Published in final edited form as:

Drug Alcohol Depend. 2013 December 15; 133(3): . doi:10.1016/j.drugalcdep.2013.08.029.

Outcome specificity in deepened extinction may limit treatment feasibility: Co-presentation of a food cue interferes with extinction of cue-elicited cocaine seeking

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Abstract

Background: We previously showed that presenting two cocaine cues simultaneously during extinction deepens the extinction of cue-elicited cocaine seeking (Kearns et al., 2012). The present study investigated whether compounding a non-drug appetitive cue with a cocaine cue would similarly deepen extinction.

Methods: In Experiment 1, tone and click were each first established as discriminative stimuli for cocaine-reinforced responding and light was a cue for food-reinforced responding. In an initial extinction phase, all stimuli were presented individually. Then, during an additional compound extinction session, rats received 8 presentations of one of the cocaine cues (counterbalanced over subjects) simultaneously with light and 8 presentations of the other cue alone. A spontaneous recovery test was used to evaluate the effectiveness of the extinction treatments. Experiment 2 was performed under conditions designed to match those of Experiment 1, except food was the reinforcer in tone and click instead of cocaine.

Results: In Experiment 1, the cocaine cue compounded with the food cue during extinction controlled greater spontaneous recovery of cocaine seeking than the cocaine cue always presented alone. In contrast, Experiment 2 demonstrated deepened extinction of responding to a food cue when both compounded cues were food cues.

Conclusions: Results suggest that deepened extinction depends on the compound presentation of cues associated with the same reinforcer. Compound presentation of cues associated with different reinforcers could lead to an enhancement of responding. Care is urged in attempts to deepen the extinction of cue-elicited drug seeking by compounding drug cues with non-drug cues.

Keywords

extinction; drug cues; cocaine; self-administration; cue exposure; stimulus compounding; rats

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Contributors All authors contributed to the design and performance of the experiment. Brendan Tunstall and David Kearns analyzed the data and wrote the manuscript. All authors have approved the final manuscript.

Conflict of Interest No conflict declared

1. INTRODUCTION

Drug cues play a central role in addiction (Volkow et al., 2012). In humans, drug cues can elicit craving for the drug (Kennedy et al., 2013; Sinha, 2013), activate the brain's reward circuits (Fotros et al., 2013; Volkow et al., 2012), and trigger relapse (Marhe et al., 2013; Prisciandaro et al., 2013). A treatment that neutralizes the power of drug cues could prove very valuable in efforts to manage addiction. Extinction has been used in such an effort. For example, the extinction-based treatment called cue-exposure therapy (Drummond et al., 1995) involves repeatedly presenting cues to addicts without the drug. This extinction should theoretically break the cue-drug association and thereby eliminate the cues' ability to influence behavior. While there have been a few studies suggesting that cue-exposure therapy may have some promise (e.g., Loeber et al., 2006; Rohsenow et al., 2001), in general, results have not been as encouraging as expected (for reviews see Conklin and Tiffany, 2002; Havermans and Jansen, 2003). A method more effective than the standard extinction procedure may help to improve outcomes.

Recently, preclinical studies have shown that there is a way of deepening the extinction of cue-elicited, drug-seeking behavior (Janak et al., 2011; Kearns et al., 2012). Deepened extinction was first reported by Rescorla (2006) in experiments involving non-drug cues. In deepened extinction, cues are first presented individually during extinction training, for several sessions. Then, the cues are presented simultaneously (i.e., compounded) during additional extinction training. We showed that even a single such compound extinction session was capable of reducing the spontaneous recovery of cue-controlled cocaine seeking by approximately 50% as compared to that produced by a cocaine cue that underwent the standard extinction treatment (Kearns et al., 2012). This suggests that the deepened extinction procedure could be a way to more effectively, and more permanently, reduce the power of drug cues over behavior.

