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ORIGINAL INVESTIGATION

Conditioned aversive responses produced by delayed, but not immediate, exposure to cocaine and morphine in male Sprague-Dawley rats

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Abstract

Rationale To determine the conditions under which tastes paired with delayed access to experimenter-delivered cocaine and morphine elicit a conditionally aversive affective state.

Objectives and methods The potential of saccharin paired with immediate access to cocaine (5, 10, 20 mg/kg, sc and ip) and delayed (30 and 10 min) access to cocaine (20 mg/kg, sc and ip) and morphine (10 mg/kg, sc) to elicit a pattern of aversive responding in the taste reactivity test (Grill and Norgren 1978a) was evaluated. Cocaine-induced aversions were compared with those produced by a moderate dose of LiCl (50 mg/kg). Finally, as an independent measure of cocaine withdrawal, the potential of exposure to saccharin paired with delayed access to cocaine to produce anxiogenic-like responding in the Light–Dark Emersion test was evaluated.

Results Immediate access to cocaine did not produce conditioned aversion at any dose. Delayed (30 or 10 min) access to sc cocaine (20 mg/kg) produced robust conditioned aversion and delayed access to ip cocaine (20 mg/kg; 30 min) and to sc morphine (10 mg/kg; 10 min) produced weaker conditioned aversion. Yawning emerged as a potential withdrawal response in rats conditioned with delayed (30 min) access to 20 mg/kg, sc, cocaine. Contextual cues did not produce conditioned aversion when paired with delayed access to sc cocaine (20 mg/kg). Finally, exposure to saccharin paired with delayed access to cocaine produced anxiogenic-like responding in the Light–Dark Emersion test.

Conclusion Our results support the contention that a conditioned aversive state develops when a taste cue comes to predict the delayed availability of drugs of abuse.

Keywords Cocaine · Negative affect · Withdrawal · Taste reactivity · Aversion · Morphine · LiCl · Rat

Cocaine abuse is accompanied by the emergence of negative affect such as dysphoria, irritability, and anhedonia. Indeed, this negative affect plays a prominent role in craving and relapse in animal models (e.g., Koob and LeMoal 1997; Solomon and Corbit 1974; Wheeler et al. 2008); the greater these negative feelings, the greater the subjective euphoric effect of subsequent cocaine administration in humans (Newton et al. 2003; Sofuoglu et al. 2003). The negative affective state can become associated with cues that trigger craving and relapse, and, consequently, the subsequent euphoric effect of the cocaine administration. Recently, using the taste reactivity test (Grill and Norgren 1978a), Wheeler et al. (2008) directly measured this negative affect in rats by demonstrating that a taste cue paired with delayed access to cocaine elicits a conditioned aversive state that is quantifiable and predicts greater subsequent cocaine intake.

The taste reactivity (TR) test (Grill and Norgren 1978a) is a direct measure of the hedonic valence of the taste stimulus (Berridge 2000). These stereotyped oromotor responses to palatable (sucrose) and unpalatable (quinine) tastes reflect not only innate reactions but also conditioned changes in affect that are dissociable from simple ingestive behavior. Rats avoid intake of a taste cue that has been paired with a drug that produces nausea such as lithium chloride (LiCl). They also display the aversive reactions of gaping, chin rubbing, and paw treading to such a conditioned aversive taste (Grill and Norgren 1978b). Rats, which cannot vomit, also avoid intake of a taste cue that has been paired with a self-administered

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drug of abuse, such as cocaine (e.g., Parker 1993), and, indeed, greater avoidance of the taste cue is associated with greater cocaine self-administration (Grigson and Twining 2002). On the other hand, when a taste cue is paired immediately with a drug of abuse, such as cocaine and morphine, rats do not subsequently respond to that taste with the aversive reactions of gaping, chin rubbing, and paw treading, although they suppress ingestion (tongue protrusions) and allow the taste to passively drip from their mouth (avoidance response) (Parker 1993, 1995).

Wheeler et al. (2008) reported that following several pairings of multiple exposures to a saccharin cue with the *delayed* opportunity to self-administer cocaine, rats eventually display conditioned gaping reactions during the waiting period. In fact, the greater the cocaine intake in the self-administration period, the greater the conditioned gaping to the saccharin during the delay. The development of gaping reactions elicited by a taste that predicted delayed access to cocaine was subsequently replicated with delayed delivery of intraperitoneal (ip) cocaine, indicating that this was not a unique effect of the potential of self-administered cocaine to recruit the mesolimbic dopamine system (e.g., Hemby et al. 1997; Stuber et al. 2005; Kippin et al. 2006). Wheeler et al. (2008) hypothesized that, "the cocaine-paired taste served as a predictive cue of cocaine's impending availability and precipitated the expression of a cocaine aversive state in learned anticipation of the future opportunity to self-administer cocaine." They suggested that the "learned cocaine aversive state" represented a state of cocaine withdrawal (a cocaine-need state). That is, the rats developed a compensatory conditioned response (Siegel and Ramos 2002) or a conditioned "b" state (Solomon and Corbit 1974) that became associated with the taste. Indeed, McDonald et al. (1997) reported that taste cues associated with naloxone-precipitated opiate withdrawal elicit conditioned gaping reactions in rats.

What is unique about the Wheeler et al. (2008, 2011) procedure is that the taste cue was intraorally delivered at 3.5-s intervals across a 30–45 min period prior to cocaine availability in a self–administration paradigm constituting a "drug waiting" period that may promote an association between the taste and the negative affective state as revealed by conditioned gaping. Parker (1993, 1995) administered cocaine by subcutaneous (sc) injection immediately following a 2-min intraoral infusion of saccharin and found no evidence of gaping to the cocaine paired saccharin solution even after five conditioning trials (spaced 72 h apart). The present series of experiments first replicated the experiments of Wheeler et al. (2008) and Parker (1993, 1995), with experimenter-delivered ip and sc cocaine and compared these results with a moderate dose of LiCl (50 mg/kg, ip) using both procedures. Cocaine was injected both sc and ip because when administered sc (relative to ip), it is more effective in producing conditioned taste avoidance (Mayer and Parker 1993; Ferrari et al. 1991), but when cocaine is administered ip (relative to sc) it is more rewarding in a place preference paradigm (Mayer and Parker 1993; Tzschentke 1998). If the potential of a conditioned cocaine waiting period to produce gaping relies on the rewarding effects of cocaine, then ip administered cocaine may be more effective in producing gaping than sc cocaine, even though sc cocaine is more likely to produce a taste avoidance response. Subsequent experiments evaluated the potential of a contextual stimulus in the absence of a taste to elicit aversive reactions followed by a delayed (30 min) injection of cocaine. We also evaluated the potential of repeated exposure to saccharin over a 10-min period of delayed access to both cocaine (20 mg/kg, sc) and another rewarding drug, morphine (10 mg/kg, sc), to produce aversive responding, as a test of generality across drugs of abuse. Finally, since cocaine withdrawal is characterized by a high state of anxiety-like responding (Kupferschmidt et al. 2012), we evaluated the potential of repeated exposure to saccharin over a 30-min period prior to cocaine (20 mg/kg, sc) to produce anxiogenic-like responding in the Light–Dark (LD) Emergence test.

Methods

Subjects

The subjects were 160 Sprague-Dawley rats (Charles River, QC). Rats were between 250 and 300 g in weight on the first conditioning trial. Rats were individually housed in a colony room on a 24-h light–dark cycle (7 AM, lights off; 7 PM, lights on) such that behavioral testing was conducted during the dark phase of the light cycle. Rats were maintained on ad libitum rat chow and water, except when indicated otherwise.

Surgery

Rats were surgically implanted with an intraoral (IO) cannula 1 week prior to conditioning. Implantation of the cannula was done while rats were under isoflurane anesthesia according to the procedures previously described (Limebeer et al. 2010). Rats underwent 3 days of post-surgical monitoring beginning the day following surgery.

