/5 min) of 0.15% saccharin following pairings with either saline (open squares) or 7 doses of LiCl expressed as moles/L (closed circles).

Cocaine-induced suppression (water-restricted)

Figure 4.

Cocaine-induced suppression of saccharin intake in water restricted rats. Mean ($\pm$ SEM) intake (ml/5 min) of 0.15% saccharin following pairings with either saline (open squares) or 8 doses of cocaine expressed in mg/kg (closed circles).

Cocaine-induced suppression (food-restricted)

Figure 5.

Cocaine-induced suppression of saccharin intake in food restricted rats. Mean ($\pm$ SEM) intake (ml/5 min) of 0.15% saccharin following pairings with either saline (open squares) or 6 doses of cocaine expressed in mg/kg (closed circles).

Cocaine-induced suppression (non-restricted)

Figure 6.

Cocaine-induced suppression of saccharin intake in non-restricted rats. Mean ($\pm$ SEM) intake (ml/5 min) of 0.15% saccharin following pairings with either saline (open squares) or 4 doses of cocaine expressed in mg/kg (closed circles).

Morphine-induced suppression (water-restricted)

Figure 7.

Morphine-induced suppression of saccharin intake in water restricted rats. Mean ($\pm$ SEM) intake (ml/5 min) of 0.15% saccharin following pairings with either saline (open squares) or 7 doses of morphine expressed in mg/kg (closed circles).

Morphine-induced suppression (food-restricted)

Figure 8.

Morphine-induced suppression of saccharin intake in food restricted rats. Mean ($\pm$ SEM) intake (ml/5 min) of 0.15% saccharin following pairings with either saline (open squares) or 7 doses of morphine expressed in mg/kg (closed circles).

Morphine-induced suppression (non-restricted)

Figure 9.

Morphine-induced suppression of saccharin intake in non-restricted rats. Mean ($\pm$ SEM) intake (ml/5 min) of 0.15% saccharin following pairings with either saline (open squares) or 7 doses of morphine expressed in mg/kg (closed circles).

Table 1

Summary of suppressive effects across drugs and restriction states

	<u>LICL</u>			<u>Cocaine</u>			<u>Morphine</u>					
	M	WR	FR	NR	mg/kg	WR	FR	NR	mg/kg	WR	FR	NR
0.002		+	-	+	1.25	-			0.5	-	+	+
0.004		+	+	+	2.5	-			1.5	-	-	+
0.009		+	+	+	5	-	-	+	3	-	-	+
0.018		+	+	+	7.5	+	+	+	5	-	+/-	+
0.037		+	+	+	10	+	+	+	15	+	+/-	+
0.075		+	+	+	15	+	+	+	30	+	+/-	+
0.15		+	+	+	20	+	+	+	60	+	+	+
					40	+	+					

WR = water restricted, FR = food restricted, NR = non-restricted, (+) = suppression, (-) = disrupted suppression, (+/-) = transient suppression