Deepened extinction is accounted for by error-correction learning theories such as the Rescorla-Wagner model (Rescorla, 2006; Rescorla and Wagner, 1972). According to these models, learning is the process whereby the discrepancy (or "prediction error") between expectation and outcome is corrected. In the case of extinction learning, presentation of the cue initially produces expectation for the reward previously associated with that cue. When the reward is not delivered, there is a prediction error. This acts to decrease reward expectation produced by the cue. This process continues until the cue no longer generates expectation for reward. Now, there is no longer a mismatch between expectation (no reward) and outcome (no reward) and, therefore, nothing to drive learning. Rescorla (2006) hypothesized that further extinction learning might be possible at this point if expectation could be restored, temporarily, over the course of additional extinction trials. He discovered that compounding two cues was a way to create such a temporary expectation and thereby produce deepened extinction.

One issue that may limit the potential clinical utility of deepened extinction as a treatment for human drug abusers is the difficulty in controlling the presentation of cues that might be compounded with the problematic target drug cue(s). While it may be possible to expose users in the clinic to small and discrete paraphernalia cues (e.g., a pipe), it may not be feasible to compound these with other relevant drug cues, such as a person or place previously associated with the drug. Further, cue-drug associations are assumed to have been formed in the user's natural environment as a by-product of the drug-taking experience. This means that the conditioning histories of many of these cues are variable or unknown. For example, while it may be assumed that there has been a consistent pairing of a crack pipe with the effects of cocaine, there may have been more irregular pairings, and

different kinds of associations formed, between broader contextual cues (e.g., a house or neighborhood) and the effects of cocaine.

A more practical treatment based on deepened extinction would need to provide the practitioner with better control over the cues that are to be presented during extinction in compound with the problematic drug cues. Of course, establishing new drug cues in the clinic would require presentation of the abused drug and this is undesirable. However, if deepened extinction could be produced by compounding the problematic drug cue(s) with other non-drug appetitive cues, a solution might be had. For example, periodic presentation of a shape on a computer screen could be established as a cue predictive of a small monetary reward. This shape should then come to elicit positive feelings, perhaps even a small “rush” or “high” that is associated with winning money. This non-drug appetitive cue could then be simultaneously presented with the target drug cue on compound extinction trials, where no money (or drug) would be presented. If the effects of the prediction error produced by the omission of reward on these compound extinction trials generalized to the drug cue, deepened extinction of the drug seeking elicited by the drug cue could be achieved.

In all of the deepened extinction studies to date, the compounded cues were previously associated with the same reinforcer. That is, either 2 drug cues (Janak et al., 2011; Kearns et al., 2012) or 2 food cues (Janak and Corbit, 2010; Rescorla, 2006; Leung et al., 2012) were compounded with each other. It is unknown whether compounding cues associated with different reinforcers will also result in deepened extinction. The present study will test this possibility in a preclinical model by investigating whether compounding a food cue with a cocaine cue will lead to the deepened extinction of the cocaine seeking elicited by the latter. Finding such an outcome would suggest that a version of deepened extinction treatment for drug abuse involving compounding non-drug cues with drug cues could be possible.

2. METHODS AND MATERIALS

2.1 Subjects

Naïve adult male Long-Evans rats served as subjects. Thirteen rats completed Experiment 1 and 12 rats completed Experiment 2. Rats were individually housed in plastic cages with wood chip bedding and metal wire tops. They were maintained at 85% of their free-feeding weights (approximately 350–450 g). The colony room where the rats were housed had a 12-h light:dark cycle with lights on at 08:00 h. Training sessions were conducted 5–7 days per week during the light phase of the light:dark cycle. Throughout both experiments, rats were treated in accordance with the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, 2011) and all procedures were approved by American University's Institutional Animal Care and Use Committee (IACUC).