Drugs

Cocaine was mixed at a concentration of 1.5 mg/ml in solution with saline (SAL) for subcutaneous injection in order to prevent skin necrosis. As well, the injection site was changed on a daily basis. There were no instances of necrosis with these precautions. Injection volumes were dependent on body weight and dose. The 5 mg/kg dose was administered at 3.3 ml/kg, 10 mg/kg at 6.7 ml/kg, and 20 mg/kg at 13.3 ml/kg. For intraperitoneal (ip) injections, cocaine was mixed at a

concentration of 5, 10, and 20 mg/ml. Volume of injection was dependent on body weight and was prepared at 1 ml/kg. In order to equate the volume of morphine and cocaine, in experiment 4, morphine was also prepared at a concentration of 1.5 ml/ml and administered at a volume of 6.7 ml/kg sc. LiCl was prepared as a 0.15 M solution in sterile water and administered at a volume of 8 ml/kg ip (50 mg/kg).

Apparatus

For taste reactivity measures, the rats were placed in a clear Plexiglas box (22.5 × 26 × 20 cm) with an opaque lid, sitting on top of a clear glass-topped table. A mirror was located under the chamber at a 45° angle in order to allow viewing of the rat ventral surface. The chamber was located in a dark room next to a 25-W light source. There was a video camera (Sony DCR-HC48; Henry's Cameras, Waterloo, ON, Canada) placed in front of the mirror to allow the trials to be recorded and scored at a later time. Trials were scored by an observer blind to the experimental groups using "The Observer" (Noldus Information Technology Inc., Leesburg, VA, USA).

For experiment 3, the distinctive context was an opaque black Plexiglas box (22.5 × 26 × 20 cm) with an opaque lid, sitting on top of a clear glass-topped table. Closed circuit cameras located beneath each chamber pointed toward the rat to allow later scoring for gaping and the activity of the rat was measured using the Ethovision software program (Noldus, Inc., NL) to measure distance (cm) traveled.

For experiment 5, the LD Emergence apparatus has been previously described by Rock et al. (2017). A video camera was mounted over the top of the light–dark box and the videotapes were analyzed by Ethovision software (Noldus Information Technology, Leesburg, VA, USA) for the duration of time spent in the light box during the 5-min test.

Taste reactivity measures

The literature on taste reactivity is somewhat inconsistent in the definition of aversive responses, especially when the responses are combined into a total aversive response score; therefore, we have chosen to individually measure each behavior described by Grill and Norgren (1978a) separately. Given that the definition of each of these behaviors is consistently applied across laboratories, such an analysis gives sufficient information to facilitate replication of results. Although Grill and Norgren (1978a, b) initially identified gaping, chin rubbing, paw treading, head shakes, face washes, forelimb flailing, and increased locomotion as aversive responses (which are often summed), a subsequent factor analysis (Parker 1995) indicated that taste reactivity responses tend to cluster into aversive responses, motoric responses, and ingestion/non-ingestion related responses. Each of the behaviors measured is described in Table 1. Aversive behaviors include gaping, chin rubbing, and paw treading. Motoric responding behaviors include active locomotion, rearing, face washing, head shakes, and forelimb flails. Ingestion/non-ingestion related responses included the positive hedonic response of tongue protrusions, the avoidance-like response of passive dripping, and avoidance of the taste (conditioned taste avoidance, CTA). As well, since the taste reactivity test provides the opportunity for detecting other orofacial reactions, we have included the response of yawning, which has been reported in animals (Nakamaura-Palacios et al. 2002; Jaw et al. 1993; Schnur et al. 1992) and humans (Bickel et al. 1988) undergoing acute withdrawal from several drugs of abuse.

Procedure

Experiment 1: immediate pairing

Experiment 1a: immediate sc cocaine All rats were adapted to the taste reactivity chamber 3 days prior to the first conditioning trial. During adaptation, the rat was placed in the taste reactivity chamber and its cannula was attached to an infusion pump (Model KDS100; KD Scientific, Holliston, MA, USA) for fluid delivery. For adaptation, reverse osmosis water was infused into the intraoral cannula at a rate of 1 ml/min for a total of 2 min.

Three days following adaptation, the first conditioning trial began. On each of four conditioning trial/testing trials, the rat was placed in the taste reactivity chamber, and its cannula was attached to the pump for the infusion of 0.1% saccharin at a rate of 1 ml/min for 2 min. The timer started when the rat made an orofacial response and stopped at 2 min. The trial was recorded for scoring at a later time. At the end of the 2-min infusion session on trials 1–3, the rat was removed from the chamber and given an immediate sc injection of saline ($n = 8$) or cocaine at a dose of 5 mg/kg ($n = 7$), 10 mg/kg ($n = 7$) or 20 mg/kg ($n = 7$). Following the injection, the rat was placed back in its home cage. The TR test was conducted on the fourth day in the same manner as the conditioning trials but with no subsequent injection.

At 16:00 on the day after the testing, the water bottles were removed from each rat's cage. At 9:00 on the following day, rats were given 0.1% saccharin solution in graduated tubes and the amounts consumed at 30, 120, 360, and 240 min were measured as a measure of CTA.

Experiment 1b: immediate ip cocaine All adaptation and conditioning procedures were conducted as outlined in experiment 1a. During the three conditioning trials, following the 2-min infusion of saccharin solution, rats were injected ip with saline ($n = 8$), or 5 mg/kg ($n = 7$), 10 mg/kg ($n = 7$), or 20 mg/kg ($n = 6$) cocaine. The drug-free test trial was

Table 1 Definition of taste reactivity test behaviors scored

Type of behavior	Behavior	Characteristics	Measure
Aversive	Gape	Wide, triangular opening of the mouth, retraction of the corners of the mouth, exposing incisors	Frequency
Aversive	Chin rub	Sustained contact of chin with floor or walls of the chamber	Frequency
Aversive	Paw tread	Quick movement of forepaws on the floor of the cage, alternating paws—while not moving forward	Frequency
Withdrawal-like response	Yawn	Elongated vertical opening of the mouth without retraction of the corners of the mouth followed by expiration of air	Frequency
Motor	Active locomotion	Forward movement—one forepaw in front of other	Duration (s)
Motor	Rear	Lifting of forepaws from the chamber floor, standing on hindlimbs	Duration (s)
Motor	Face wash	Grooming of the face with forelimbs	Duration (s)
Motor	Head shake	Rapid side-to-side movement (shaking) of the head	Frequency
Motor	Forelimb flail	Swinging of the forelimbs	Frequency
Positive hedonic	Tongue protrusion	2-s bouts of rhythmic protrusions of the tongue	Frequency
Avoidance-like	Passive drips	Dripping of solution from the mouth	Frequency

conducted on the fourth day and the CTA test on the following day as outlined in experiment 1a.

Experiment 1c: immediate ip LiCl All adaptation and conditioning procedures were conducted as outlined in experiment 1a. During the three conditioning trials, following the 2-min infusion of saccharin, the rats were injected ip with saline (n = 8) or 50 mg/kg (8 ml/kg 0.15 M) LiCl (n = 8). The drug-free test trial was conducted on the fourth day and CTA test on the following day as outlined in experiment 1a.

Experiment 2: delayed (30-min) saccharin pairing with cocaine (20 mg/kg, sc and ip) and LiCl (50 mg/kg, ip)

All rats were adapted to the taste reactivity procedure 3 days prior to the first conditioning trial. During adaptation, the rat was placed in the taste reactivity chamber and its cannula was attached to an infusion pump (Model KDS100; KD Scientific, Holliston, MA, USA) for fluid delivery. During the adaptation trial, each rat was intraorally infused with reverse osmosis water on each of 30 trials for 10 s/trial at the rate of 1.2 ml/min every min over 30 min.