2.2 Apparatus

Training took place in six operant chambers. Each chamber was 20 cm high, 23 cm long, and 18 cm wide and had aluminum front and rear walls, white translucent plastic side walls, a clear plexiglass ceiling, and a grid floor. A response lever and food trough were located on the front wall of the chamber. A click stimulus (approximately 15 clicks/s and 64–78 dB), created by a BRS CL-201 click generator and amplified by a BRS AA-201 amplifier, was presented through a 20-cm diameter speaker located in an enclosure mounted approximately 21 cm above the chamber. A tone stimulus (approximately 4500 Hz and 64–66 dB) was delivered by a model SC628HR Sonalert (Mallory Sonalerts, Indianapolis, IN) that was mounted on top of the chamber ceiling. A light stimulus was provided by two 15-cm, 25-W, 120-VAC tubular light bulbs located 10 cm outside each of the side walls of the chamber. These light bulbs were operated at 103 VAC. Each chamber was housed inside a sound

attenuation chest (Weiss, 1970) that had a continuously operating ventilation fan. A shielded houselight provided a low level of continuous illumination during all sessions. Experimental procedures were controlled by Med-Associates software (Med-PC, St. Albans, VT) running on a PC located in an adjacent room.

Cocaine (provided by the National Institute on Drug Abuse) in a saline solution at a concentration of 2.56 mg/ml was infused at a rate of 3.19 ml/min by 10-ml syringes driven by Harvard Apparatus (South Natick, MA) or Med-Associates (St. Albans, VT) syringe pumps located outside of the sound attenuation chests. Tygon tubing extended from the 10-ml syringes to a 22-gauge rodent single-channel fluid swivel and tether apparatus (Alice King Chatham Medical Arts, Hawthorne, CA) that descended through the ceiling of the chamber. Cocaine was delivered to the subject through Tygon tubing that passed through the metal spring of the tether apparatus. This metal spring was attached to a plastic screw cemented to the rat's head to reduce tension on the catheter.

2.3 Procedure: Experiment 1

The present procedure was similar to that previously used by Kearns et al. (2012) except that one of the S^D s – the light – was established as an S^D for food-reinforced responding rather than cocaine-reinforced responding.

2.3.1. Lever-press acquisition

With the light stimulus continuously illuminated, rats were trained to lever press for food pellets (45 mg, Rodent Grain-Based Diet, BioServ, Frenchtown, NJ) in sessions lasting approximately 60 min. Food pellets were available for lever pressing on a fixed-ratio 1 (FR-1) schedule and were also presented non-contingently every 120 s if a lever press was not made. Rats were trained until 100 responses were emitted within a session.

2.3.2. Light/no-light discrimination training

During 2-h sessions, light S^D components lasting 180 s on average (range: 150–210 s) were presented, during which food pellets were available for lever pressing according to a variable-interval (VI) 15-s schedule. These light components alternated with S^A components lasting 60 s on average (range: 30–90 s) where the light was off and responding did not result in food pellet delivery. A 30-s response correction contingency operated during the final 30 s of each S^A component. According to this contingency, a 30-s S^A -termination clock was reset to zero by a response. This contingency was designed to reduce response rates during S^A components. Rats were trained with the schedule parameters described above for 1 session. Then, the VI schedule that operated during S^D components was increased to VI 60 s and sessions were continued until a minimum of 4 further sessions were completed and until rats responded at least 3 times faster in light than in light-off for 2 consecutive sessions.

2.3.3. Surgery

Rats were then surgically prepared with chronic indwelling jugular vein catheters, using a modification of the procedure originally developed by Weeks (1962). In brief, under ketamine (60 mg/kg) and xylazine (10 mg/kg) anesthesia, approximately 3 cm of Silastic tubing (0.044 mm i.d., 0.814 mm o.d.) was inserted into the right jugular vein. This Silastic tubing was connected to 8 cm of vinyl tubing (Dural Plastics; 0.5 mm i.d., 1.0 mm o.d.) that was passed under the skin around the shoulder and exited the back at the level of the shoulder blades. The vinyl tubing was threaded through a section of Tygon tubing (10 mm long, 4 mm diameter) that served as a subcutaneous anchor. Six stainless steel jeweler's screws were implanted in the skull, to which a 20-mm plastic screw was cemented with

dental acrylic. Catheters were flushed daily with 0.1 ml of a saline solution containing 1.25 U/ml heparin and 0.08 mg/ml gentamycin.