Three days following adaptation, the first conditioning trial began. On each of four conditioning trial/testing trials, the rat was placed in the taste reactivity chamber and its cannula was attached to the pump for the infusion of 0.1% saccharin and infused according to the same schedule as water during adaptation. After the 30th infusion, on conditioning trials 1–4, the rat was removed from the chamber and given an immediate injection of saline (n = 6 half sc/half ip), 20 mg/kg, sc, cocaine (n = 6), 20 mg/kg, ip, cocaine (n = 6), or 50 mg/kg (8 ml/kg 0.15 M) LiCl (n = 6). Following the injection, the rat was placed back in its home cage. The TR test trial occurred 24 h after the final conditioning trial. The rats were given a CTA test 24 h after the test trial as in experiment 1.

Experiment 3: delayed (30 min) context pairing with cocaine

In experiment 2, rats were exposed to saccharin in the TR chamber, which is distinct from their home cage. Therefore, to ensure that the aversive taste reactions were elicited by the taste rather than by the conditioning context, experiment 3 evaluated the potential of a distinctive context to elicit aversive reactions following repeated pairings with cocaine upon removal from the context 30 min later. Limebeer et al. (2008) have shown that rats will learn to gape to a distinctive context (even in the absence of a taste) if they experience LiCl-induced nausea while in that context.

The rats received four conditioning trials, one per day on consecutive days, followed by a drug-free test trial 24 h later. On each trial, they were placed in the distinctive context for 30 min. They were then immediately removed from the chamber and given an injection of either saline (n = 8) or 20 mg/kg sc cocaine (n = 8). On the test trial, the behavior of the rat was recorded with closed circuit cameras located beneath each chamber and the image was sent to Ethovision software to measure distance (cm) traveled, which was analyzed in 5-min intervals across the 30-min test. As well, the behaviors of gaping, chin rubbing, paw treading, and yawning were scored from the videotapes.

Experiment 4: delayed (10 min) saccharin pairing with cocaine or morphine

The procedures of experiment 4 were similar to those of experiment 2 except that the rats were injected with sc saline (n = 8), 20 mg/kg sc cocaine (n = 7), or 10 mg/kg sc morphine (n = 8) 10 min following repeated 10-s (every minute) saccharin exposures in the TR test. This provides a control for amount of saccharin exposure on the development of conditioned aversive responses because the total amount of saccharin infused was 2 ml (as in experiment 1) across the 10-min trials rather than 6 ml (as in experiment 2) across the 30-min trials. As well in the CTA test, there was no 30-min measure taken.

Experiment 5: assessment of anxiogenic-like responding following exposure to saccharin previously paired with delayed access to cocaine

A total of 14 rats were conditioned as in experiment 2 such that half (n = 7) received three daily conditioning trials with repeated saccharin infusions (10 s) every minute for 30 min prior to cocaine (sc) and the other half (n = 7) prior to saline (sc). On the following day, the rats received saccharin as during conditioning, but after 30 min were placed in the dark corner of the LD emersion box facing away from the opening between the two chambers and the movement of the rat was tracked during the 5-min test. An additional two groups of rats received three daily unpaired home cage injections of cocaine (n = 8) or saline (n = 8) and on the next day were given the same LD test described above, to evaluate the effect of cocaine exposure on anxiogenic-like behavior 24 h later.

Data analysis

The primary measures to be compared across experiments included the aversive reactions of gaping, chin rubbing, paw treading, the positive hedonic reaction of tongue protrusions, the avoidance behaviors of passive drips, and CTA. In experiments 1 and 4, the frequency or duration of each TR behavior above on each conditioning/testing trial for each group was entered into a mixed-factors analysis of variance (ANOVA). As well, the frequency or duration of the motoric TR behaviors displayed on the final TR test trial were analyzed as single factor ANOVAs. To evaluate CTA in experiments 1, 2, and 4, the mean cumulative amount (ml) of saccharin solution consumed at each interval of testing for each group was entered into a mixed-factors ANOVA. In experiment 2, the TR test trial data for the entire 30-min test trial was evaluated at each 5-min interval, which included summed reactions during and between saccharin infusions. The 5-min interval scores for each behavior on the test trial for each group were entered into a mixed-factors ANOVA. The total frequency or duration of each motoric TR behavior displayed during the 30-min test trial was analyzed as single-factor ANOVA. In experiment 3, the number of gapes and the distance traveled per 5-min interval for each group were entered into a mixed-factors ANOVA. In experiment 5, the TR behaviors of gaping, chin rubbing, paw treading, yawning, passive drips and tongue protrusions, and the time (s) spent in the lit box by the rats conditioned with delayed cocaine and the rats conditioned with delayed saline were compared by t tests. As well, the time (s) spent in the lit box by the home cage cocaine and home cage saline groups were compared by a t test. Significance was defined as $p < 0.05$.

Results

Experiment 1: immediate conditioning

When administered immediately following 2-min exposures to saccharin, cocaine (sc or ip) did not produce any of the aversive behaviors of gaping, chin rubbing, or paw treading across the four conditioning/testing trials at any dose. In contrast, the moderate dose of LiCl produced conditioned gaping, chin rubbing, and paw treading across trials. As previously reported (Parker 1993), and as is also seen with LiCl, cocaine delivered sc suppressed hedonic tongue protrusions and produced CTA across all doses and at 20 mg/kg increased passive dripping. When cocaine was delivered ip, only 20 mg/kg suppressed tongue protrusions, increased passive dripping, and produced a CTA. Among the motoric responses, the groups conditioned with 20 mg/kg, sc, cocaine showed more head shakes on the test trial than those conditioned with saline. In addition, LiCl conditioned rats showed less face washing (often considered to be an aversive reaction) than saline conditioned rats, but they did not differ in any other motoric behavior across trials.

Experiment 1a: immediate sc cocaine Figure 1 presents the mean (± SEM) number or duration of gaping, chin rubbing, paw treading, tongue protrusions, and passive drips, as well the CTA measured for the groups conditioned with sc cocaine. Yawning is not depicted in any table or figure of experiment 1a–c because the rats did not display yawning. The 4 × 4 mixed-factors ANOVA for the behaviors of gaping, chin rubbing, and paw treading revealed no significant effects.

The 4 × 4 mixed-factors ANOVA for tongue protrusions revealed a significant group effect, $F(3, 25) = 4.6$, $p < 0.01$, and an effect of trial, $F(3, 75) = 6.0$, $p < 0.01$. An analysis of each trial revealed that the groups significantly differed only on trial 3, $F(3,28) = 5.7$, $p = 0.004$ and trial 4 $F(3, 28) = 12.5$, $p < 0.001$; subsequent Bonferroni tests revealed that all sc cocaine doses produced suppressed tongue protrusions on trials 3 and 4 relative to group saline (p's < 0.05).