2.3.4. Tone/no-tone and click/no-click discrimination training

After 5-7 days of recovery in the homecage, rats were trained to self-administer cocaine on a 3-component multiple schedule. During tone or click S^D components lasting 60 s on average (range: 50 - 70 s), cocaine infusions (0.5 mg/kg/inf) were available for lever pressing according to a VI 60-s schedule. These S^D components alternated with S^Δ components lasting 120 s on average (range: 90 - 150 s) where all stimuli were absent and lever pressing did not result in cocaine. Each of the tone and click S^D components were equally likely to follow an S^Δ component, with the restriction that there were no more than two consecutive S^D components of the same type. A 30-s response correction contingency operated during the final 30 s of each S^Δ component as described in section 2.3.2. Sessions lasted 2 h. Rats were trained with the schedule parameters described above until they responded at a rate at least 3 times faster in tone and in click components than in S^Δ components for 2 consecutive sessions.

2.3.5. Terminal baseline discrimination training

In this phase, light S^D components, where lever presses were reinforced by food according to a VI 60-s schedule, were added to the training schedule described above, so that rats were now trained on a 4-component multiple schedule. Cocaine (0.5 mg/kg/inf) continued to be available for lever pressing during tone and click S^D components on a VI 60-s schedule, and no reinforcement could be obtained during all-stimuli-off S^Δ components. The order of presentation of S^D components was randomized with the restriction that there be no more than 2 consecutive S^D s of the same type. Sessions lasted 2 hrs. Rats were trained on this terminal baseline procedure for a minimum of 2 sessions and until (1) response rates during tone, click, and light S^D components were at least 3 times higher than in S^Δ components, and (2) response rates in the tone and the click were within 20% of each other when averaged over the final 2 sessions. Care was taken to equate tone and click response rates because these were the stimuli that would be compared with each other in testing. If necessary, the amplitude of the click or tone was slightly adjusted during training on an individual-subject basis so that rates were comparable. Similarly, if a rat persisted in responding during S^Δ components, the length of the response-correction contingency was increased on an individual-subject basis up to a maximum of 300 s.

2.3.6 Extinction Phase 1

Rats next received an extinction phase where the tone, click, and light stimuli were each presented separately. Each stimulus was presented 8 times per session (as in Kearns et al., 2012). Each tone, click, or light component lasted 45 s and was separated by a 135-s period where all stimuli were off. Lever pressing did not produce cocaine or food pellets at any time and the response-correction contingency in S^Δ was discontinued. Rats were trained on this procedure for 3 sessions.

2.3.7 Extinction Phase 2: Stimulus compounding

The auditory stimuli were used to compare the effectiveness of the deepened extinction treatment with the standard extinction treatment. For half the rats, the tone was compounded with the light during this phase, while the click was always presented individually. For the other half of the rats, the roles of the tone and the click were reversed. Using the notation of Rescorla, the auditory stimulus that was compounded with the light was designated Stimulus X and the auditory stimulus that was always presented alone was designated Stimulus Y (the light was designated Stimulus A).

Rats were exposed to one stimulus compounding session in this phase. The session began with half of a session like those in Extinction Phase 1. That is, Stimulus X, Y, and A were presented individually 4 times each. This was followed by 8 presentations each of the AX compound (the light and one of the auditory stimuli presented simultaneously) and Stimulus Y (presented individually). Presentations of AX components and Y components lasted 45 s each and were separated by all-stimuli-off periods lasting 135 s. As before, lever pressing during this session not produce cocaine or food pellets at any time.