For the behavior of passive drips, the 4 × 4 mixed-factors ANOVA revealed significant effects of group, $F(3, 25) = 6.9$,

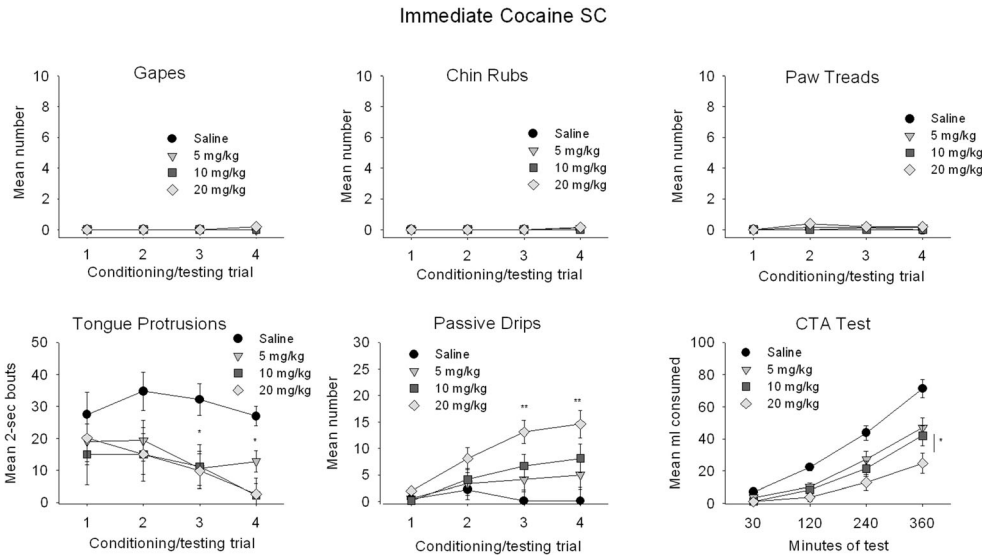

Fig. 1 Mean (± SEM) frequency or duration (s) of primary TR measures and CTA in experiment 1a with immediate sc cocaine. Asterisks indicate a group difference from saline; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

$p < 0.001$, and a group × trial interaction, $F(9, 75) = 2.4$, $p = 0.019$. The groups differed on conditioning trial 3, $F(3, 25) = 6.4$, $p = 0.002$, and on the test trial, $F(3, 25) = 5.4$, $p = 0.005$; subsequent Bonferroni tests revealed that on each of these trials, group 20 mg/kg sc cocaine displayed more passive drips than group saline ($p < 0.01$). Finally, the ANOVA for the CTA revealed significant effects of time $F(3, 75) = 185.3$, $p < 0.001$, group × time $F(9, 75) = 6.8$, $p < 0.001$, and a main effect of group $F(3, 25) = 10.6$, $p < 0.001$. Subsequent Bonferroni post hoc comparisons of the group effect revealed that all three doses of cocaine suppressed saccharin intake across the 6 h CTA test relative to group saline (p's < 0.05) and the cocaine doses did not differ from one another at any interval of testing.

Of the motoric behaviors assessed depicted in Table 1 (active locomotion (s), rearing (s), face washing (s), head shakes (f), forelimb flails (f)), the single-factor ANOVA for experiment 1a revealed only a significant effect for head shakes, $F(3, 25) = 6.4$, $p < 0.01$, with the group conditioned with 20 mg/kg, sc, cocaine showing more ($p < 0.01$) head shakes than group saline or 10 mg/kg, sc cocaine (data not depicted).

Experiment 1b: immediate ip cocaine Figure 2 presents the mean (±SEM) number or duration of the primary TR reactions measured in experiment 1b with ip cocaine. The 4 × 4 ANOVA for the behaviors of gaping, chin rubbing, and paw treading revealed no significant effects.

The 4 × 4 mixed-factors ANOVA for the number of tongue protrusions revealed only a significant main effect of trials, $F(3, 72) = 3.0$, $p = 0.037$. To evaluate each trial separately, single-factor ANOVAs revealed that groups only differed on trial 4, $F(3, 27) = 4.3$, $p < 0.015$; Bonferroni post hoc comparison tests revealed that group 20 mg/kg, ip, cocaine displayed significantly fewer tongue protrusions on the final test trial than groups saline or 5 mg/kg cocaine (p's < 0.05).

The 4 × 4 mixed-factors ANOVA for the number of passive drips revealed a significant effect of group, $F(3, 24) = 6.0$, $p < 0.001$; trial, $F(3, 24) = 5.7$, $p = 0.004$; and a group × trial interaction, $F(9, 72) = 6.0$, $p < 0.001$. To evaluate the interaction, single-factor ANOVAs for each trial revealed a group effect only on trials 3 and 4 (p's < 0.01); subsequent Bonferroni post hoc comparison tests revealed that group 20 mg/kg ip cocaine displayed more passive drips than group saline or 5 mg/kg ip cocaine on each of these trials (p's < 0.025). Finally, the ANOVA for the CTA revealed significant main effects of group, $F(3, 24) = 7.3$, $p < 0.001$; time, $F(3, 72) = 157.1$, $p < 0.001$; and a group × time interaction, $F(9, 72) = 3.2$, $p < 0.01$. Bonferroni post hoc comparisons of the group effect revealed that a dose of 20 mg/kg, ip, cocaine suppressed saccharin intake overall relative to saline and 5 mg/kg, ip, cocaine ($p < 0.05$). Bonferroni tests for each interval also revealed that at 30 min and at 120 min only, group 10 mg/kg, ip, cocaine drank less than group saline ($p < 0.05$), but this was overcome with further exposure to saccharin.

The single-factor ANOVA for the motoric responses assessed in experiment 1b on the final test trial revealed no significant effects (data not depicted).

Experiment 1c: immediate ip LiCl Figure 3 presents the mean (± SEM) number or duration of the primary TR behaviors displayed by the groups in experiment 1c. For the behaviors of gaping, chin rubbing, and paw treading, the 2 × 4 ANOVAs revealed a significant main effect of group (gapes, $F(2, 21) = 29.2$, $p < 0.001$; chin rubs, $F(2, 21) = 20.5$, $p < 0.001$; paw treads, $F(2, 21) = 6.7$, $p = 0.006$), trials (gapes, $F(3, 63) = 32.7$, $p < 0.001$; chin rubs, $F(3, 63) = 16.7$, $p < 0.001$; paw treads, $F(3, 63) = 14.1$, $p < 0.001$), and a group × trials interaction (gapes, $F(3, 63) = 10.1$, $p < 0.001$; chin rubs, $F(3, 63) = 224.9$, $p < 0.001$; paw treads, $F(3, 63) = 5.2$, $p < 0.001$). Subsequent independent t tests revealed that group LiCl

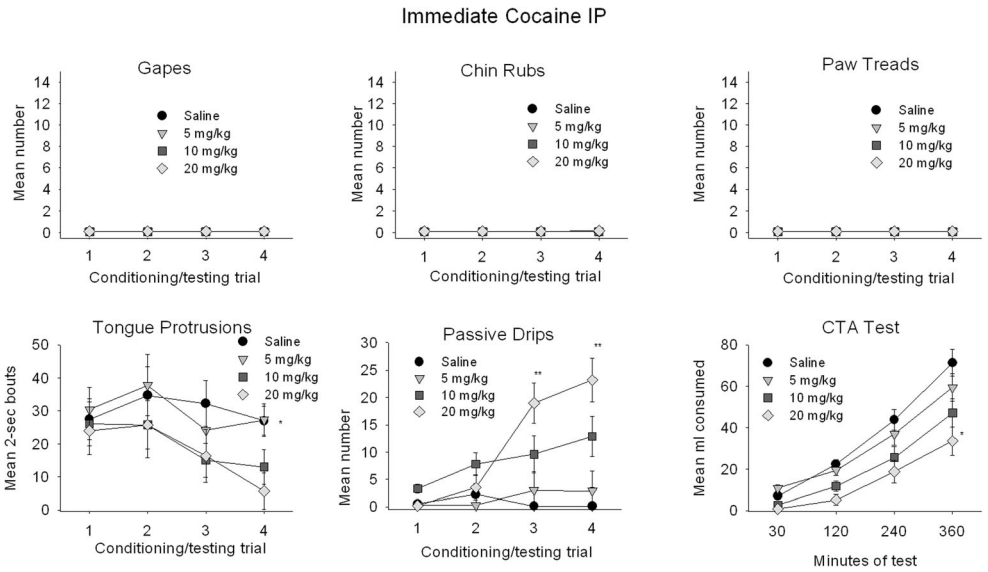

Fig. 2 Mean (± SEM) frequency or duration (s) of each of primary TR measures and CTA in experiment 1b with immediate ip cocaine. Asterisks indicate a group difference from saline; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

displayed significantly more gaping and chin rubbing on trials 2–4 (p's < 0.001) and displayed more paw treading on trials 3–4 (p's < 0.05) than group saline.