2.3.8 Spontaneous recovery test

A spontaneous recovery test was used to evaluate the effectiveness of the extinction of responding elicited by Stimuli X and Y. Following the final Extinction Phase 2 session, rats were left in their homecages for approximately 2 weeks (range = 13-16 days). The test session was like an extinction session in that lever pressing did not produce cocaine or food pellets at any time and 45-s stimulus components alternated with 135-s periods where all stimuli were off. The test consisted of 4 presentations each of the tone (T) and the click (C) in the order T-C-T-C-C-T-C-T. Because the tone and the click were counterbalanced in their roles as Stimulus X and Y over subjects, this meant that the order of presentation of Stimuli X and Y was also counterbalanced over subjects.

2.4. Procedure: Experiment 2

Because the opposite of deepened extinction was observed in Experiment 1, a second experiment was conducted to help in understanding this result. In Experiment 1, response rates to light – the food cue – were significantly higher than response rates to click or tone – the cocaine cues. In previous experiments reporting deepened extinction, the response rates to the compounded stimuli have been comparable. Rescorla (2006) has suggested that deepened extinction might not occur if A of the AX compound is a stronger stimulus than X. Thus, the outcome of Experiment 1 could have been due to the difference in rates between A and X. Alternatively, it may be the case that the response rate difference was unimportant and the failure to find deepened extinction in Experiment 1 was due to the fact that the compounded stimuli were previously associated with different reinforcers.

Experiment 2 was performed to aid in deciding between these alternatives. The design was the same as that used in Experiment 1, except that tone, click, and light were all food cues. The schedule of food reinforcement operating in light (VI 60-s) was richer than that in tone or click (VI 180-s) so that light would control a significantly higher response rate, as it did in Experiment 1. (see Figure 1). Rats in Experiment 2 did not have surgery. All other aspects of the procedure were the same as in Experiment 1. If deepened extinction were observed in Experiment 2, then the notion that difference in response rates between the compounded cues in Experiment 1 prevented deepened extinction could be ruled out.

2.5 Data analysis

For all statistical tests, $\alpha = 0.05$. Response rate was the primary measure of interest for all training phases, extinction phases, and for the test in both experiments. Repeated measures analyses of variance (ANOVAs) and/or paired samples *t*-tests, where appropriate, were used to compare response rates across stimulus conditions. The Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995) was used to control $\alpha = 0.05$ for families of multiple *t*-tests.

3. RESULTS

3.1. Experiment 1

3.1.1. Discrimination training—Rats required 1-3 sessions to acquire the lever-press response with the light on continuously. They then had a mean total of 36.8 (± 1.2 SEM) total discrimination training sessions (including the terminal baseline sessions and all previous discrimination training sessions). Figure 1 shows the mean ($\pm$ SEM) response rates (responses/min) in Stimuli X, Y, A and in S^A components averaged over the last two sessions on the terminal baseline schedule. A repeated measures ANOVA performed on X, Y, and A response rates revealed a significant effect of stimulus condition ($F[2,24] = 32.2, p < 0.001$). Responding to Stimulus A (the light) was significantly higher than responding to either X or Y ($t[12]s = 5.7, ps < 0.001$), but, most importantly, X and Y did not differ from each other ($t[12] = 0.3, p = 0.3$).

3.1.2. Extinction Phase 1—The left-hand section of Figure 2 presents mean ($\pm$ SEM) response rates in X and Y during the three Extinction Phase 1 sessions. Responding declined to less than 1 response per min in both stimuli by the third session. Mean responses per min during Stimulus A (not presented in figure) over these sessions declined from 10.5 (± 1.3 SEM) to 3.4 (± 0.5 SEM). A 3×3 (Stimulus $\times$ Session) repeated measures ANOVA performed on X, Y, and A response rates confirmed that there was a significant main effect of Stimulus ($F[2,24] = 54.5, p < 0.001$) and Session ($F[2,24] = 17.2, p < 0.001$), as well as a significant interaction ($F[4,48] = 13.5, p < 0.001$). Subsequent paired-sample t-tests indicated that, on each of the 3 sessions, responding to A was significantly higher ($t[12]s = 5.2, ps < 0.001$) than to X or Y, while rates to X and Y were not different from each other ($t[12]s = 0.8, ps = 0.4$). Mean response rates during all-stimuli-off periods were less than 0.5 responses per min during all sessions.