The 2 × 4 ANOVA for the positive hedonic reaction of tongue protrusions revealed significant main effects of group, $F(2, 21) = 16.9$, $p < 0.001$; trials, $F(3, 63) = 6.7$, $p = 0.001$; and a group by trials interaction, $F(3, 63) = 7.0$, $p < 0.001$; group LiCl displayed fewer tongue protrusions than group saline on trials 2–4 (p's < 0.01).

For the reaction of passive dripping, the ANOVA revealed significant main effects of group, $F(2, 21) = 17.7$, $p < 0.001$; trials, $F(3, 63) = 16.7$, $p < 0.001$; and a group × trials interaction, $F(3, 63) = 84.0$, $p = 0.002$; group LiCl displayed enhanced passive dripping on trials 2–4 (p's < 0.05). Finally for the CTA, depicted in the bottom left corner of Fig. 2, the 2 × 4 mixed-factors ANOVA revealed significant main effects of group, $F(1, 14) = 62.7$, $p < 0.001$; interval, $F(1, 14) = 83.4$, $p < 0.001$; and a group × interval interaction, $F(1, 14) = 70.2$, $p < 0.001$; group LiCl displayed suppressed intake in the CTA test at all intervals relative to group saline ($p < 0.01$).

The independent t tests for the motoric responses on the TR trial in experiment 1c revealed only that group LiCl displayed significantly *less* face washing than group saline, $t(14) = 3.3$; $p < 0.01$; no other behaviors differed between the groups (data not depicted).

Experiment 2: delayed (30 min) saccharin pairing with cocaine (20 mg/kg, sc and ip) and LiCl (50 mg/kg, ip)

The primary TR aversive behaviors (gaping, chin rubbing, paw treading) as well as the potential withdrawal-related measure of yawning, the positive hedonic reactions of tongue protrusions, and the neutral reaction of passive drips display

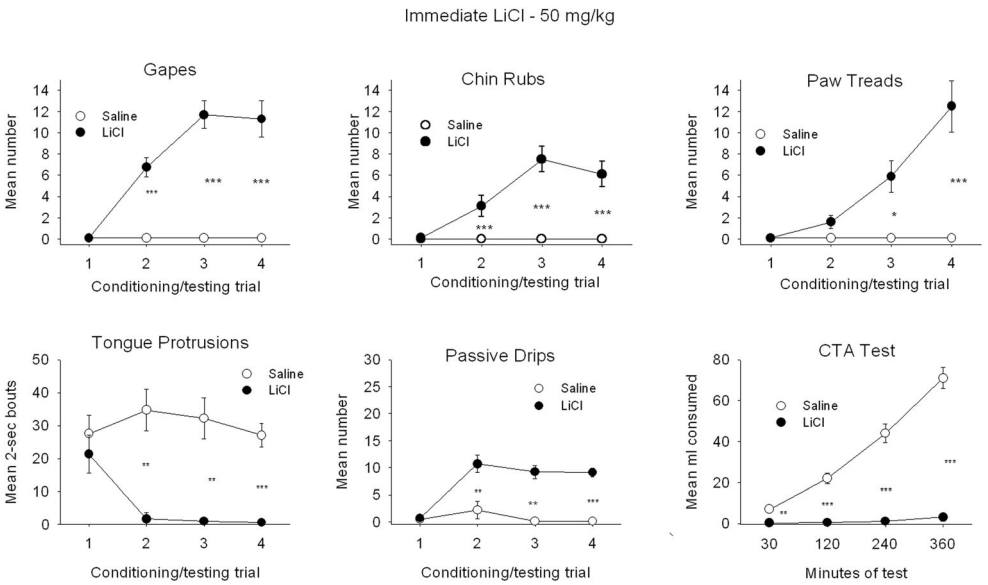

Fig. 3 Mean (± SEM) frequency or duration of primary TR measures and CTA in experiment 1c with immediate LiCl (50 mg/kg, ip). Asterisks indicate a group difference from saline; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

by the various groups on the final TR test trial across 5-min intervals are depicted in the upper section of Fig. 4. As well in the lower right-hand corner of Fig. 4, the CTA measure across intervals is depicted. When saccharin predicted the delayed delivery of sc cocaine or LiCl, rats displayed conditioned gaping and chin rubbing which was more pronounced during the early intervals of testing. However, only LiCl conditioned rats displayed the aversive behavior of paw treading. Only rats conditioned with sc cocaine displayed the withdrawal-like response of yawning. Rats conditioned with delayed access to cocaine (both ip and sc) or LiCl displayed suppressed tongue protrusions; however, only cocaine sc and LiCl produced enhanced passive dripping. Finally, all three drug groups showed suppressed consumption relative to group saline in the CTA test and did not differ from one another overall. Both sc cocaine and LiCl suppressed face washing and sc cocaine produced more head shaking than LiCl on the final test trial. The results of the analysis of each behavior is described below.

Aversive TR responses For the gaping measure, the 4 × 6 mixed-factors ANOVA of the number of gapes per 5-min interval for the various groups revealed a significant effect of group, $F(3, 20) = 4.8$, $p = 0.011$. Bonferroni pairwise comparison tests for the main effect across time revealed that both cocaine sc and LiCl produced significantly more gaping than saline (p's < 0.05), but not cocaine ip. Because we predicted that ip cocaine would produce conditioned gaping under conditions of delay (Wheeler et al. 2008), a less conservative LSD (LSD) comparison test revealed that ip cocaine also enhanced gaping overall ($p < 0.05$). The analysis also revealed significant effects of time, $F(5, 100) = 7.6$, $p < 0.001$ and a group × time interaction, $F(15, 100) = 3.9$, $p < 0.001$. Bonferroni post hoc comparison tests revealed that during the first and second 5-min intervals, both groups cocaine sc and LiCl displayed more gaping than group saline (p's < 0.05). Group cocaine ip did not differ from any other group at any interval. For chin rubbing, the ANOVA revealed an effect of time, $F(5, 100) = 45.4$, $p < 0.001$, and a group × time interaction, $F(15, 100) = 2.7$, $p = 0.002$. Bonferroni tests revealed that during the first 5-min interval, both LiCl and sc cocaine produced more chin rubbing than either ip cocaine or saline (p's < 0.05). For paw treading, the ANOVA revealed a main effect of group, $F(3, 20) = 5.4$, $p = 0.007$, with subsequent Bonferroni post hoc tests on the main effect indicating that group LiCl displayed more paw treading than any other group (p's < 0.05). The effect of time, $F(5, 100) = 3.1$, $p < 0.05$, and the group × time interaction, $F(15, 100) = 2.2$, $p = 0.011$, were also significant, with Bonferroni tests revealing that LiCl produced more paw treading than all treatments during all intervals but the fourth.

Withdrawal-like response The withdrawal-like response (B) of yawns are depicted in the upper right-hand corner of Fig. 4. The ANOVA revealed only a significant main effect of group, $F(3, 20) = 9.5$, $p < 0.001$; subsequent Bonferroni tests revealed that cocaine sc produced significantly more yawns over the 30-min test than any other treatment (p's < 0.01). This may represent a conditioned withdrawal response to cocaine that is also seen with opiates (e.g., Schnur et al. 1992; Bickel et al. 1988).