3.1.3. Extinction Phase 2: Stimulus compounding—The two bars in the middle section of Figure 2 present mean ($\pm$ SEM) response rates during the 8 presentations of the AX compound and during the final 8 presentations of stimulus Y during Extinction Phase 2 session. As expected, rats responded more to the AX compound than to Y alone ($t[12] = 4.6, p = 0.001$). Mean response rates during all-stimuli-off periods were less than 0.1 responses per min.

3.1.4. Spontaneous recovery test—Rats in Experiment 1 spent a mean of 13.8 days (range: 13-16) in their homecages prior to the spontaneous recovery test. Mean ($\pm$ SEM) response rates to X and Y on this test are presented in the right-hand section of Figure 2 (rats made on average less than 0.5 responses per min during all-stimuli-off periods). Rats responded significantly more to stimulus X than to Stimulus Y ($t[12] = 2.4, p < 0.05$).

3.2. Experiment 2

3.2.1. Discrimination training

Rats required 1-2 sessions to acquire the lever-press response with the light on continuously. They then had a mean of 18.0 (± 0.7 SEM) total discrimination training sessions (including terminal baseline sessions and all previous discrimination sessions). Figure 1. shows the mean ($\pm$ SEM) response rates (responses/min) in Stimuli X, Y, A and in S^A components averaged over the last two sessions on the terminal baseline schedule. A repeated measures ANOVA performed on X, Y, and A response rates revealed a significant effect of stimulus condition ($F[2,22] = 29.1, p < 0.001$). As in Experiment 1, responding to A was significantly higher than responding to either X or Y ($t[11]s > 5.2, ps < 0.001$) but responses rates to X and Y did not differ from each other ($t[11] = 1.1, p = 0.3$).

3.2.2. Extinction Phase 1

As shown in the left section of Figure 3, responding in X and Y declined across the three Phase 1 extinction sessions and responding was comparable across the two stimuli. Mean responses per min during Stimulus A (not presented in figure) over these sessions declined from 11.9 (± 2.6 SEM) to 5.6 (± 1.3 SEM). A 3×3 (Stimulus $\times$ Session) repeated measures ANOVA performed on X, Y, and A response rates confirmed that there was a significant main effect of Stimulus ($F[2,22] = 11.7, p < 0.001$) and Session ($F[2,22] = 19.9, p < 0.001$), as well as a significant interaction ($F[4,44] = 4.5, p < 0.005$). Subsequent paired-sample *t*-tests indicated that, on each of the 3 sessions, responding to A was significantly higher ($t[11]s = 2.8, ps = 0.02$) than to X or Y, while rates to X and Y were not different from each other ($t[11]s = 1.0, ps = 0.3$). Mean response rates during all-stimuli-off periods were less than 0.5 responses per min during all sessions.

3.2.3. Extinction Phase 2: Stimulus compounding

The two bars in the middle section of Figure 3 present mean ($\pm$ SEM) response rates during the 8 presentations of the AX compound and during the final 8 presentations of stimulus Y during the Extinction Phase 2 session. As expected, rats responded significantly more to the AX compound than to Y alone ($t[11] = 4.0, p < 0.005$). Mean response rate during all-stimuli-off periods were less than 0.1 responses per min.

3.2.4. Spontaneous recovery test

Rats in Experiment 2 spent a mean of 13.9 days (range: 13 to 16) in their homecages prior to the spontaneous recovery test. The two bars in the right section of Figure 3 present mean ($\pm$ SEM) response rates in X and Y during the spontaneous recovery test (rats made on average 0.5 responses per min during all-stimuli-off periods). Now, opposite to the result of Experiment 1, rats responded significantly more to Y than to X ($t[11] = 2.3, p < 0.05$).

4. DISCUSSION

In Experiment 1, compounding a food cue with a cocaine cue during extinction did not produce deepened extinction of the cocaine seeking elicited by the cocaine cue, but instead led to heightened cocaine seeking on a spontaneous recovery test. In contrast, in Experiment 2, which was conducted under the same conditions and with the same stimuli used in Experiment 1, compounding a food cue with another food cue produced deepened extinction, thereby replicating the results of previous studies that compounded 2 food cues (Janak and Corbit, 2010; Rescorla, 2006, Experiments 2-4). These results, taken together with previous studies showing that deepened extinction occurs when 2 cocaine cues (Kearns et al., 2012; Janak et al., 2011) are presented simultaneously during extinction indicate that deepened extinction depends on the compounded cues having been previously associated with the same reinforcer (or US).

The theoretical basis for deepened extinction is the prediction error resulting from the overexpectation for reward during compound cue extinction. That deepened extinction of the response elicited by the cocaine cue was not observed in Experiment 1 suggests that co-presentation of the food cue did not produce a prediction error during the compound extinction trials that affected the cocaine cue. Thus, this outcome is not consistent with the view that cues within a motivational class (appetitive vs. aversive) have functionally comparable effects (Dickinson and Dearing, 1979; Weiss et al., 1996). Instead, the results of Experiment 1 are more consistent with studies showing that the associations that animals learn are highly outcome specific, even when both outcomes are within the appetitive class (Balleine and Ostlund, 2007; Bonardi, 1989; Colwill and Triola, 2002; Corbit et al., 2007; Delamater et al., 2003; Lombas et al., 2008; Rescorla, 1990). The most surprising result in

Experiment 1 was that there was significantly more spontaneous recovery of responding to the cocaine cue extinguished in compound with the food cue as compared to the cocaine cue always presented on its own in extinction. The simultaneous presentation of A (the food cue) with X (a cocaine cue) may have somehow protected X-elicited responses from the extinction they would have undergone if X was presented alone (as Y was). For example, if A were a more salient stimulus than X, it may have diverted attention from X during the compound AX trials and prevented the further extinction of X-elicited responses. That is, A may have overshadowed X during the AX extinction trials. That response rates to A were higher than to X during training and extinction provides some support for the notion that A was the more salient stimulus.

There are two reasons, however, why this overshadowing account seems unlikely. First, by the time the AX compound was presented, X had already been presented individually during 3 prior extinction sessions. It would seem that relatively little additional extinction learning would have occurred during individual X presentations in a fourth extinction session. Thus, there should not have been much extra extinction learning for A to interfere with on the compound extinction session. Second, in Experiment 2 overshadowing of X by A would also be expected during AX extinction trials, since A in that experiment also controlled a significantly higher response rate by virtue of signaling a richer schedule of reinforcement. But, in contrast to the outcome of Experiment 1, co-presentation of A with X in Experiment 2 resulted in the deepened extinction of X-elicited responding.

The formation of within-compound associations (Cunningham, 1981; Holland and Ross, 1981; Rescorla, 1982; Williams et al., 1986) may better explain the greater responding to X than to Y on the spontaneous recovery test in Experiment 1. During the AX extinction trials rats could have learned an association between X (the cocaine cue) and A (the food cue). Previous experiments (Williams et al., 1986) on within-compound associations have shown that they can be formed with as few as 8 compound cue presentations, which is exactly how many there were in Experiment 1. If rats learned an X-A association on the compound extinction session, then presentation of X – but not Y – on the spontaneous recovery test would have retrieved the representation of A. Because A previously controlled a relatively high response rate, its retrieval during presentation of X would be expected to boost response rates. This could explain the higher response rates to X than to Y on the test in Experiment 1. Rats in Experiment 2 may have also learned a within-compound association, but the deepening of the extinction of X-elicited responding in that experiment appears to have more than offset any potential increase in responding that X's retrieval of A may have produced. Future experiments that prevent or otherwise neutralize potential within-compound associations could lead to the development of a procedure that produces an even greater deepened extinction effect.