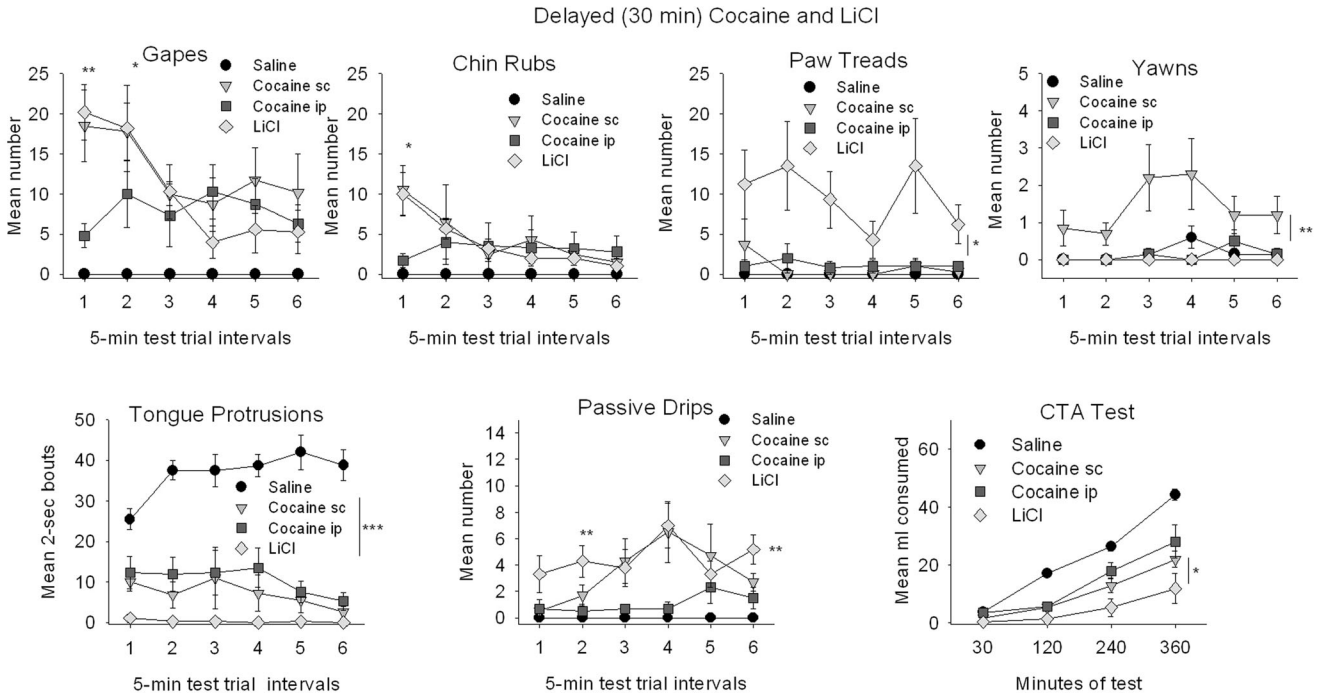

Fig. 4 Mean (± SEM) frequency or duration (s) of primary TR measures and CTA in experiment 2 with 30 min delayed cocaine (20 mg/kg, either sc or ip) and LiCl (50 mg/kg, ip). Asterisks indicate a group difference from saline; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Tongue protrusions, passive drips, and CTA For tongue protrusions, the ANOVA revealed a significant main effect of group, $F(3, 20) = 33.5$, $p < 0.001$, and a group × time interaction, $F(15) = 3.2$, $p = 0.015$; subsequent Bonferroni tests revealed that during each interval, group saline showed more tongue protrusions than all other groups (p's < 0.01), which did not differ from one another at any interval. For passive drips, the ANOVA revealed a significant main effect of group, $F(3, 20) = 7.3$, $p = 0.002$; time, $F(5, 100) = 3.7$, $p = 0.004$; and a group × time interaction, $F(15, 100) = 2.0$, $p < 0.02$. Bonferroni tests on each interval revealed that group LiCl (p's < 0.05) displayed more passive drips on intervals 2, 4, and 6 than group saline or group cocaine ip. Only at interval 4 did cocaine sc (p's < 0.05) show more passive drips than group saline or cocaine ip. For the CTA measure, the 4 × 4 ANOVA for each cumulative drinking measure revealed a significant main effect of group, $F(3, 20) = 16.1$, $p < 0.001$; Bonferroni tests overall revealed that group saline drank more saccharin solution overall than any other group (p's < 0.025). As well, group LiCl had a greater overall CTA than group cocaine ip ($p < 0.05$), but not cocaine sc. There was also a significant effect of time, $F(3, 60) = 108.9$, $p < 0.001$, and a group × time interaction, $F(9, 60) = 6.3$, $p < 0.001$; subsequent Bonferroni tests revealed that although the groups did not differ in the initial 30 min of intake, during interval 120 min all groups drank less saccharin than group saline (p's < 0.001), but during intervals 240 and 360 min, only groups LiCl ($p < 0.001$) and cocaine sc (p's < 0.025) continued to drink significantly less than group saline.

Motoric responses The one-way ANOVA of the TR test motoric responses in experiment 2 revealed a significant group effect for the behaviors of active locomotion, $F(3, 20) = 3.8$, $p = 0.026$; face washing, $F(3, 20) = 7.6$, $p = 0.001$; head shakes, $F(3, 20) = 6.7$, $p = 0.003$; and forelimb flails, $F(3, 20) = 3.6$, $p = 0.03$. Subsequent Bonferroni comparison tests revealed that the group conditioned with cocaine ip were more active than the group conditioned with cocaine sc or LiCl (p's < 0.05); no group differed from group saline in active locomotion. Groups LiCl ($p < 0.01$) and cocaine sc ($p < 0.05$) displayed significantly less face washing than group saline or cocaine ip (p's < 0.01). Group LiCl displayed significantly fewer head shakes than group cocaine sc or cocaine ip (p's < 0.05), but not group saline. Finally, group cocaine ip displayed more forelimb flails than group saline (p's < 0.05), but no other groups differed (data not depicted).

Experiment 3: delayed (30 min) context pairing with cocaine

None of the aversive primary TR behaviors (gapes, chin rubs, and paw treads) nor activity measures revealed any differences between the cocaine and saline groups. The 2 × 6 mixed-factors ANOVA for gaping, chin rubbing, paw treading, and yawning were not significant. Therefore, the conditioned aversive effects evident in experiment 2 were the result of conditioning to the flavor, not the context. The mean distance traveled during each 5-min interval of the final test trial were entered into a 2 × 6 mixed-factors ANOVA which revealed only a significant main effect of time, $F(5, 70) = 4.6$, $p < 0.001$, with all rats more active during the first 5-min interval (data not depicted).

Experiment 4: delayed (10 min) saccharin pairing with cocaine or morphine

Figure 5 presents the mean frequency or duration of the primary TR measures during each conditioning trial in experiment 4. Delayed access to cocaine and morphine (but to a lesser extent) produced conditioned gaping in rats even when the delay was only 10 min, and this effect (with cocaine) began after a single conditioning trial. However, the aversive behaviors of chin rubbing and paw treading were not produced. Both cocaine and morphine produced suppressed consumption in the CTA test and cocaine, but not morphine, enhanced passive dripping across trials.

For the behavior of gaping, the ANOVA across the five trials revealed a significant effect of group, $F(2, 20) = 7.9$, $p = 0.003$. Because we predicted that delayed access to a rewarding drug would produce aversive gaping, LSD comparison tests were used to reveal that both cocaine ($p < 0.001$) and morphine ($p = 0.028$) produced more gaping than saline conditioned groups. The trial effect, $F(4, 80) = 12.4$, $p < 0.001$, and the group × trial interaction, $F(8, 80) = 5.3$, $p < 0.001$, were also significant. Subsequent Bonferroni tests on each trial revealed that the cocaine conditioned group displayed more gaping than group saline on trials 2 ($p < 0.05$), 4, and 5 (p's < 0.001), but group morphine did not differ from group saline on any trial. There were no significant group differences in chin rubbing or paw treading.

For the response of yawning, although all of the rats that yawned were in the cocaine group, the ANOVA revealed no significant effects across the trials. Since yawning was seen in the cocaine conditioned group in experiment 2 following a 30-min taste exposure predominately during intervals 3–4 (minutes 10–20), it is likely that the lack of significant yawning in this group here is a function of the shorter duration (10 min) of exposure to the taste.

For tongue protrusions, the ANOVA revealed only a significant effect of trial, $F(4, 80) = 10.5$, $p < 0.001$. For passive dripping, the ANOVA revealed only a significant main effect of group, $F(2, 20) = 6.7$, $p = 0.006$; Bonferroni tests revealed that group cocaine displayed more passive dripping across trials than did group saline or group morphine (p's < 0.025). Finally, the mean cumulative amount of saccharin consumed in the CTA test at 120, 240, and 360 min was entered into a

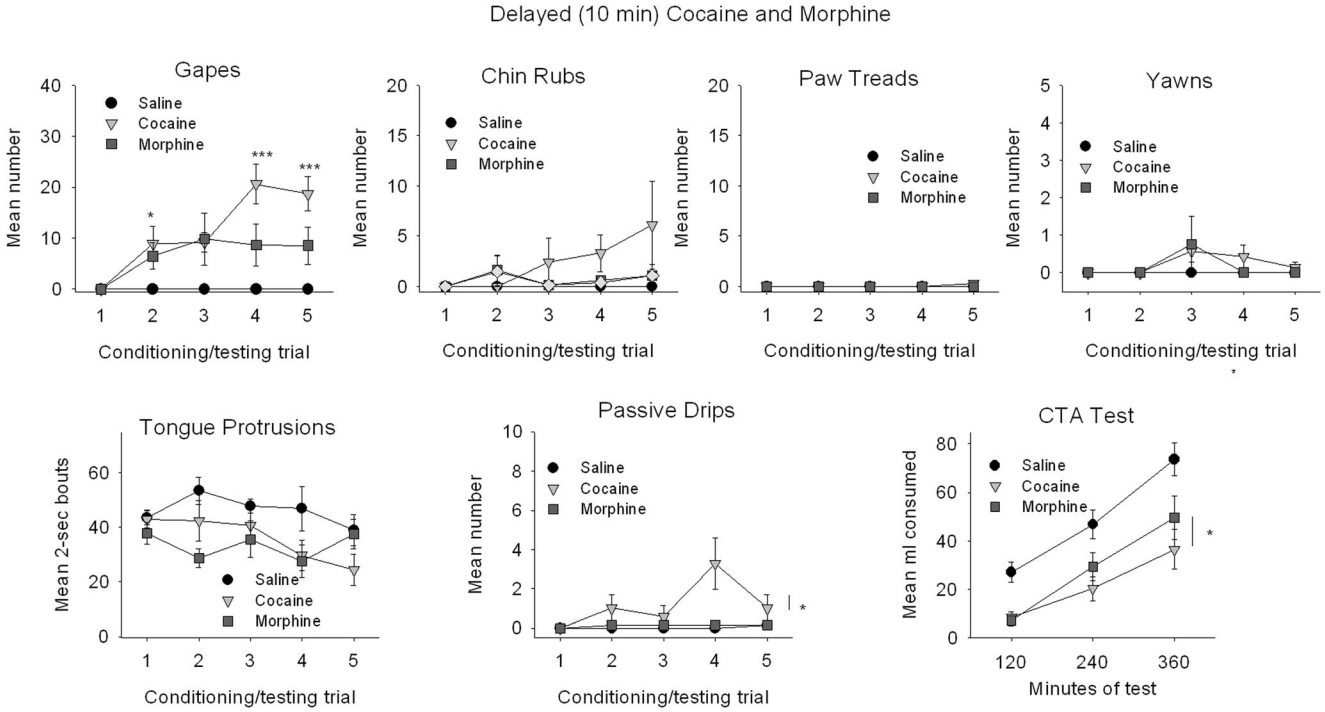

Fig. 5 Mean (± SEM) frequency or duration primary TR measures and CTA in experiment 4 with 10 min delayed cocaine (20 mg/kg, sc) or morphine (10 mg/kg, sc). Asterisks indicate a group difference from saline; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

3 × 3 mixed-factors ANOVA, which revealed a significant effect of group, $F(2, 20) = 6.4$, $p = 0.007$; Bonferroni tests indicated that both group cocaine and morphine drank less saccharin overall than group saline. Groups cocaine and morphine did not significantly differ in their intake of saccharin solution. There was also a significant effect of interval of drinking, $F(2, 40) = 116.6$, $p < 0.001$, but no group by interval interaction.

The one-way ANOVA for each motoric behavior on the final TR test trial in experiment 4 revealed a significant group effect only for the behavior of forelimb flails, $F(2, 20) = 4.3$, $p = 0.028$, but subsequent Bonferroni tests revealed no significant difference among the groups.

Experiment 5: assessment of anxiogenic-like responding following exposure to saccharin previously paired with delayed access to cocaine

Rats given 10-s infusions of saccharin every min for 30 min prior to an injection of cocaine displayed anxiogenic-like responding when compared to the saline conditioned rats. This was not simply due to experience with cocaine, as the home cage rats injected with cocaine or saline did not differ in the light–dark emergence test. Table 2 presents the mean (± SEM) number of seconds spent in the lit box. The rats in group Sac → Delayed Cocaine spent significantly less time in the lit box than the rats in group Sac → Delayed Saline, $t(12) = 2.35$, $p = -0.037$; however, group Home Cage Cocaine and Home Cage Saline did not differ from one another, $t(14) = 0.86$. Table 3 presents the mean (± SEM) number of the TR behaviors scored during the 30-min session of saccharin exposure prior to the LD test. Group Delayed Cocaine displayed more gapes, $t(12) = 2.3$, $p < 0.5$; more yawns, $t(12) = 2.2$, $p < 0.05$; and fewer 2-s bouts of tongue protrusions, $t(12) = 2.6$, $p < 0.05$, than Group Delayed Saline. None of the other behaviors (or any motoric behaviors) differed among the groups.

Discussion

It has long been understood that rats will avoid a taste paired with rewarding drugs including cocaine (Booth et al. 1997; Goudie et al. 1977; Hunt and Amit 1987; Ferrari et al. 1991; Mayer and Parker 1993; Grigson 1997). Indeed, cocaine also produces the avoidance-like behaviors of suppressed tongue protrusions and enhanced passive dripping in the taste reactivity test (e.g., Parker 1993, 1995). However, when cocaine is immediately paired with a taste, it does not produce a conditioned "aversion" characterized by the aversive taste reactivity

Table 2 Mean (± SEM) seconds spent in open lit box in experiment 5

Group Sac → Delayed Saline ($n = 7$)	Group Sac → Delayed Cocaine ($n = 7$)
183.6 (± 5.2) s	165.3 (± 5.8) s
Group Home Cage Saline ($n = 8$)	Home Cage Cocaine ($n = 8$)
107.1 (± 8.7) s	110.7 (± 18.4) s

Table 3 Mean (± SEM) number of TR behaviors during 30-min test infusion in experiment 5

Group Sac → Delayed Saline		Group Sac → Delayed Cocaine
Gape	0.0	29.4 (± 12.8)*
Chin Rub	0.0	8.6 (± 5.4)
Yawn	0.6 (± 0.1)	5.4 (± 2.2)*
Paw tread	0.0	0.6 (± 0.4)
Passive drip	0.0	3.6 (± 2.6)
Tongue protrusions	180.6 (± 27.1)	99.6 (± 16.5)*

*$p < 0.05$

measures of gaping, chin rubbing, and paw treading that are produced by emetic drugs, such as LiCl (Parker 1993, 1995). Here, we replicate and extend this finding in rats that received daily conditioning trials with both ip and sc cocaine. During brief taste reactivity conditioning/testing trials with saccharin followed immediately by cocaine (either sc or ip), rats passively drip the taste from their mouths and suppress ingestive tongue protrusions during intraoral delivery (replicating Parker 1993, 1995), as they show with a LiCl-paired flavor. Yet, unlike the LiCl-paired flavor, rats do not display the aversive responses of gaping, chin rubbing, or paw treading during an intraoral exposure to this cocaine-paired flavor. This was not likely due to the LiCl simply being more potent because while LiCl appeared to support a stronger CTA than cocaine in experiment 1, it did not do so in experiment 2. Cocaine sc (20 mg/kg), but not LiCl, did enhance head shakes (often considered to be an aversive reaction) on the final test trial. On the contrary, like what was reported by Wheeler et al. (2008, 2011), rats given several saccharin exposures during each of four 30-min sessions prior to exposure to cocaine developed the aversive behaviors of gaping and chin rubbing; however, the remaining aversive behavior of paw treading was not apparent. Both LiCl and cocaine sc actually suppressed face washing (initially identified by Grill and Norgren 1978a, and often included by others, as an aversive response). When the delay was decreased to 10 min in experiment 4, both sc cocaine and morphine produced gaping reactions, albeit the effect of cocaine was more robust than morphine, but neither produced the aversive reactions of chin rubbing or paw treading.