In the present study, rats in Experiment 1 had approximately twice as many training sessions (36.8 vs. 18.0) as did the rats in Experiment 2. In both experiments, rats were trained until they met the same stimulus control criteria. Though the subjects in Experiment 1 needed more sessions to achieve the required level of discriminative performance, responding in stimuli X, Y, and A relative to S^{Δ} responding was comparable across experiments by the end of training (see Figure 1). Results from our earlier deepened extinction paper (Kearns et al., 2012) also suggest that the number of sessions required to reach criterion in Experiment 1 of the present study was not responsible for the failure to observe deepened extinction. In Experiment 2 of Kearns et al. (2012), rats required a mean of 29.4 (± 3.5 SEM) sessions when trained on a procedure similar to that used in Experiment 1 of the present study except that responding in light was maintained by cocaine rather than food. In that earlier experiment, the deepened extinction effect was observed even though a relatively large number of training sessions was required. This suggests that the difference between the

outcomes of Experiments 1 and 2 of the present study were due to the identities of the reinforcers that previously maintained responding in the stimuli compounded during extinction, rather than to the difference in number of training sessions.

In summary, compounding a non-drug appetitive cue with a cocaine cue during extinction did not deepen the extinction of the cocaine seeking elicited by the cocaine cue, but instead augmented cue-elicited responding. It appears that for deepened extinction of cue-elicited cocaine seeking to occur, an increased expectation specifically for cocaine is necessary during extinction, rather than a more general increase in reward expectation or simply an increased response rate. This seems to suggest that temporarily generating a desire for a drug in a controlled setting will be crucial for successful deepened extinction treatments of drug-cue-elicited drug seeking. However, the present results suggest that great care must be taken in evaluating such treatments designed to enhance extinction before using them in humans. Other procedures also designed to enhance extinction have sometimes had the opposite of their intended effect. For example, while some studies have found that administration of d-cycloserine (DCS) facilitated extinction of drug cues (e.g., Torregrossa et al., 2010), another study found that DCS *increased* the power of cocaine cues (Lee et al., 2009). Likewise, while some studies have found that interspersing extinction trials within a retrieval-reconsolidation window enhanced the extinction of cue-elicited drug seeking (Xue et al., 2012), others have found *increased* drug seeking (Millan et al., 2013). Thoroughly testing cue-targeted treatments and optimizing procedural parameters within pre-clinical models (e.g., as in Buffalari et al., 2013) will be essential for maximizing the benefits, and minimizing potential risks, of such treatments before they are used in humans.

Acknowledgments

Role of Funding Source This research was supported by Award Number R01DA008651 from the National Institute on Drug Abuse (NIDA). The content is solely the responsibility of the authors and does not necessarily represent the official views of NIDA or the National Institutes of Health. NIDA did not play a role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decisions to submit the paper for publication.

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Figure 1. Experiment 1 and 2 baseline training data. Data presented are the mean ($\pm$ SEM) response rates (responses per min) in Stimuli A (black bars), X (white bars), Y (horizontal stripes), and all-stimuli-off (vertical stripes) components averaged over the final 2 training sessions.

Figure 2.

Experiment 1 extinction and test results. Data presented are the mean ($\pm$ SEM) response rates (responses per min) in Stimuli X (black bars) and Y (white bars) during the 3 sessions of Extinction Phase 1, the Extinction Phase 2 session, and spontaneous recovery test. * indicates $p < 0.05$.

Figure 3.

Experiment 2 extinction and test results. Data presented are the mean ($\pm$ SEM) response rates (responses per min) in Stimuli X (black bars) and Y (white bars) during the 3 sessions of Extinction Phase 1, the Extinction Phase 2 session, and spontaneous recovery test. * indicates $p < 0.05$.