Another behavior that is rarely reported in a taste reactivity context is that of yawning, which has been reported as a withdrawal response to opiates and other drugs in animals (Nakamaura-Palacios et al. 2002; Jaw et al. 1993; Schnur et al. 1992) and humans (Bickel et al. 1988). Here, we report that rats respond to delayed access to cocaine, but not immediate access to cocaine or immediate or delayed access to LiCl, with an orofacial yawning reaction (prolonged elongated vertical opening of the mouth followed by expiration of air) which is characteristically very different than gaping (wide opening triangular shape open mouth exposing bottom incisors). This yawning response occurred between minutes 10 and 20 of the TR test during the 30-min delayed access to cocaine in experiment 2, but was not significant in experiment 4 with only a 10-min delay. Interestingly, yawning is a response that rats display to dopamine (D2, D3) agonists, such as quinpirole (e.g., Collins et al. 2005) or to serotonin 2c (5-HT_{2c}) agonists, such as Loracserin (e.g., Serafine et al. 2015). Here, this response emerged only in rats conditioned with long delayed (but not short delayed—10 min) access to cocaine (sc) and may represent a withdrawal response to cocaine as an indirect measure of a dysregulated dopamine and/or serotonin system.

The results of experiment 5 provide an independent assessment of the conditioned withdrawal produced by exposure to saccharin previously paired with delayed access to cocaine. Rats experiencing cocaine withdrawal display anxiogenic-like responding in preclinical models (Hu et al. 2016; Oliveriera Citó Mdo et al. 2012; Kupferschmidt et al. 2012), including the LD emersion test (Costall et al. 1990). Indeed, rats exposed to saccharin previously paired with delayed access to cocaine spent less time in the open lit box than rats exposed to saccharin previously paired with delayed access to saline, a pattern of anxiogenic-like responding. This difference was not simply the result of 24 h withdrawal from three daily exposures to cocaine because home cage cocaine exposed rats did not differ from home cage saline exposed rats in time spent in the lit open box.

Our findings are therefore consistent with those of Wheeler et al. (2008, 2011) who suggested that such delayed exposure to cocaine produces an aversive state of withdrawal that becomes associated with the taste of saccharin and thus producing gaping reactions, as rats also show to a flavor paired with opiate withdrawal (McDonald et al. 1997). Several lines of evidence such a contention. Using fast-scan voltammetry, Wheeler et al. (2011) observed decreased mesolimbic dopamine concentrations to a taste cue that signaled delayed cocaine availability, but this switched to elevated mesolimbic dopamine concentrations to taste cues signaling imminent cocaine delivery in a self-administration session. As well, under conditions of delayed access to cocaine, NAc neurons display a quinine-like (opposite of a sucrose-like) excitability pattern (Roitman et al. 2005; Wheeler and Carelli 2009) during saccharin exposure. Interestingly, the excitatory response profile and the aversive taste reactivity are inversely correlated with the latency to make the first press for cocaine. These findings suggest that the switch in NAc activity from inhibitory to excitatory during infusions of the cocaine-paired taste reflects the learned association between the taste and the negative affective state of withdrawal that drives the increased motivation to consume cocaine when available. Furthermore, rats given 20 min access to saccharin which had been paired with morphine displayed suppressed DA signaling compared with rats conditioned with saline (Grigson and Hajnal 2007). Finally,

presentation of a taste cue predictive of delayed access to cocaine produced an elevated intracranial self-stimulation threshold (Wheeler et al. 2011). These findings collectively indicate a pronounced dampening of the DA system during exposure to a taste that signaled delayed cocaine or morphine availability.

Carelli and West (2013) hypothesize that the increased motivated behavior for cocaine following this learned association may be a consequence of the development of a negative affective state that is a consequence of delayed drug availability. Koob and colleagues (Ahmed and Koob 1998) argue that chronic cocaine self-administration alters the rat's hedonic set point, reducing responsiveness to rewarding stimuli, as a modified version of the opponent process theory (Solomon and Corbit 1974). This allosteric regulation reduces the hedonic set point by increasing the function of the brain "anti-reward" system, thereby increasing tolerance to the hedonic effects of cocaine (see also Siegel and Ramos 2002). Wheeler et al. (2008) found that this negative state may be alleviated by drug loading; that is, rats that show the strongest aversions to delayed cocaine-paired taste also showed the greatest self-administration of cocaine. Thus, during this waiting period, the rat experiences an aversive state that includes the onset of conditioned anxiety, craving, and/or withdrawal (a cocaine-need state). Nyland and Grigson (2013) provided some direct evidence for a withdrawal interpretation. Following several days of pairing taste exposure with the delayed opportunity to self-administer cocaine, rats were exposed to the cocaine-associated taste followed by an injection of naloxone which can precipitate withdrawal not only from morphine but also from cocaine, as measured by body weight loss. The cocaine group had a significant loss in body weight 2 h after naloxone administration and the greater the weight loss, the greater the subsequent cocaine self-administration. These findings suggest that avoidance of the taste results from the development of an aversive conditioned withdrawal state that develops when the taste cue comes to predict the delayed availability of the drug.

The failure to see conditioned aversive responses to saccharin when paired with immediate access to cocaine cannot simply be attributed to less exposure to the saccharin because in experiment 3, the rats received the same amount of saccharin exposure as in experiment 1 (2 ml), but the saccharin signaled a waiting period of 10 min. Thus, the saccharin became a cue for the delayed availability of cocaine and morphine in experiment 3 producing gaping reactions. This finding extends the effect to another rewarding drug and supports Grigson's (1997) model of reward comparison, as well. Indeed, as has also been shown by Colechio et al. (2014), the development of a negative affective state produced by delayed availability to cocaine appears to develop even after only a single conditioning trial.

There is an issue, however, that remains somewhat unclear. If saccharin acquires conditioned aversive effects because it comes to predict the delayed delivery of a highly desired drug, then it is not clear why sc cocaine, which was ineffective in producing a conditioned place preference (Mayer and Parker 1993, see also Tzschentke 1998), is more effective in producing a negative conditioned affective state than is ip cocaine, which is more effective in producing a CPP. As well, sc cocaine was more effective than 10 mg/kg sc morphine in producing conditioned aversion with a 10-min delay, yet morphine consistently produces a conditioned place preference (CPP) at this dose at the sc route of administration (e.g., Mueller et al. 2002; Tzschentke 1998).

Here, we have analyzed each of the behaviors separately that were initially identified in the TR test by Grill and Norgren (1978a). Many researchers in the field have taken shortcuts of only measuring certain of these behaviors, such as gaping, or combining across selective behaviors providing total aversive score. For instance, the behavior of face washing has been combined with gaping as an aversive measure; however, the results of experiments 1c and 2 clearly show that face washing is decreased (not increased) by pairings of saccharin with LiCl or delayed access to sc cocaine. Clearly the responses of face washing, head shaking, forelimb flailing, and general activity are not consistently aversive responses, as seen here and discussed by Parker (1995). It will be important for future researchers to avoid composite scores that may not reflect a common process.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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